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# Proinsulin to insulin ratio is associated with incident type 2 diabetes but not with vascular complications in the KORA F4/FF4 study

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## ABSTRACT

**Introduction** We investigated the association of the proinsulin to insulin ratio (PIR) with prevalent and incident type 2 diabetes (T2D), components of the metabolic syndrome, and renal and cardiovascular outcomes in the population-based Cooperative Health Research in the Region of Augsburg (KORA) F4 study (2006–2008)/FF4 study (2013–2014).

**Research design and methods** The analyses included 1514 participants of the KORA F4 study at baseline and 1132 participants of the KORA FF4 study after a median follow-up time of 6.6 years. All-cause and cardiovascular mortality as well as cardiovascular events were analyzed after a median time of 9.1 and 8.6 years, respectively. The association of PIR with T2D, renal and cardiovascular characteristics and mortality were assessed using logistic regression models. Linear regression analyses were used to assess the association of PIR with components of the metabolic syndrome.

**Results** After adjustment for sex, age, body mass index (BMI), and physical activity, PIR was associated with prevalent (OR: 2.24; 95% CI 1.81 to 2.77;  $p < 0.001$ ) and incident T2D (OR: 1.66; 95% CI 1.26 to 2.17;  $p < 0.001$ ). PIR was associated with fasting glucose ( $\beta$  per SD:  $0.11 \pm 0.02$ ;  $p < 0.001$ ) and HbA1c ( $\beta$ :  $0.21 \pm 0.02$ ;  $p < 0.001$ ). However, PIR was not positively associated with other components of the metabolic syndrome and was even inversely associated with waist circumference ( $\beta$ :  $-0.22 \pm 0.03$ ;  $p < 0.001$ ), BMI ( $\beta$ :  $-0.11 \pm 0.03$ ;  $p < 0.001$ ) and homeostatic model assessment of insulin resistance ( $\beta$ :  $-0.22 \pm 0.02$ ;  $p < 0.001$ ). PIR was not significantly associated with the intima-media thickness (IMT), decline of kidney function, incident albuminuria, myocardial infarction, stroke, cardiovascular or all-cause mortality.

**Conclusions** In the KORA F4/FF4 cohort, PIR was positively associated with prevalent and incident T2D, but inversely associated with waist circumference, BMI and insulin resistance, suggesting that PIR might serve as a biomarker for T2D risk independently of the metabolic syndrome, but not for microvascular or macrovascular complications.

## INTRODUCTION

Vascular complications and a roughly doubled risk for all-cause mortality pose a great burden to patients with type 2 diabetes (T2D).<sup>1</sup>

## Significance of this study

### What is already known about this subject?

► Elevated proinsulin may derive from compensatory hyperinsulinemia in insulin-resistant states and/or from inefficient proinsulin processing or premature proinsulin release, indicating an insulin secretion defect. To correct for compensatory hyperinsulinemia, the proinsulin to insulin ratio instead of proinsulin was suggested. Whether the proinsulin to insulin ratio is independently associated with incident type 2 diabetes and cardiovascular complications is still under debate.

### What are the new findings?

► The proinsulin to insulin ratio was associated with an increased risk of incident type 2 diabetes in the Cooperative Health Research in the Region of Augsburg F4 study (2006–2008)/FF4 study (2013–2014).  
► The proinsulin to insulin ratio was not positively related to other components of the metabolic syndrome and was even inversely associated with waist circumference, body mass index and insulin resistance.  
► The proinsulin to insulin ratio was not associated with the intima-media thickness as measure of prevalent subclinical atherosclerosis, nor with cardiovascular or all-cause mortality.  
► The proinsulin to insulin ratio was not associated with decline of kidney function or incident albuminuria.

Gerald Reaven suggested that in T2D, insulin resistance causes compensatory hyperinsulinemia that will lead to the metabolic syndrome and finally results in cardiovascular disease.<sup>2</sup> In line, the risk for cardiovascular disease has been shown to be doubled in insulin-resistant compared with insulin-sensitive individuals with pre-diabetes.<sup>3</sup>

Recently, the awareness for the heterogeneity of T2D has increased and efforts have been undertaken to define subgroups of T2D

## Significance of this study

## How might these results change the focus of research or clinical practice?

- An elevated proinsulin to insulin ratio is an independent risk factor for the development of type 2 diabetes. Due to the lacking or even inverse association with other components of the metabolic syndrome and anthropometric measures, the proinsulin to insulin ratio might be an interesting candidate marker for lean individuals at risk for type 2 diabetes. Future research should focus on the question whether the proinsulin to insulin ratio represents a prognostic marker to identify patients with diabetes at a comparably lower risk for diabetes-associated complications.

that may differ in terms of pathophysiology, treatment requirements and prognosis. Up to now, T2D was mainly regarded as a consequence of obesity-induced insulin resistance as part of the metabolic syndrome.<sup>4</sup> However, lean individuals constitute a substantial proportion of patients with T2D and the pathophysiology of T2D in this group is still under debate.<sup>5</sup> A deeper knowledge on the underlying pathophysiology and outcomes of such subtypes would enable a more personalized treatment and surveillance of patients with T2D. A new concept put forward by Ahlqvist *et al* uses six variables (glutamate decarboxylase antibodies, age at diagnosis, body mass index (BMI), HbA1c and homeostatic model assessment 2 estimates of  $\beta$ -cell function and insulin resistance) to assign adult-onset T2D into five clusters.<sup>6</sup> Among them, three clusters mainly include participants with a low BMI: cluster 1, referred to as severe autoimmune diabetes and characterized by positive glutamate decarboxylase antibodies (GAD) antibodies; cluster 2, referred to as severe insulin-deficient diabetes (SIDD); and cluster 5, referred to as mild age-related diabetes. The second cluster (SIDD) is characterized by low insulin secretion rather than insulin resistance.<sup>6</sup> This subtype, according to Gerald Reaven, might be less likely to develop cardiovascular complications.<sup>2</sup>

Before the manifestation of diabetes, insulin alone is not able to discern healthy insulin-sensitive individuals from persons with a defect in insulin secretion as in both phenotypes insulin secretion is low. Proinsulin may be a marker for a leaner diabetes risk phenotype. Under physiological conditions, virtually all proinsulin is cleaved at residues 32–33 and 65–66 to produce C peptide and insulin. Only a small amount of intact proinsulin is released into the circulation along with the 32–33 split proinsulin and the 65–66 split proinsulin.<sup>7</sup> Hyperproinsulinemia indicates a pathological state that may arise from inefficient proinsulin processing within the  $\beta$ -cell secretory granula or the premature release of proinsulin.<sup>8</sup>

Because high proinsulin secretion also appears along with high insulin secretion in compensatory hyperinsulinemia, the proinsulin to insulin ratio (PIR) is used to differentiate disproportionally elevated proinsulin from compensatory hyperinsulinemia. Several studies have

shown that a high PIR indicates disturbed insulin secretion.<sup>9–11</sup> PIR has been connected to a primary defect in insulin secretion already in healthy individuals<sup>12</sup> and associates with T2D in a prospective manner.<sup>13–17</sup> Hence, PIR appears to be suitable to detect insulin deficiency already before the onset of T2D.

Our hypothesis was that PIR is associated with prevalent and incident T2D, but not with other components of the metabolic syndrome, the intima-media thickness (IMT) as marker for prevalent subclinical atherosclerosis,<sup>18</sup> or cardiovascular or renal events in the population-based Cooperative Health Research in the Region of Augsburg (KORA) F4 study (2006–2008)/FF4 study (2013–2014).

## METHODS

## Study participants and definition of variables

The KORA F4 (2006–2008) and FF4 (2013–2014) cohort studies are follow-up examinations of the population-based KORA S4 study (1999–2001). Recruitment and eligibility criteria for the KORA studies, study design, standardized sampling methods and data collection (medical history, medication, anthropometric and blood pressure measurements) have been described elsewhere.<sup>19,20</sup> All participants gave written informed consent before taking part. Total proinsulin was measured in the first 1567 participants of the KORA F4 study. Participants with a diabetes type other than type 2 or unknown glucose tolerance status (n=25) and participants with T2D treated with insulin (n=28) were excluded from the analyses. Of the remaining 1514 participants, 66 died before the follow-up examination and 316 declined participation in the FF4 survey or could not be contacted. After exclusion of participants with prevalent T2D in the baseline examination (n=91), the study sample in the longitudinal F4/FF4 examination comprised 1001 participants for the analysis of incident T2D. Participants with missing covariates for other outcomes were excluded from the respective analyses. The numbers of participants included in each analysis are given in the results tables and illustrated in online supplementary figure 1. The median (first quartile; third quartile) follow-up time was 6.6 (6.4; 6.8) years.

Criteria for a clinically diagnosed diabetes mellitus were a validated medical diagnosis or current self-reported use of glucose-lowering agents. After an overnight fasting period, all participants without clinically diagnosed diabetes underwent a standard 75g oral glucose tolerance test. Newly diagnosed diabetes, impaired glucose tolerance (IGT), impaired fasting glucose (IFG), and normal glucose tolerance (NGT) were defined according to the 1999 WHO diagnostic criteria based on both fasting and postchallenge glucose values (T2D:  $\geq 7.0$  mmol/L fasting and/or  $\geq 11.1$  mmol/L 2-hour glucose; IFG:  $\geq 6.1$  and  $< 7.0$  mmol/L fasting glucose; IGT:  $\geq 7.8$  and  $< 11.1$  mmol/L 2-hour glucose). Pre-diabetes was defined as IFG and/or IGT.

Metabolic syndrome was defined according to the International Diabetes Federation definition as presence of at least three of the following five criteria: (1) elevated waist circumference (waist circumference  $\geq 94$  cm in men and  $\geq 80$  cm in women); (2) fasting triglycerides  $\geq 1.7$  mmol/L and/or use of fibrates or nicotinic acid; (3) high-density lipoprotein (HDL) cholesterol  $< 1.0$  mmol/L in men or  $< 1.3$  mmol/L in women and/or use of fibrates or nicotinic acid; (4) fasting glucose  $\geq 5.6$  mmol/L and/or use of glucose-lowering medication; (5) elevated blood pressure (systolic blood pressure  $\geq 130$  mm Hg and/or diastolic blood pressure  $\geq 85$  mm Hg and/or use of antihypertensive medication, given that the participants were aware of being hypertensive).

Arterial hypertension was defined as a systolic blood pressure  $\geq 140$  mm Hg and/or a diastolic blood pressure  $\geq 90$  mm Hg, and/or intake of antihypertensive medication, given that the participants were aware of being hypertensive.

Leisure time physical activity was assessed with two separate questions concerning leisure time sport activity in winter and in summer (cycling included). Possible answers were: (1)  $> 2$  hours, (2) 1–2 hours, (3)  $< 1$  hour, and (4) none per week. Participants who had a total score  $< 5$ , obtained by summing the numbers (1)–(4) from the winter and summer questions, were classified as ‘physically active’.

Total mortality and cardiovascular mortality (International Classification of Diseases Ninth Revision codes 390–459 and 798) were ascertained by regularly checking the vital status of the participants through the population registries. Death certificates were obtained from the local health authorities. Incident non-fatal myocardial infarction occurring until the age of 75 years was assessed by surveillance through the local myocardial infarction registry. Incident non-fatal myocardial infarction occurring in participants  $> 74$  years or residing outside the study area, as well as non-fatal stroke were assessed by postal follow-up questionnaires. Using data from participants’ hospital records and their attending physicians, all self-reported incident stroke cases and myocardial infarction cases occurring outside the study area or in persons  $> 74$  years and the date of diagnosis were validated. Stroke and myocardial infarction were pooled to a combined end point with the only first event taken into account in case of several events. Participants with prevalent stroke ( $n=34$ ) or prevalent myocardial infarction ( $n=41$ ) or missing data on incident stroke and myocardial infarction ( $n=84$ ) were excluded from the analyses regarding the outcome of incident myocardial infarction or stroke. The follow-up time (median (first quartile; third quartile)) was 9.1 (8.8; 9.4) years for total and cardiovascular mortality and 8.6 (8.1; 9.0) years for incident stroke/myocardial infarction.

### Laboratory measurements

Blood samples were collected after an overnight fast of at least 8 hours and were kept at room temperature until centrifugation. Plasma was separated immediately, serum after 30 min. Plasma and serum samples were assayed

immediately or stored at  $-80^{\circ}\text{C}$ . Blood glucose levels were assessed using the hexokinase method (GLU Flex; Dade Behring, Marburg, Germany). HDL cholesterol was measured with enzymatic methods (CHOD-PAP; Dade Behring). Triglycerides were measured by an enzymatic color test (GPO-PAP method, TGL Flex; Dade Behring). Serum creatinine was determined with a modified Jaffe test (Krea Flex; Dade Behring). Insulin was measured by an electrochemiluminescence immunoassay on a Cobas e602 instrument (Roche Diagnostics, Mannheim, Germany). HbA1c was measured in hemolyzed whole blood using the cation-exchange high-performance liquid chromatographic, photometric VARIANT II TURBO HbA1c Kit—2.0 assay on a VARIANT II TURBO Hemoglobin Testing System (Bio-Rad Laboratories, Hercules, USA). Intact proinsulin (frozen EDTA plasma) was measured by ELISA (intact human proinsulin ELISA, Cat No EZHIPI-17K, Linco Research, St Charles, MO). Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation (2009) based on serum creatinine.<sup>21</sup> Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as fasting insulin (in pmol/L)  $\times$  fasting glucose (in mmol/L)  $\div 135$  in all participants except those with antidiabetic treatment.

### Measurement of IMT

Ultrasound measurement (Sonoline G, 10 MHz transducer; Siemens Medical Solutions, Munich, Germany) of both common carotid arteries (CCA) was performed using a validated protocol<sup>22</sup> as previously described.<sup>23</sup> Optimal images of the right and left CCAs far wall were recorded on DVD videotapes. IMT measurements were performed off line over a length of 10 mm beginning at 0–5 mm of the dilatation of the distal CCA using an automated edge detection reading system (Prowin software, Medical Technologies International, USA). We used the average of the measurements of three frozen images from both the left and right CCAs to calculate artery thickness of the distal CCA ((mean left+mean right)/2). One certified reader measured all IMT scans. Reproducibility studies for intersonographer ( $n=30$  IMT measurements) and inter-reader variability ( $n=50$  IMT measurements) revealed coefficients of variations of 1.9% and 3.0% with Spearman correlation coefficients of  $\geq 0.89$ .

### Statistical analyses

Characteristics of the study participants were compared between participants with NGT and with pre-diabetes or T2D, respectively, using t-tests in case of approximately normally distributed variables. Mann-Whitney U tests were performed for variables with skewed distributions. Binomial proportions were compared with  $\chi^2$  tests. The associations of PIR with the outcomes of interest were assessed in logistic regression models in case of categorical dependent variables and in linear regression models in case of continuous dependent variables. Continuous variables were transformed to a Gaussian distribution by



the probability integral transformation followed by an inverse transform sampling and were used as in calculations per 1 SD. In multivariable logistic and linear regression analyses, the associations of PIR with the respective dependent variables were adjusted for covariates in different models: model 1: age (continuous), sex and physical activity (active/inactive); model 2: model 1 plus BMI (continuous); model 3: model 2 plus arterial hypertension. Smoking status (never/former/current), alcohol consumption (no; moderate (men 0.1 to <40 g/day and women 0.1 to <20 g/day); high (men  $\geq$ 40 g/day and women  $\geq$ 20 g/day/high)), low-density lipoprotein, HDL and triglycerides were not found to be associated with PIR after adjustment for sex, age and BMI and were therefore not included in the models. Pre-existing cases at baseline were excluded from the longitudinal incidence analyses. The level of statistical significance was set at 5% (two sided). The calculations were performed using the statistical environment R, V.3.6.0.

## RESULTS

### Study population characteristics

Table 1 displays the baseline characteristics of the total study population and stratified by diabetes status. PIR was higher in participants with T2D ( $p<0.001$ ). Baseline characteristics of participants who progressed to T2D during the follow-up time versus non-progressors are shown in online supplementary table 1. PIR was elevated in participants who developed incident T2D in comparison to non-progressors ( $p<0.001$ ).

### Association of PIR with T2D and glycemic traits

An increased PIR was associated with a higher T2D prevalence (OR per SD 2.24; 95% CI 1.81 to 2.77), a higher T2D incidence (OR: 1.66; 95% CI 1.26 to 2.17), and with incident pre-diabetes after adjustment for sex, age, BMI and physical activity (table 2).

Cross-sectionally, PIR was directly related to HbA1c and fasting glucose (table 3). Interestingly, PIR was inversely associated with HOMA-IR. This inverse association was present in normoglycemic, pre-diabetic and diabetic participants with the weakest association in pre-diabetic individuals (online supplementary table 2).

Proinsulin alone was strongly associated with prevalent (OR 4.15; 95% CI 3.14 to 5.48) and incident T2D (OR 3.72; 95% CI 2.55 to 5.42) after adjustment for sex, age, BMI and physical activity.

### Association of PIR with components of the metabolic syndrome and BMI

In linear regression analysis with the components of the metabolic syndrome (table 4), PIR was directly associated with elevated fasting glucose and inversely associated with an elevated waist circumference. In line, PIR was inversely associated with BMI after adjustment for sex, age and physical activity ( $\beta$ :  $-0.11\pm0.03$ ;  $p<0.001$ ). PIR was not associated with the other components of the metabolic syndrome (elevated blood pressure, elevated

triglycerides, and reduced HDL cholesterol) after adjustment for sex, age and physical activity.

In contrast, proinsulin alone was strongly directly associated with all components of the metabolic syndrome (online supplementary table 3).

### Lack of an association of PIR with IMT, decline of renal function, cardiovascular events, cardiovascular mortality and all-cause mortality

Cross-sectionally, PIR was not significantly associated with IMT after adjustment for sex, age, BMI, arterial hypertension and physical activity (online supplementary table 4). In the longitudinal analysis, PIR was not associated with the incidence of a urinary albumin to creatinine ratio  $\geq 30$  mg/g and of an eGFR  $<60$  mL/min/1.73 m<sup>2</sup> (online supplementary table 5). Further, an increased PIR was not related to the combined cardiovascular end point comprising non-fatal and fatal myocardial infarctions and stroke, nor to cardiovascular or all-cause mortality (table 5).

The association of proinsulin alone with IMT (online supplementary table 4), decline of kidney function and incident albuminuria (online supplementary table 5), the combined cardiovascular end point, cardiovascular mortality and all-cause mortality (online supplementary table 6) were stronger in the crude analyses ( $p<0.001$  for each observation) compared with the associations of PIR with the respective parameters. However, significance of these associations disappeared after multivariable adjustment, except for all-cause mortality, which remained significantly associated with proinsulin (HR 1.35; 95% CI 1.07 to 1.70;  $p=0.012$ ).

## DISCUSSION

In the population-based KORA F4/FF4 cohort, we showed that the PIR is positively associated with prevalent and incident T2D, but not with other components of the metabolic syndrome. In cross-sectional analyses we observed an association of PIR with HbA1c, fasting glucose and T2D. A further recent cross-sectional study by Nakamura *et al* described an association of fasting proinsulin with fasting glucose and T2D that was stronger than the association of PIR and the proinsulin to C peptide ratio.<sup>24</sup> We confirmed a stronger association of proinsulin alone compared with PIR with prevalent and incident T2D. Nonetheless, PIR was independently related to a higher risk for incident pre-diabetes and T2D in our study. These results are in line with previous studies describing an association of PIR with incident T2D.<sup>13–17</sup> However, other studies found no association of PIR with incident diabetes.<sup>25–27</sup> These divergent findings might be explained by the shorter follow-up time of 24 months in the Hoorn study<sup>25</sup> and the lower incidence of T2D in the study of Wareham *et al*.<sup>26</sup>

Interestingly, PIR associated inversely with HOMA-IR and anthropometric measures (BMI and waist circumference) in our study cohort, which fits the hypothesis

**Table 1** Characteristics of the study participants at KORA F4, overall and stratified for diabetes status\*

	Total study cohort		Normal glucose tolerance		Pre-diabetes		P value†		Type 2 diabetes		P value‡	
n	1514		1091		275		–		148		–	
Male sex, n (%)	736 (49)		501 (46)		148 (54)		0.02\$		87 (59)		0.004\$	
Age (years)	56.6±12.9		53.5±12.4		63.9±10.8		<0.001¶		66.3±9.2		<0.001¶	
BMI (kg/m <sup>2</sup> )	27.4±4.6		26.4±4.2		29.3±4.6		<0.001¶		30.7±4.5		<0.001¶	
Waist circumference (cm)	93.0±13.5		89.9±12.7		99.4±12.4		<0.001¶		103.9±11.1		<0.001¶	
HbA1c (%)	5.4 (5.2; 5.6)		5.3 (5.1; 5.5)		5.6 (5.3; 5.8)		<0.001**		6.2 (5.9; 6.7)		<0.001**	
HbA1c (mmol/mol)	35.5 (32.2; 38.8)		34.4 (32.2; 36.6)		37.7 (34.4; 39.9)		<0.001**		44.3 (40.7; 49.7)		<0.001**	
Arterial hypertension, n (%)††	590 (39)		314 (29)		160 (58)		<0.001\$		116 (78)		<0.001\$	
Physically inactive, n (%)	631 (42)		410 (38)		138 (50)		<0.001\$		83 (56)		<0.001\$	
Proinsulin (pmol/L)	3.0 (2.0; 4.8)		2.5 (1.8; 3.7)		4.3 (2.9; 6.7)		<0.001**		7.0 (4.8; 11.2)		<0.001**	
Insulin (pmol/L)	53.4 (38.4; 78.0)		48.0 (34.8; 66.0)		72.0 (50.5; 108.0)		<0.001**		90.0 (60.0; 132.0)		<0.001**	
Proinsulin to insulin ratio	0.055 (0.039; 0.078)		0.052 (0.038; 0.072)		0.059 (0.042; 0.083)		0.002**		0.078 (0.054; 0.119)		<0.001**	
Previous stroke, n (%)	34 (2)		18 (2)		7 (3)		0.46\$		9 (6)		0.002\$	
Previous myocardial infarction, n (%)	41 (3)		17 (2)		10 (4)		0.048\$		14 (9)		<0.001\$	

\*Mean±SD, median (first quartile; third quartile), or number of participants (proportion in %).

†The p value is related to the null hypothesis of no difference between participants with normal glucose tolerance and pre-diabetes.

‡The p value is related to the null hypothesis of no difference between participants with normal glucose tolerance and type 2 diabetes.

\$χ<sup>2</sup> test.

¶T-test.

\*\*Mann-Whitney U test.

††Defined as systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg and/or use of antihypertensive medication, given that the participants were aware of being hypertensive.

BMI, body mass index; KORA F4, Cooperative Health Research in the Region of Augsburg F4 (2006–2008) study.

**Table 2** ORs (95% CI) for prevalent pre-diabetes (vs normal glucose tolerance) and type 2 diabetes (vs no type 2 diabetes), as well as with incident pre-diabetes (vs non-progressors to pre-diabetes) and incident type 2 diabetes (vs non-progressors to type 2 diabetes) as dependent variables and proinsulin to insulin ratio as independent variable (per SD): results of logistic regression models

Prevalent pre-diabetes (yes: n=275; no: n=1091)	Prevalent type 2 diabetes (yes: n=148; no: n=1366)	Incident pre-diabetes (yes: n=135; no: n=677)	Incident type 2 diabetes (yes: n=79; no: n=922)
Without adjustment			
1.23 (1.07 to 1.40)**	2.26 (1.87 to 2.73)***	1.24 (1.03 to 1.50)*	1.58 (1.24 to 2.01)***
Adjustment for sex, age, BMI, physical activity (model 2)			
1.10 (0.94 to 1.29)	2.24 (1.81 to 2.77)***	1.27 (1.03 to 1.57)*	1.66 (1.26 to 2.17)***

\*p<0.05; \*\*p<0.01; \*\*\*p<0.001.  
BMI, body mass index.

of an insulin-deficient T2D subtype that constitutes a low HOMA-IR (normal to high insulin sensitivity), but a high PIR (defect in insulin secretion). Our observation of an association of PIR with glucose metabolism that is independent of the metabolic syndrome is supported by a recent study showing increased proinsulin secretory ratios in non-obese participants with T2D compared with obese participants with T2D in response to a glucose-potentiated arginine test.<sup>28</sup> In turn, high HOMA-IR, indicating insulin resistance (eg, in overweight or obesity), may initially be related to a low PIR in pre-diabetes or NGT, resulting from a compensatory hypersecretion of insulin, whereas proinsulin levels initially remain constant, leading to a decreased or normal PIR.<sup>17 29 30</sup> At a later stage, when  $\beta$ -cell failure and T2D become apparent, proinsulin levels will increase disproportionately, resulting in an elevated PIR. Hence, for insulin-resistant individuals, an elevated PIR may be found in manifest T2D, but not in pre-diabetes. In line, in our study, PIR was strongly elevated in diabetic participants, but was not associated with prevalent pre-diabetes in the regression analysis. Interestingly, however, PIR was associated with incident pre-diabetes, indicating that the underlying  $\beta$ -cell defect may be detectable in a very early stage before the progression to pre-diabetes, when insulin resistance-driven hyperinsulinemia might temporarily obscure the association. In line, the inverse

association between PIR and HOMA-IR was present in all glucose tolerance groups, but was lowest in participants with pre-diabetes, constituting the group with the highest proportion of primarily insulin-resistant participants with high insulin levels and preserved  $\beta$ -cell function with relatively low proinsulin levels. In contrast to our results, two recent cross-sectional studies found a positive association of PIR with HOMA-IR in diabetic participants<sup>31</sup> and in obese Egyptians.<sup>32</sup> However, these results were not corrected for possible confounders.

Apart from the direct association with elevated fasting glucose and the inverse association with an elevated waist circumference, PIR was not related to the other components of the metabolic syndrome, namely elevated triglycerides, reduced HDL cholesterol and elevated

**Table 4** Cross-sectional association estimates between proinsulin to insulin ratio and components of the metabolic syndrome adjusted for sex, age and physical activity (model 1):  $\beta$  coefficients $\pm$ SE from linear regression models are given per SD

	Adjusted $\beta$ $\pm$ SE	P value
Elevated waist circumference* (yes: n=1011; no: n=503)	-0.22 $\pm$ 0.03	<0.001
Elevated triglycerides† (yes: n=371; no: n=1143)	0.08 $\pm$ 0.08	0.21
Reduced HDL cholesterol‡ (yes: n=276; no: n=1238)	-0.08 $\pm$ 0.07	0.23
Elevated fasting glucose§ (yes: n=463; no: n=1051)	0.30 $\pm$ 0.06	<0.001
Elevated blood pressure¶ (yes: n=740; no: n=774)	0.03 $\pm$ 0.06	0.62

\*Defined as  $\geq 80$  cm in women and  $\geq 94$  cm in men.

†Defined as  $\geq 1.7$  mmol/L and/or intake of fibrates or nicotinic acid.

‡Defined as  $< 1.0$  mmol/L in men and  $< 1.3$  mmol/L in women and/or intake of fibrates or nicotinic acid.

§Defined as  $\geq 5.6$  mmol/L and/or intake of antidiabetic medication.

¶Defined as systolic blood pressure  $\geq 130$  mm Hg and/or diastolic blood pressure  $\geq 85$  mm Hg and/or use of antihypertensive medication, given that the participants were aware of being hypertensive.

HDL, high-density lipoprotein.

**Table 3** Cross-sectional association estimates between proinsulin to insulin ratio and continuous glycemic traits:  $\beta$  coefficients $\pm$ SE from linear regression models are given per SD proinsulin to insulin ratio

HbA1c	Fasting glucose	HOMA-IR
n (without/with diabetes) 1366/148	n (without/with diabetes) 1366/148	n (without/with diabetes) 1366/82
Without adjustment		
0.29 $\pm$ 0.02***	0.18 $\pm$ 0.02***	-0.12 $\pm$ 0.03***
Adjustment for sex, age, BMI, physical activity (model 2)		
0.21 $\pm$ 0.02***	0.11 $\pm$ 0.02***	-0.22 $\pm$ 0.02***

\*\*\*p<0.001.

BMI, body mass index; HOMA-IR, homeostatic model assessment of insulin resistance.

**Table 5** HRs (95% CI) for overall mortality, cardiovascular mortality, non-fatal and fatal stroke, and non-fatal myocardial infarction or coronary death in dependence on proinsulin to insulin ratio (per SD)

	All-cause mortality	Cardiovascular mortality	Non-fatal or fatal stroke, myocardial infarction, or coronary death
n (non-cases)†	1391	1459	1262
n (cases)‡	123	55	93
Without adjustment			
Total study cohort	1.57 (1.31 to 1.88)***	1.74 (1.33 to 2.27)***	1.33 (1.09 to 1.63)**
Adjustment for sex, age, BMI, arterial hypertension, physical activity (model 3)			
Total study cohort	1.16 (0.96 to 1.42)	1.22 (0.90 to 1.64)	1.01 (0.81 to 1.26)

\*\*p&lt;0.01; \*\*\*p&lt;0.001.

†Number of participants without events.

‡Number of events.

BMI, body mass index.

blood pressure, which is in line with a study by Pivatto *et al* showing that proinsulin and insulin alone, but not PIR, were associated with the metabolic syndrome.<sup>33</sup> In sum, these data indicate that the diabetes risk phenotype with an increased PIR is not primarily characterized by obesity, the metabolic syndrome, or insulin resistance.

Despite the increased risk of prevalent and incident T2D, PIR was not associated with IMT, incident albuminuria  $\geq 30$  mg/g creatinine, decline of kidney function below an eGFR of 60 mL/min/1.73 m<sup>2</sup> and cardiovascular events and mortality even without adjustment for glucose tolerance status. This is in line with the literature on PIR and diabetic complications.<sup>34–35</sup> It has to be noted that several studies found an association between proinsulin itself and cardiovascular complications.<sup>36–39</sup> However, as outlined above, only PIR is corrected for an insulin resistance-driven hyperinsulinemia, whereas proinsulin alone will increase with increasing HOMA-IR.<sup>40</sup> Thus, proinsulin is associated with insulin resistance,<sup>41</sup> which may partly explain the association of proinsulin with cardiovascular events and mortality,<sup>42</sup> since a high HOMA-IR has been shown to predict cardiovascular disease.<sup>43</sup> Consequently, proinsulin alone may be associated with cardiovascular disease, but only PIR is suitable to detect a more insulin-sensitive but insulinopenic subtype of T2D risk, which possibly does not confer as strongly an increased risk for cardiovascular complications as the insulin-resistant T2D subtype. In our study, proinsulin alone was associated with all-cause mortality after multivariable adjustment, whereas the associations with IMT, the combined cardiovascular end point and cardiovascular mortality, which were highly significant in the crude analyses, lost significance after multivariable adjustment, supporting the view that proinsulin is not an independent cardiovascular risk factor in the KORA F4 cohort.

In a recent cross-sectional study, PIR was higher in participants with diabetic nephropathy compared with diabetic participants without nephropathy.<sup>31</sup> However, long-standing T2D may provoke higher PIR irrespective of the underlying pathophysiology (insulin deficient vs

insulin resistant), and a higher PIR in that study does not necessarily point to a more insulin-sensitive phenotype anymore. In insulin-resistant individuals with overt T2D, PIR might mainly indicate severity of dysglycemia, which probably is connected to nephropathy, and the study did not consider these possible confounders.

### Strengths and limitations

The findings of our study are based on a large and well-characterized prospective study. Glucose tolerance status was determined by oral glucose tolerance test at baseline and follow-up visits. However, the KORA study included nearly exclusively Caucasians. Hence, our results may not apply to other ethnic groups. We used HOMA-IR instead of the gold standard methods (ie, hyperinsulinemic-euglycemic clamp) to measure insulin resistance. However, HOMA-IR is a widely used marker and can be considered as a reliable index for insulin resistance for clinical research purposes.<sup>44</sup> The follow-up time for mortality and cardiovascular events was about 9 years. With a mean age of 56 years at baseline, cardiovascular events might occur at a higher age and therefore a longer follow-up time might be necessary. In sum, 123 deaths (about 8% of the cohort) and 93 first cardiovascular events were reported in this study. We cannot exclude that, with a longer follow-up time, we would have detected further events, which might have altered some of our results.

### CONCLUSION

In conclusion, our results support the notion that PIR is elevated in a T2D risk subphenotype that is characterized by lower BMI and higher insulin sensitivity and that does not confer a higher rate of renal or cardiovascular events. Future studies focusing on lean individuals at high risk for T2D should further examine the value of PIR as prospective biomarker for incident pre-diabetes, T2D and secondary complications.

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