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Diabetes & Metabolism 39 (2013) 330–336

Original article

Fasting insulin at baseline influences the number of cardiometabolic risk factors and R-R interval at 3 years in a healthy population: The RISC Study

Z. Pataky [a,*], A. Golay [a], M. Laville [b], E. Disse [b], A. Mitrakou [c], C. Guidone [d], R. Gabriel [e], E. Bobbioni-Harsch [a], on behalf of the RISC Investigators

[a] *Service of Therapeutic Education for Chronic Diseases, WHO Collaborating Centre, University Hospitals of Geneva and University of Geneva, Geneva, Switzerland*
[b] *Centre de Recherche en Nutrition Humaine Rhône-Alpes, Pierre-Bénite, France*
[c] *Diabetes-Metabolism unit, Department of Clinical Therapeutics, University of Athens, Medical School, Alexandra Hospital, Athens, Greece*
[d] *Department of Internal Medicine, Catholic University, Rome, Italy*
[e] *Hospital Universitario La Paz, Madrid, Spain*

Received 13 December 2012; received in revised form 23 May 2013; accepted 26 May 2013

Abstract

Aim. – This was a cross-sectional and longitudinal study of factors contributing to the number of cardiometabolic risk factors, common carotid artery intima–media thickness (CCA-IMT) and R-R interval in clinically healthy subjects without diabetes.

Methods. – Anthropometric and cardiometabolic parameters were measured in the Relationship between Insulin Sensitivity and Cardiovascular Disease (RISC) Study cohort at baseline ($n = 1211$) and 3 years later ($n = 974$). At baseline, insulin sensitivity was assessed by the euglycaemic clamp technique. The CCA-IMT was echographically measured and the R-R interval was electrocardiographically evaluated at baseline and at the 3-year follow-up.

Results. – Higher baseline BMI, fasting insulin and tobacco use as well as greater changes in BMI and fasting insulin but lower adiponectin levels, were associated with a greater number of cardiometabolic risk factors at the 3-year follow-up independently of insulin sensitivity (all $P < 0.02$). The CCA-IMT increased with the number of cardiometabolic risk factors ($P = 0.008$), but was not related to fasting insulin, whereas higher fasting insulinaemia and its 3-year changes were significantly associated with a smaller R-R interval ($P = 0.005$ and $P = 0.002$, respectively). These relationships were independent of baseline age, gender, BMI, adiponectin, insulin sensitivity, tobacco use and physical activity.

Conclusion. – In clinically healthy subjects, fasting insulinaemia, adiponectin and lifestyle parameters are related to the presence of one or two cardiometabolic risk factors before criteria for the metabolic syndrome are met. These results underline the importance of fasting insulinaemia as an independent cardiometabolic risk factor at an early stage of disease development in a healthy general population.

Keywords: Lifestyle; Fasting insulin; Cardiometabolic risk factors

Résumé

L'insulinémie à jeun influence le nombre de facteurs de risque cardiométabolique et l'interval R-R dans une population saine : l'étude RISC.

But. – Examiner les contributeurs de facteurs de risque cardiométabolique dans une population cliniquement saine et sans diabète.

Méthodes. – Les paramètres anthropométriques et cardio-métaboliques ont été mesurés dans la cohorte Relationship between Insulin Sensitivity and Cardiovascular Disease (RISC) au temps 0 ($n = 1211$) et à trois ans ($n = 974$). La sensibilité à l'insuline a été mesurée par la méthode de clamp hyperinsulinémique–euglycémique et l'épaisseur de la paroi de l'artère carotide interne (CCA-IMT) a été mesurée par l'échographie. L'intervalle R-R a été mesuré par un électrocardiogramme.

Résultats. – Au temps 0, une indice de masse corporelle (IMC) élevée, l'hyperinsulinémie à jeun et la consommation du tabac, une augmentation de l'IMC et de l'insulinémie à jeun, ainsi que des taux d'adiponectine basses à trois ans ont été associé à l'augmentation

* Corresponding author. Service of Therapeutic Education for Chronic Diseases, WHO Collaborating Centre, Gabrielle Perret-Gentil 4, CH-1211 Geneva 14, Switzerland. Tel.: +41 2 23 729 722; fax: +41 2 237 297 15.
E-mail address: zoltan.pataky@hcuge.ch (Z. Pataky).

1262-3636/$ – see front matter
http://dx.doi.org/10.1016/j.diabet.2013.05.008

du nombre de facteurs de risque cardiométabolique à trois ans, indépendamment de la sensibilité à l'insuline ($P < 0{,}02$ pour tous). La CCA-IMT augmentait avec l'augmentation du nombre de facteurs de risque cardiométabolique ($P = 0{,}008$). L'hyperinsulinémie au temps 0 et ses modifications à trois ans ont été associée à l'interval R-R ($P = 0{,}005$, 0,002, respectivement). Ces associations ont été indépendantes de l'âge, sexe, IMC, adiponectine, sensibilité à l'insuline, consommation du tabac et activité physique au temps 0.

Conclusion. – Dans une population saine, l'insulinémie à jeun, l'adiponectine et le mode de vie sont associés à la présence d'un ou de deux facteurs de risque cardiométabolique, avant que les critères du syndrome métabolique se réunissent. Nos résultats confirment l'hyperinsulinémie à jeun comme facteur de risque cardiométabolique indépendant.

Mots clés : Mode de vie ; Insulinémie à jeun ; Facteurs de risque cardio-métabolique

1. Introduction

The association between body weight and cardiometabolic morbidity and mortality has been widely described in the literature [1–3]. The metabolic syndrome, defined as the concomitant presence of three out of five cardiometabolic risk factors, has been recognized as an intermediary step between uncomplicated obesity and the presence of cardiometabolic diseases [4–6]. More recently, however, several authors have suggested that the presence of any cardiometabolic risk factor, even before the establishment of the metabolic syndrome, should be taken into account in the prevention of cardiometabolic diseases [7–9]. Furthermore, an increasing number of cardiometabolic risk factors have been linearly linked to an increased risk of all-cause and cardiovascular mortality over a 14-year follow-up period [10], thus, supporting the role played by individual cardiometabolic risk factors in the occurrence of cardiometabolic diseases. As the importance of the number of cardiometabolic risk factors has also been well established by these studies, it is relevant to investigate which factors can influence this number.

According to some authors, fasting hyperinsulinaemia has been suggested to contribute to insulin resistance [11] and the development of type 2 diabetes [12,13]. Indeed, a cross-sectional relationship was found between fasting insulinaemia and cardiometabolic risk factors in the Relationship between Insulin Sensitivity and Cardiovascular Disease (RISC) Study cohort [14].

The primary aim of the present study was to evaluate the impact of baseline fasting insulinaemia, insulin sensitivity, adiponectin and lifestyle parameters on the number of cardiometabolic risk factors at 3 years of follow-up. The secondary aim was to investigate the possible association between fasting insulinaemia and the number of metabolic syndrome components at baseline and (i) subclinical atherosclerotic disease, as measured by intima–media thickness (IMT) of the common carotid artery (CCA) at years, and (ii) the autonomic nervous system evaluated by heart rate, as measured by the R-R interval at 3 years.

The RISC Study cohort included a clinically healthy population with neither diabetes nor overt cardiovascular disease (CVD). This allowed for the investigation of the number of cardiometabolic risk factors before the presence of overt cardiometabolic disease.

2. Methods

2.1. Study population

The present investigation examined a clinically healthy population with no diabetes or any other overt diseases from the RISC Study, the rationale and design of which has been published elsewhere [15]. In brief, participants were recruited at 21 centers from the local populations of 13 European countries, according to the following inclusion criteria: any gender; age between 30 and 60 years; and clinically healthy. Exclusion criteria were the presence of chronic diseases, overt CVD, carotid stenosis greater than 40%, and treatment for hypertension, diabetes and/or dyslipidaemia. In addition, after screening, only those with blood pressure lower than 140/90 mmHg, plasma cholesterol lower than 7.8 mmol/L, triglycerides lower than 4.6 mmol/L, and fasting and 2-h glucose lower than 7.0 mmol/L and lower than 11.1 mmol/L, respectively, were enrolled. The cohort was examined at time 0 and after a 3-year follow-up period. At both time points, body weight was measured, and the body mass index (BMI) calculated as the ratio between body weight (kg) and height (m^2). At baseline, insulin sensitivity was assessed by the hyperinsulinaemic–euglycaemic clamp technique. IMT at the level of the CCA segment as well as the R-R interval were measured at both baseline and at 3 years (1211 subjects at baseline and 974 at follow-up).

The study protocol had the approval of the relevant local ethics committees. The participants were informed of the aims of the study and gave their written consent.

2.2. Definition of cardiometabolic risk factors

Cardiometabolic risk factors were defined according to the International Diabetes Federation guidelines for diagnosis of the metabolic syndrome [16]: specifically, fasting glucose greater or equal to 5.6 mmol/L; fasting triglycerides greater or equal to 1.7 mmol/L; high-density lipoprotein (HDL) cholesterol less than 1.03 mmol/L and less than1.29 mmol/L in men and women, respectively; blood pressure greater or equal to 130/85 mmHg; and waist circumference greater or equal to 94 cm in men and greater or equal to 80 cm in women.

2.3. Insulin sensitivity

Insulin sensitivity was evaluated by hyperinsulinaemic–euglycaemic clamp [17] at time 0. Insulin was continuously infused at a rate of 240 pmol/min/m^2, and a glucose solution (20%) was infused at variable rates to maintain a constant plasma glucose concentration between 4.5 and 5.5 mmol/L. Plasma glucose was measured at 5- to 10-min intervals to ensure that it remained within the target glucose concentration. The steady-state period (for calculation of insulin sensitivity) was between 80 to 120 min. Insulin sensitivity, measured during the steady-state period, was expressed as mmol/kg fat free mass (FFM)/min/pmol insulin (ins) and reported as M/I (insulin) values. FFM was evaluated using a bio-impedance balance (Tanita International Division, West Drayton, Middlesex, UK).

2.4. Carotid artery ultrasound imaging, R-R interval and blood pressure measurements

CCA-IMT imaging, performed at both baseline and follow-up, followed a validated protocol [18]. Longitudinal B-mode images were taken of the right and left CCAs from anterior, lateral and posterior angles. The same reader evaluated images from all the 21 participating centres. CCA frames during diastole were selected to provide images of the near and far walls of the intima–media complex. Frames were digitized and analyzed using a medical image processing (MIP) system [19] (National Research Council Institute of Clinical Physiology, Pisa, Italy). Lines were drawn along the lumen–intima and media–adventitia interfaces, with IMTs then computed as an average of several measurements. A 30-s longitudinal B-mode image was taken of the right CCA and the concomitant blood pressure measured using a sphygmomanometer cuff. An automatic contour detection algorithm determined the average minimum and maximum CCA diameter.

The R-R interval, a parameter associated with autonomic neuropathy, was derived from a 10-min electrocardiography (ECG) recording and expressed in ms [20].

Sitting blood pressure was the median value of three measurements taken with an OMRON 705CP blood pressure monitor (OMRON Healthcare Europe, Hoofddorp, The Netherlands).

2.5. Analytical methods

Plasma samples were analyzed at different core laboratories according to the analysis to be performed (all given analyses were done in the same laboratory for all the samples). In Odense, Denmark, glucose was determined by the glucose oxidase technique (Cobas Integra; Hoffmann-La Roche, Basel, Switzerland) while insulin was measured by fluoroimmunoassay (AutoDELFIA insulin kit; Wallac Oy, Turku, Finland). Plasma adiponectin was determined by a previously described time-resolved immunofluorometric assay [21]. Total and HDL cholesterol and triglycerides were measured by an enzymatic colorimetric method for a modular system (Hoffmann-La Roche). LDL cholesterol concentrations were calculated by the Friedewald formula [22].

2.6. Lifestyle parameter assessment

Information was collected on the socioeconomic status of the study participants and their partners, their personal medical history and family history of CVD, stroke, hypertension and diabetes in first-degree relatives, smoking status and alcohol intake. The lifestyle questionnaire was prepared in English, then translated into 11 languages for the study and back-translated into English to ensure homogeneity. At baseline, all the participants were fitted with a Computer Science Applications (CSA) Actigraph [now Manufacturing Technology, Inc. (MTI), Fort Walton Beach, FL, USA] attached to a waist belt for 3–7 days. The Actigraph is a single-channel recording accelerometer capable of continuous data collection. Data were summed over 1-min periods and expressed as counts per minute (cpm).

2.7. Statistical analysis

At baseline, the participants were classified according to the number of cardiometabolic risk factors they presented, ranging from 0 to 5. Due to null distribution, data were log-transformed (insulin, adiponectin, M/I value, tobacco use, alcohol consumption and physical activity) and the differences between subgroups were investigated by factorial analysis of variance (ANOVA) followed by Fisher's post-hoc test. In Figs. 1 and 2 and Supplementary data, Fig. SI, values are presented as means ± SD. Independent contributors to the number of cardiometabolic risk factors were evaluated by multiple regression analysis after adjusting for recruitment center.

At the 3-year follow-up, independent contributors to the number of cardiometabolic risk factors, CCA-IMT and R-R interval were evaluated by multiple regression analysis. All statistical analyses were performed using STATA v.10 software (StataCorp LP, College Station, TX, USA). The alpha level was set at 0.05.

3. Results

3.1. Characteristics of the study population

At baseline (Table 1), 1211 volunteers (613 normal weight and 598 overweight/obese, 531 men and 680 women) with a mean age of 44 ± 8 years (range: 29–61 years) and mean BMI of 25.4 ± 4.0 kg/m^2 (range: 17–44 kg/m^2) were examined. A total of 974 subjects (503 normal weight and 471 overweight/obese, 432 men and 542 women) completed the 3-year follow-up.

3.2. Cross-sectional analyses

As shown in Fig. 1, when the participants were stratified according to the number of cardiometabolic risk factors at baseline, the mean fasting insulin levels differed between strata (ANOVA $P < 0.0001$, Fig. 1a), as did also adiponectin

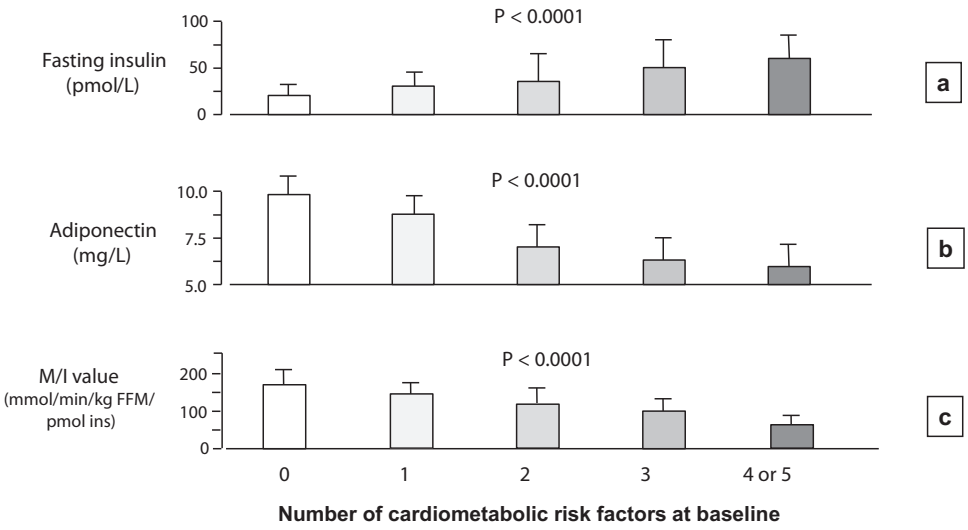

Fig. 1. Fasting insulin (a), adiponectin (b) and M/I values (c) in the RISC Study cohort, stratified according to the number of cardiometabolic risk factors at baseline (*P* values by ANOVA).

($P < 0.0001$, Fig. 1b). Insulin sensitivity (M/I value, Fig. 1c) was lower, the more cardiometabolic risk factors there were ($P < 0.0001$). Fisher's post-hoc analysis showed that fasting insulin values were significantly different between each cardiometabolic risk factor category ($P < 0.0001$).

Of the evaluated lifestyle parameters, tobacco use ($P = 0.002$) and alcohol intake ($P = 0.03$) as well as physical activity ($P = 0.0007$) were also associated with the number of cardiometabolic risk factors (Supplementary data, Fig. SIa–c).

In addition, the number of cardiometabolic risk factors was associated with both the CCA-IMT ($P < 0.0001$) and R-R interval ($P = 0.009$; Supplementary data, Fig. SII a and b).

3.3. Longitudinal analyses

BMI, fasting insulin, adiponectin and tobacco use all significantly and independently contributed to the number of cardiometabolic risk factors at the 3-year follow-up (Table 2) after additional adjustments for study center. In contrast, the M/I value, alcohol consumption and physical activity were not associated with the number of risk factors.

Δ BMI and Δ fasting insulin were both significantly ($P < 0.0001$) associated with the number of cardiometabolic risk factors at the 3-year follow-up after adjusting for age, gender, baseline BMI, fasting insulin, M/I value, adiponectin, tobacco use, physical activity and study center.

However, no significant association was seen between the evolution of cardiometabolic risk factors and changes in CCA-IMT, whereas the Δ R-R interval was significantly linked to both Δ number of cardiometabolic risk factors ($P = 0.02$) and Δ fasting insulin ($P = 0.002$; data not shown here).

On multiple regression analysis (Table 3) after adjusting for age, gender, BMI, fasting insulin, M/I value, lifestyle parameters and study center, the number of cardiometabolic risk factors at follow-up remained significantly associated with CCA-IMT ($P = 0.008$) while fasting insulin significantly ($P = 0.005$) contributed to the R-R interval.

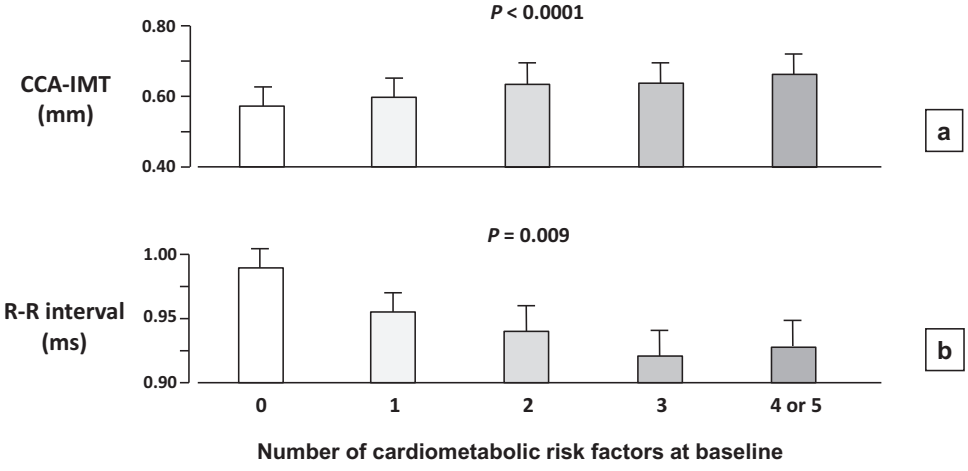

Fig. 2. Common carotid artery intima–media thickness (a) and R-R interval (b) at baseline in the RISC cohort, stratified according to the number of cardiometabolic risk factors at baseline (*P* values by ANOVA).

Table 1
Baseline characteristics of the study population ($n = 1211$ unless otherwise stated).

Variables	Mean (SD)
Age (years)	43.7 (8.3)
Gender (male/female)	531/680
Body mass index (kg/m^2)	25.4 (4.0)
Waist circumference (cm)	86.2 (12.7)
Fasting glucose (mmol/L)	5.0 (0.5)
Total cholesterol (mmol/L)	4.8 (0.8)
HDL cholesterol (mmol/L)	1.4 (0.4)
LDL cholesterol (mmol/L)	2.9 (0.8)
Triglycerides (mmol/L)	1.0 (0.5)
Fasting insulin (pmol/L)	31.9 (21.3)
Adiponectin (mg/L)	8.5 (3.8)
M/I value (mmol/min/kg FFM/mmol ins)	142.9 (72.1)
CCA-IMT (mm)	0.600 (0.085)
R-R interval (s)	0.955 (0.152)
Tobacco use (g/day)	8.1 (11.9)
Physical activity (cpm, $n = 752$)	368.7 (176.6)
Alcohol consumption (g/week)	71.0 (83.4)

HDL: high-density lipoprotein; LDL: low-density lipoprotein; CCA-IMT: common carotid artery intima–media thickness; cpm: counts per minute.

Table 2
Independent contributors to the number of cardiometabolic risk factors at the 3-year follow-up (multiple regression analysis adjusted for study center).

Independent variables	Regression coefficients	P value
Age (years)	0.019	< 0.0001
Gender (male = 1, female = 2)	0.161	0.07
Body mass index (kg/m^2)	0.097	< 0.0001
Δ Body mass index (kg/m^2)	0.129	< 0.001
Fasting insulin (pmol/L)	0.014	< 0.0001
Δ Fasting insulin (pmol/L)	0.009	< 0.0001
Adiponectin (mg/L)	−0.039	0.002
M/I value (mmol/min/kg FFM/mmol ins)	−0.137	0.20
Tobacco use (g/day)	0.007	0.02
Physical activity (cpm)	−0.001	0.29
Alcohol consumption (g/week)	0.001	0.73

cpm: counts per minute

4. Discussion

Our present study in a clinically healthy general population has revealed that at baseline, higher fasting insulin was linearly associated with the number of cardiometabolic risk factors that, in turn, influenced the CCA-IMT (Figs. 1 and 2). Previous investigations had shown the high prevalence of cardiometabolic risk factors in the higher BMI categories [23–25] as well as the impact of lifestyle parameters [26,27]. Our present study has demonstrated that both fasting insulin and lifestyle were already related to the presence of one or two cardiometabolic risk factors even before criteria for the metabolic syndrome were fulfilled (Fig. 1 and Table 2). Indeed, this early relationship also applied to the normal and near-normal BMI classes.

However, our results showed that baseline insulin sensitivity was no longer associated with the number of cardiometabolic risk factors once the model was adjusted for BMI and fasting insulin, as indicated by multiple regression analysis (Table 2). Yet, fasting insulin remained independently associated with the number of cardiometabolic risk factors at year 3, which is consistent with other reports from the RISC Study [14,28]. This result further confirms that fasting insulinaemia and insulin sensitivity, although strongly linked, may play independent roles in the pathophysiology of cardiometabolic diseases. Such consistent demonstration of the predominant role of insulinaemia, in contrast to insulin resistance, is one of the more original findings of the RISC Study.

It has been suggested that hyperinsulinaemia could be the primary lesion in type 2 diabetes and that the inability to respond to a mixed meal before bariatric surgery is due to the already elevated basal insulin secretion [29]. Roux-en-Y surgery may serve as a model for studying concepts of diabetes onset from a pathophysiological point of view. Recent data have shown that, after bypass surgery, fasting insulin was independent of the effects of fasting glucose, fasting free fatty acids and insulin sensitivity in non-diabetic subjects 1 year after gastric bypass and in weight-stable conditions [29,30]. Our present study has shown that, in a clinically healthy population, fasting insulin was

Table 3
Independent baseline contributors to common carotid artery intima–media thickness (CCA-IMT) and R-R interval at a 3-year follow-up (multiple regression analysis adjusted for study center).

Independent variables	Dependent variables			
	CCA-IMT		R-R interval	
	Regression coefficient	P value	Regression coefficient	P value
Age (years)	0.005	< 0.0001	0.001	0.69
Gender (male = 1, female = 2)	−0.024	0.001	−0.078	< 0.0001
Cardiometabolic risk factor number	0.010	0.008	−0.009	0.13
Body mass index (kg/m^2)	0.002	0.10	−0.001	0.81
Δ Body mass index (kg/m^2)	0.004	0.08	−0.010	0.01
Fasting insulin (pmol/L)	−0.001	0.80	−0.001	0.005
Δ Fasting insulin (pmol/L)	−0.001	0.08	−0.001	0.002
Adiponectin (mg/L)	−0.001	0.97	−0.001	0.34
M/I value (mmol/min/kg FFM/mmol ins)	0.001	0.59	0.001	0.41
Tobacco use (g/day)	0.001	0.05	−0.001	0.61
Physical activity (cpm)	−8.20E-6	0.68	0.001	0.005

cpm: counts per minute

significantly associated with the number of cardiometabolic risk factors independently of insulin sensitivity, BMI, adiponectin and lifestyle parameters (tobacco use, alcohol consumption and physical activity). This association did not change even after further adjustments for both fasting and 2-h plasma glucose.

By demonstrating the concomitant occurrence at baseline of enhanced thickness of the carotid artery wall and heart rate, both of which are influenced by the number of cardiometabolic risk factors [20,31], our results further highlight the importance of the number of cardiometabolic risk factors in CVD risk.

Longitudinally, our findings failed to demonstrate any impact of cardiometabolic risk factor modification on the evolution of CCA-IMT, which may have been due to the short follow-up period. Nevertheless, in the results from Koskinen et al. [31], while CCA-IMT progression was influenced by baseline cardiometabolic risk factors, it was not affected by changes in the number of cardiometabolic risk factors during a 6-year follow-up.

On the other hand, the R-R interval at year 3 was strongly associated with fasting insulin at baseline and Δ fasting insulin. It is well known that an acute rise in plasma insulin concentration shifts the cardiac autonomic nervous system balance in a dose-dependent manner towards sympathetic activation, even in healthy subjects [32,33]. Muscelli et al. [34] demonstrated that the acute effects of insulin on sinus node activity were similar between lean and obese insulin-resistant subjects, and unrelated to insulin resistance in glucose metabolism. Also, a smaller R-R interval has been shown to be associated with conventional cardiometabolic risk factors, particularly diabetes, obesity and the metabolic syndrome [35], as well as fasting plasma glucose and triglycerides [36]. Furthermore, the R-R interval has been found to be predictive of cardiac mortality and is therefore a means of identifying those at high risk of cardiovascular death among patients with or without diabetes [37] and in the general population [38].

Recently, it was demonstrated that massive weight loss had beneficial effects on heart rate variability in morbidly obese patients independently of insulin sensitivity [20]. Our present study has shown, for the first time, that heart rate is regulated by the number of cardiometabolic risk factors and by fasting insulin levels.

Taken altogether, our present results and those from the literature suggest several conclusions. The first and most relevant is that the cardiometabolic profile is related to fasting insulinaemia independent of insulin sensitivity. Second, the relationship between the number of cardiometabolic risk factors and cardiovascular parameters, such as the CCA-IMT and R-R interval clearly indicates that each cardiometabolic risk factor can affect the cardiovascular profile even in the early stages and in a clinically healthy population.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

Acknowledgements

The RISC Study was supported by EU grant no. QLG1-CT-2001-01252 and an additional grant from AstraZeneca (Sweden). Locally, the Geneva centre received the support of the Hans Wilsdorf Foundation and the Swiss Life Insurance Foundation.

Appendix A. Supplementary data

Supplementary data (Fig. S1 and Table SI) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.diabet.2013.05.008.

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