

Clinical correlates of plasma insulin levels over the life course and association with incident type 2 diabetes: the Framingham Heart Study

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ABSTRACT

Introduction Insulin is a glucose-lowering hormone that affects carbohydrate, lipid, and protein metabolism. Limited data exist on the correlates of insulin levels over the life course in healthy community-dwelling individuals.

Research design and methods Using multilevel modeling of multiple serial observations over 21 years, we assessed the longitudinal correlates of fasting insulin and the cross-sectional correlates of fasting and 2-hour (2h, post 75 g glucose challenge) plasma insulin concentrations in 2140 relatively healthy Framingham Heart Study participants without diabetes (61% women; mean age, 42 years). We used multivariable-adjusted Cox regression to relate glycaemic markers (fasting and 2h-insulin, fasting glucose, 2h-glucose, and hemoglobin A_{1c}) to the risk of type 2 diabetes during follow-up.

Results Over the life course, fasting insulin concentrations were inversely associated with age, male sex, and physical activity, whereas waist circumference, the total/high-density lipoprotein (HDL) cholesterol ratio, and blood triglycerides were positively associated with insulin levels ($p < 0.005$ for all). Male sex (inversely related) and the total/HDL cholesterol ratio (positively related) emerged as the most important cross-sectional correlates of 2h-insulin ($p < 0.005$ for all). All markers were associated with higher risk of type 2 diabetes (352 cases, median follow-up 18 years, $p < 0.001$ for all).

Conclusions We observed common and distinct correlates of fasting and 2h-insulin levels. Our findings highlight a potential role of insulin in lipid and lipoprotein metabolism. Furthermore, fasting and 2h-insulin are critical markers of future diabetes risk. Further studies are needed to confirm our findings.

INTRODUCTION

Insulin is a proteo-hormone affecting carbohydrate, lipid, and protein metabolism.¹ One of its primary functions is to lower blood glucose levels by stimulating the uptake and storage of glucose in adipocytes and peripheral muscles.² As a critical anabolic hormone,

Significance of this study

What is already known about this subject?

- Insulin production and secretion are regulated by various factors, with blood glucose levels having a strong impact. Prior analyses identified *cross-sectional* associations of fasting insulin concentrations; for example, with obesity, lipids, and physical activity.
- Mounting evidence exists that the metabolic determinants of fasting and 2-hour (2h) postload plasma glucose and insulin may be different.

What are the new findings?

- We observed that lipid traits and sex were significantly associated with both, fasting and 2h insulin levels.
- Age, physical activity and waist circumference were longitudinal correlates of fasting plasma insulin, but not of 2h insulin after adjusting for 2h glucose.
- During a median follow-up of 18 years, fasting and 2h insulin levels displayed strong associations with incident type 2 diabetes.

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

- Our results underscore the relevance of insulin levels not only for glucose homeostasis but also for lipid and lipoprotein metabolism. This might impact the clinical management of individuals with insulin resistance, the metabolic syndrome and diabetes.

insulin also promotes cell growth.¹ Impaired insulin production or secretion and a reduced response to the physiological actions of insulin (insulin resistance) are important pathomechanisms underlying the presence of type 2 diabetes mellitus. We are observing an increasing trend in the incidence of type 2 diabetes in the USA and worldwide.³

Insulin resistance and the associated elevated circulating insulin levels (hyperinsulinemia) confer higher risks of type 2

diabetes⁴ and for cardiovascular disease (CVD) events,⁵ but the underlying mechanisms remain unclear. Understanding physiological, clinical and biochemical correlates influencing interindividual variation in insulin concentrations over the life course is important in order to elucidate factors that may contribute to hyperinsulinemia. Insulin production and secretion are regulated by different dietary components and hormones, with glucose levels having a strong impact.⁶ Prior studies have assessed clinical and biochemical correlates of fasting insulin concentrations in the community, but most used a cross-sectional design.^{7–9} Data on the longitudinal assessment of insulin over the life course in individuals free of diabetes are scant.^{10–13} Furthermore, the correlates of 2-hour (2h) postload (postprandial) insulin levels have less commonly been investigated in community-based settings.⁹ Of note, there is mounting evidence that the metabolic determinants of impaired fasting glucose and impaired glucose tolerance (characterized by normal fasting glucose, but elevated 2h-postload glucose) are different.¹⁴ However, studies focusing on the metabolic differences between fasting versus 2h-insulin are relatively scarce.⁹

In the present investigation, we examined the cross-sectional and longitudinal correlates of fasting plasma insulin concentrations using data from participants of two generations of the community-based Framingham Heart Study (FHS). Additionally, we evaluated the cross-sectional correlates of insulin concentrations obtained 2 hours after a 75 g oral glucose challenge. Given the availability of repeated measurements of insulin over time and the deep phenotyping of our samples, they can offer important information regarding a range of factors that may influence circulating insulin levels. Lastly, we assessed the associations of fasting insulin levels, 2h-insulin concentrations, and standard glycemic markers (fasting glucose, 2h-glucose, hemoglobin A_{1c} (HbA_{1c})) with the risk of developing new-onset type 2 diabetes during a follow-up period of approximately 20 years.

We hypothesized that insulin levels will vary with age, differ between men and women, and will be associated with different cardiometabolic risk factors over the life course. Furthermore, we hypothesized that higher fasting and 2h-insulin levels will be associated with an increased risk of type 2 diabetes.

RESEARCH DESIGN AND METHODS

Study sample

The eligibility criteria, recruitment and design of the FHS have been described previously.¹⁵ Our *base sample* consisted of 8230 FHS participants from the Offspring cohort (n=4135) and the Third Generation (Gen 3; n=4095) (online supplemental figure 1). Participants were eligible for the present investigation if they had attended at least one of examination cycles 5 (1991–1995), 7 (1998–2001), 8 (2005–2008) or 9 (2011–2014) of the Offspring cohort, or at least one of examination

cycles 1 (2002–2005) or 2 (2008–2011) of the Gen 3 cohort. From the base sample, we excluded individuals with prevalent type 2 diabetes (n=483), prevalent CVD (n=465), and those with missing insulin values (n=10), resulting in an overall *sample* of n=7272 (online supplemental figure 1). We defined the following subsamples for analyses:

- ▶ To assess the *longitudinal correlates* of fasting plasma insulin, we created a healthy sample from the overall sample by excluding individuals with dyslipidemia (n=2502), hypertension (n=1458), abdominal obesity (n=750), and smoking (n=422), resulting in a sample size of 2140 individuals, who contributed 3116 person-observations (*sample 1*). More specifically, our analyses included 718 individuals with two observations (insulin measurements), 69 individuals with three observations and 40 individuals with more than three observations. The average time from the initial exam to the follow-up exam was 3 years. For participants with multiple follow-up exams, the average follow-up time was 8 years.
- ▶ To assess the *cross-sectional correlates* of fasting plasma insulin and of 2h-insulin, respectively, we used data from individuals of the healthy *sample 1* who attended examination cycle 5 of the Offspring cohort (n=565, *sample 2*) because 2h-insulin levels were only available at that examination cycle. We used this same sample (*sample 2*) to assess the cross-sectional correlates of fasting plasma insulin to facilitate a comparison with the correlates of 2h-plasma insulin levels.
- ▶ Finally, to evaluate the associations of different glycemic markers, that is, fasting plasma insulin, 2h-insulin, fasting plasma glucose, 2h-glucose, and HbA_{1c}, with the incidence of type 2 diabetes on follow-up, we created a third sample as follows: from the *overall sample* (n=7272), we included only those participants who attended examination cycle 5 because 2h-insulin and 2h-glucose were only available at Offspring exam 5 (n=3078, *sample 3*).

All participants provided written informed consent. The corresponding authors had full access to all results and take responsibility for their integrity.

Assessment of insulin, glucose, and HbA_{1c}

Participants had blood drawn after an overnight fast. At examination cycle 5 of the Offspring cohort, participants additionally underwent a 75 g oral glucose challenge. They had blood drawn for the assessment of glucose and insulin 2 hours after the glucose challenge. A detailed list of the assays used to measure insulin, glucose and HbA_{1c} at the different examination cycles in the Offspring and Gen 3 cohorts is provided in online supplemental table 1. Given the varying methods for insulin assessment across examination cycles within each cohort, insulin values were standardized (mean=0; SD=1) within each examination cycle for both cohorts.

HbA_{1c} was measured by high-performance liquid chromatography assays standardized to Diabetes Control and

Complication Trial values¹⁶ with intra-assay and interassay coefficients of variation (CVs) lower than 3%.

Assessment of covariates

At each FHS examination, standardized interviews were conducted by trained personnel obtaining information on sociodemographic characteristics and lifestyle factors and on medical history and medications used by the participants. Height (in meters) and weight (in kilograms) were measured, and body mass index (BMI) was calculated (kg/m^2). Blood pressure (BP) was measured twice on the left arm of the seated participants with a mercury column sphygmomanometer and a cuff of an appropriate size; the average of the two readings was used as the BP at that examination cycle. Blood C reactive protein (CRP), total cholesterol, and high-density lipoprotein (HDL) cholesterol concentrations were obtained by standard enzymatic methods. Physical activity was assessed using a standardized questionnaire; for this investigation, we used the Physical Activity Index¹⁷ which is a weighted score that takes into account the time during a typical day spent sleeping, sedentarily, or in light, moderate or heavy activity.¹⁷

Outcome of interest

FHS participants are under regular surveillance for the incidence of outcomes, including type 2 diabetes. For this study, the incidence of type 2 diabetes was assessed from Offspring examination cycle 5 (1991–1995) through Offspring examination cycle 9 (2011–2014). Type 2 diabetes was defined as fasting plasma glucose level ≥ 126 mg/dL or the self-reported use of hypoglycemic medications.¹⁸

Statistical analyses

Longitudinal tracking of fasting plasma insulin

In our primary analyses, fasting plasma insulin was log-transformed to normalize its distribution and then standardized within each exam to account for the different assays used to measure insulin levels. We used multi-level modeling in sample 1 (SAS Proc Mixed) with an unstructured covariance matrix to assess the longitudinal correlates of fasting plasma insulin levels (dependent variable). Random intercept and slope for age were examined for all models to reflect the different starting values and slopes for insulin in each participant. We also investigated possible non-linear relations between age and insulin by examining the quadratic effect of age (by fitting an age-squared term). Models included the following eligible correlates: age, sex, waist circumference, systolic BP, the total/HDL cholesterol ratio, triglycerides, CRP, and the Physical Activity Index. We chose waist circumference over BMI, because the former better reflects cardiometabolic risk, including the risk of developing diabetes, than BMI.¹⁹ We also included fasting plasma glucose or HbA_{1c} among the candidate correlates (separate model for each) in order to account for glycemic status. We evaluated possible non-linear relations between fasting plasma

glucose and 2h-glucose in relation to insulin by including a squared term for the respective covariate in the models. Additionally, we assessed biologically plausible interactions of sex with fasting plasma insulin, fasting plasma glucose, and HbA_{1c} by including corresponding interaction terms in the models. As a secondary analysis, we reran our longitudinal correlates analysis without standardizing the log-transformed fasting plasma insulin and instead included 'assay method' as a random effect in the multilevel mixed model to account for any unobserved confounding between assay methods.

Cross-sectional correlates of fasting plasma insulin and 2h-insulin

Given that 2h-insulin was measured only at Offspring examination cycle 5, we used a multivariable-adjusted linear regression model (in sample 2) to assess the cross-sectional correlates of 2h-insulin and of fasting plasma insulin (dependent variables; separate model for each). Candidate correlates were age, sex, waist circumference, systolic BP, the total/HDL cholesterol ratio, triglycerides, Physical Activity Index, and fasting plasma insulin (for the 2h-insulin models only). We also included fasting plasma glucose or HbA_{1c} among the candidate correlates (separate model for each) to account for glycemic status.

Associations of insulin and glycemic markers with incident type 2 diabetes

We generated cumulative probability curves using the Kaplan-Meier method to display the unadjusted associations of fasting plasma insulin, 2h-insulin, fasting plasma glucose, 2h-glucose, and HbA_{1c} (separate curve for each marker, each marker dichotomized at the median) with the incidence of type 2 diabetes.

To account for potential confounders, we used multivariable-adjusted Cox proportional hazards regression models (in sample 3) to relate each of the markers (independent variables all at Exam 5; separate model for each marker) to the incidence of type 2 diabetes (dependent variable through Exam 9). We adjusted these models for age, sex, smoking status, waist circumference, hypertension status, total/HDL cholesterol ratio, triglyceride levels, lipid-lowering medication, and the Physical Activity Index. Finally, using sample 3, we performed a stepwise backward selection procedure within the Cox model, forcing in all covariates mentioned above, to evaluate which of the five markers (fasting plasma insulin, 2h-insulin, fasting plasma glucose, 2h-glucose, and HbA_{1c}), revealed the strongest association with incident type 2 diabetes. Stepwise backward selection of the markers in the model was based on entry and exit criteria of $p < 0.05$.

We evaluated possible non-linear relations for insulin and glucose in relation to the incidence of diabetes by adding the respective glycemic marker as a quadratic term. Since 2h-insulin was indicative of a non-linear association, we performed a sensitivity analysis for the stepwise backward selection by adding the quadratic term for 2h-insulin and our results remained consistent (data not shown).

Table 1 Baseline characteristics of study samples

	Overall sample		Healthy sample*	
	(n=7272)		(n=2140)	
	Men (n=3295)	Women (n=3977)	Men (n=841)	Women (n=1299)
Age, years	47±12	47±12	43±12	42±11
Systolic blood pressure, mm Hg	124±15	118±18	116±10	108±11
Diastolic blood pressure, mm Hg	78±9	73±10	74±8	69±8
Hypertensive treatment, %	12%	11%	N/A	N/A
Body mass index, kg/m ²	27.9±4.3	26.2±5.6	25.4±2.6	22.7±2.8
Waist circumference, cm	98.6±11.6	87.8±14.7	90.9±6.7	78.1±6.5
Fasting plasma insulin, pmol/L	28 (22,39)	26 (21,34)	25 (19,35)	23 (18,30)
2h-insulin, pmol/L‡	N/A	N/A	28 (16,45)	30 (20,45)
Fasting plasma glucose, mg/dL	97.0±8.5	91.9±8.9	94.5±7.3	88.3±6.6
2h-glucose, mg/dL‡	N/A	N/A	96 (81,109)	92 (79,107)
Hemoglobin A _{1c} , %†	5.3±0.6	5.3±0.6	5.2±0.5	5.2±0.5
Hemoglobin A _{1c} , mmol/mol†	34±6.3	34±6.5	34±4.9	33±5.2
Total cholesterol, mg/dL	197±36	196±37	169±21	169±20
HDL cholesterol, mg/dL	46±13	60±16	50±12	63±14
Triglycerides, mg/dL	114 (78,172)	94 (67,135)	75 (60,101)	70 (55,91)
Dyslipidemia treatment, %	8%	4%	N/A	N/A
C reactive protein, mg/L	1.0 (0.5,2.2)	1.3 (0.5,3.5)	0.7 (0.3,1.4)	0.7 (0.3,1.7)
Physical Activity Index	35.2 (32,40.7)	34.2 (31,38)	35.5 (32.5,40.7)	35.1 (32,39.2)
Current smokers, %	17%	17%	N/A	N/A

N/A indicates not available/not applicable.

*The healthy sample consists of non-smoking participants free of prevalent diabetes, cardiovascular disease, dyslipidemia, hypertension, and abdominal obesity.

†Hemoglobin A_{1c} was only available in the Offspring cohort and Gen 3, exam 2 participants.

‡2h-insulin and 2h-glucose were only available at Offspring cohort examination cycle 5. Continuous measures are presented as mean±SD or median (Q1, Q3).

HDL, high-density lipoprotein.

All analyses were performed with SAS V.9.4 (Cary, North Carolina, USA), and a two-sided p value of <0.05 was considered statistically significant.

RESULTS

Baseline characteristics of the overall and the healthy subsample (consisting of non-smoking participants free of prevalent diabetes, CVD, dyslipidemia, hypertension, and abdominal obesity) are displayed in [table 1](#). We observed the expected differences in the cardiovascular and metabolic profiles between the two samples.

Cross-sectional and longitudinal correlates of fasting plasma insulin

On multivariable adjustment, fasting plasma insulin levels were inversely associated with age, male sex (indicating higher levels in women), and the Physical Activity Index; whereas waist circumference, the total/HDL cholesterol ratio, and triglyceride levels were positively associated with fasting plasma insulin levels over the life course ([table 2](#)). When we replaced fasting plasma glucose with HbA_{1c}, results

were relatively similar, except for systolic BP which became associated, and physical activity was no longer associated with fasting plasma insulin in our longitudinal analyses ([table 2](#)). Our results remained consistent when we used non-standardized fasting plasma insulin as the outcome variable and included ‘assay method’ as a random effect in the model (online supplemental table 2).

In cross-sectional analyses, waist circumference, the total/HDL cholesterol ratio, and circulating triglyceride concentrations were positively associated with fasting plasma insulin concentrations in a model that included fasting plasma glucose (Model 1; [table 3](#)). When we replaced fasting plasma glucose with HbA_{1c}, only triglyceride levels were significantly associated with fasting plasma insulin in cross-sectional analyses ([table 3](#)). We did not observe statistically significant interactions of glycemic status (fasting plasma glucose or HbA_{1c}) and sex for fasting plasma insulin (p>0.05). The quadratic term for fasting plasma glucose was not statistically significantly related to fasting plasma insulin (online supplemental table 3).

Table 2 Longitudinal correlates of fasting plasma insulin

Potential correlates	Model 1 (adjusted for fasting plasma glucose)		Model 2 (adjusted for HbA _{1c})	
	β (SE)	P value	β (SE)	P value
Age, years	-0.11 (0.02)	<0.0001	-0.10 (0.03)	0.001
Male sex	-0.48 (0.06)	<0.0001	-0.35 (0.08)	<0.0001
Waist circumference, cm	0.24 (0.03)	<0.0001	0.31 (0.04)	<0.0001
Systolic blood pressure, mm Hg	0.02 (0.02)	0.39	0.07 (0.03)	0.02
Total/HDL cholesterol ratio, mg/dL	0.08 (0.02)	0.002	0.11 (0.03)	0.004
Triglycerides, mg/dL	0.18 (0.02)	<0.0001	0.17 (0.03)	<0.0001
C reactive protein, mg/L	0.03 (0.02)	0.09	0.03 (0.03)	0.28
Physical Activity Index	-0.01 (0.003)	0.004	-0.002 (0.004)	0.53
Fasting plasma glucose, mg/dL	0.25 (0.02)	<0.0001	—	—
HbA _{1c} , %	—	—	0.06 (0.03)	0.054

β indicates the regression coefficient; SE indicates standard error of β. Fasting plasma insulin was log-transformed and standardized within each exam. The beta coefficients indicate the change in standardized log-fasting plasma insulin per 1-SD increment in the continuous variables, per 1-unit increment of the Physical Activity Index, or presence versus absence of categorical variables (n=2140).

Bold indicates p<0.05.

HbA_{1c}, Hemoglobin A_{1c}; HDL, high-density lipoprotein.

Cross-sectional correlates of 2h-insulin

Male sex (inversely related), the total/HDL cholesterol ratio, and fasting insulin levels (positive relations) were significantly associated with 2h-insulin (table 3), regardless of adjusting for fasting plasma glucose or 2h-glucose. The quadratic term for 2h-glucose, but not for fasting

plasma glucose, was statistically significantly related to 2h plasma insulin (online supplemental table 3). However, even after adding the quadratic term for 2h-glucose, the coefficient estimates of all variables in the model remained essentially unchanged (online supplemental table 3 compared with table 3).

Table 3 Cross-sectional correlates of fasting and 2-hour (2h) plasma insulin

Potential correlates	Fasting plasma insulin				2-hour plasma insulin			
	Model 1 (adjusted for fasting plasma glucose)		Model 2 (adjusted for HbA _{1c})		Model 1 (multivariable-adjusted, including fasting plasma insulin and fasting plasma glucose)		Model 2 (multivariable-adjusted, including fasting plasma insulin and 2h-glucose)	
	β (SE)	P value	β (SE)	P value	β (SE)	P value	β (SE)	P value
Age, years	-0.01 (0.04)	0.82	-0.02 (0.05)	0.72	0.10 (0.04)	0.02	-0.01 (0.03)	0.78
Male sex	-0.14 (0.12)	0.25	0.02 (0.15)	0.89	-0.59 (0.12)	<0.0001	-0.50 (0.10)	<0.0001
Waist circumference, cm	0.17 (0.06)	0.004	0.08 (0.07)	0.26	0.002 (0.06)	0.97	0.03 (0.05)	0.56
Systolic blood pressure, mm Hg	0.02 (0.04)	0.66	0.02 (0.05)	0.78	0.06 (0.04)	0.19	-0.001 (0.04)	0.97
Total/HDL cholesterol ratio, mg/dL	0.12 (0.05)	0.02	0.12 (0.06)	0.06	0.15 (0.05)	0.003	0.12 (0.04)	0.003
Triglycerides, mg/dL	0.15 (0.05)	0.001	0.17 (0.06)	0.004	0.04 (0.05)	0.42	-0.02 (0.04)	0.62
Physical Activity Index	-0.005 (0.01)	0.44	-0.001 (0.01)	0.90	0.0003 (0.01)	0.97	-0.004 (0.01)	0.46
Fasting plasma glucose, mg/dL	0.10 (0.04)	0.01	—	—	0.06 (0.04)	0.15	—	—
Log-fasting plasma insulin, pmol/L	—	—	—	—	0.41 (0.04)	<0.0001	0.37 (0.03)	<0.0001
HbA _{1c} , %	—	—	0.05 (0.05)	0.36	—	—	—	—
2h-glucose, mg/dL	—	—	—	—	—	—	0.53 (0.03)	<0.0001

β indicates the regression coefficient; SE indicates standard error of β. The regression coefficients indicate the change in standardized log-fasting plasma insulin and log-2h-plasma insulin per 1-standard deviation increment in the continuous variables, per 1-unit increment of the Physical Activity Index, or presence vs absence of categorical variables (n=565).

Bold indicates p<0.05.

HbA_{1c}, hemoglobin A_{1c}; HDL, high-density lipoprotein.

Table 4 Association of glycemic markers with incident type 2 diabetes (separate model for each marker)

	N events/N at risk	HR * (95% CI)	P value
Fasting plasma insulin, pmol/L	352/2819	1.36 (1.21 to 1.52)	<0.0001
2h-insulin, pmol/L	344/2750	1.33 (1.23 to 1.44)	<0.0001
Fasting plasma glucose, mg/dL	350/2792	2.76 (2.46 to 3.11)	<0.0001
2h-glucose, mg/dL	345/2755	1.91 (1.74 to 2.09)	<0.0001
Hemoglobin A _{1c} , % (mmol/mol)	254/2043	1.27 (1.16 to 1.39)	<0.0001

Bold indicates $p < 0.05$.

*Models were adjusted for age, sex, current smoking, waist circumference, hypertension status, total/HDL cholesterol ratio, triglyceride levels, lipid-lowering medication, and the Physical Activity Index. Fasting plasma insulin was log-transformed. All glycemic markers were standardized (mean=0, SD=1). Thus, the HRs are given per 1-SD increment in the glycemic marker. 2h, 2-hour; HR, Hazard Ratio.

Association of all insulin and glucose markers with type 2 diabetes

The median follow-up time for the incidence of type 2 diabetes was 18 years. During the follow-up period, between 254 (for HbA_{1c}; table 4) and 352 participants (for fasting plasma insulin) developed new-onset type 2 diabetes. All markers (fasting plasma insulin, 2h-insulin, fasting plasma glucose, 2h-glucose, HbA_{1c}) were positively associated with time to type 2 diabetes (online supplemental figure 2; table 4). Using a backward stepwise selection procedure with all five glycemic markers in the same model, only fasting blood glucose and 2h-glucose levels remained significantly associated with type 2 diabetes (online supplemental table 4).

CONCLUSIONS

Using multiple observations from two generations of the FHS, we assessed longitudinal and cross-sectional correlates of fasting plasma insulin levels and cross-sectional correlates of 2h-insulin levels in relatively healthy study participants (eg, non-smoking individuals, free of diabetes, CVD, dyslipidemia, hypertension, and abdominal obesity). We also analyzed the association of a broad spectrum of glycemic markers (fasting plasma insulin, 2h-insulin, fasting plasma glucose, 2h-glucose, and HbA_{1c}) with incident type 2 diabetes over a median follow-up of 18 years.

Principal findings

First, we identified common correlates of fasting and 2h-insulin levels. Specifically, we observed that lipid traits (triglyceride levels and/or the total/HDL cholesterol ratio) and sex (higher values in women) were significantly associated with *both* fasting (longitudinally and/or cross-sectionally) and 2h-insulin levels. Second, age and physical activity (both inverse), and waist circumference (positive) were significant correlates of the *fasting* plasma insulin, but not 2h-insulin after adjusting for 2h-glucose. Third, fasting and 2h-glucose levels displayed the strongest associations (of all glycemic markers evaluated) with the risk for incident type 2 diabetes.

Comparison with the literature

Distinct correlates of fasting and 2h-insulin concentrations

Over the life course, age, physical activity, and waist circumference were associated with fasting insulin concentrations, but these traits were not associated with 2h-insulin in cross-sectional analyses that adjusted for 2h-glucose. These differences are interesting because fasting and postprandial glucose and insulin levels reflect different physiological processes and have distinct metabolic implications.¹⁴ As an example, 2h-insulin concentrations reflect in part the pancreatic response to a glucose challenge; the lack of association of 2h-insulin with age and physical activity may suggest that this response does not seem to change with age and is not impacted substantially by physical activity.

Fasting insulin is in part determined by baseline insulin production and by insulin sensitivity vs insulin resistance in the periphery. It is conceivable that the physiological actions of insulin, for example, in the peripheral muscles, are improved by regular physical activity, which would explain the association of fasting insulin with physical activity, particularly in our longitudinal analyses over the life course.

The association of fasting insulin with waist circumference is consistent with the notion that abdominal obesity is an essential correlate of insulin resistance and hyperinsulinemia.²⁰ Overall, our observations suggest that lifestyle measures, such as physical activity, may have a more profound effect on baseline (fasting) rather than postprandial (2h) insulin levels, and particularly over more extended periods; this premise warrants further investigation.

Common correlates of fasting and 2h-insulin concentrations

In our healthy community-based sample, the total/HDL cholesterol ratio and/or triglyceride levels were positively associated with 2h-insulin (cross-sectional) and fasting insulin concentrations in cross-sectional and longitudinal analyses. Similar positive associations of fasting plasma insulin with triglyceride levels and inverse associations with HDL cholesterol were also reported in children and young adults in prior studies.^{7,21} This constellation resembles the lipid abnormalities frequently observed in type 2 diabetes and in the metabolic syndrome, commonly

referred to as ‘diabetic dyslipidemia’: high triglyceride levels, low HDL levels and high low-density lipoprotein (LDL) cholesterol levels. The pathophysiology of this condition has been comprehensively reviewed elsewhere.²² Indeed, insulin resistance and insulin seem to play a pivotal role in developing these lipid alterations.²² Insulin promotes lipogenesis (triglyceride synthesis) and inhibits lipolysis in adipocytes,^{20–23} and affects the activity of different enzymes involved in lipid and lipoprotein metabolism, including the hormone-sensitive lipase and lipoprotein lipase.^{20–24} The fact that we observed consistent associations of these lipid traits with fasting and 2h-insulin levels in our sample highlights the significance of insulin not only for glucose hemostasis but also for lipid and lipoprotein metabolism in healthy individuals.²³

On a similar note, men had lower levels of fasting insulin over the life course and lower 2h-insulin levels than women. Differences between women and men with regard to fasting and post glucose challenge insulin and glucose levels have been reported in prior studies,^{25–26} and a recent genome-wide analysis reported sex differences in their association with fasting insulin levels for defined genetic variants.²⁵

Association of glycemic markers with incident type 2 diabetes

Glycemic markers such as fasting glucose,²⁷ 2h-glucose²⁸ and HbA_{1c},²⁹ and different insulin measures (including concentrations of insulin and proinsulin),³⁰ have been related to the incidence of diabetes. Investigators have also evaluated whether a combination of these markers improved the prediction of type 2 diabetes.^{27–28} In most scenarios, higher or above-threshold levels of two or more such markers conferred higher risks for incident type 2 diabetes as compared with individuals who had elevated levels on only one marker.^{27–28} Consistent with these observations, all five markers evaluated in our sample (fasting plasma insulin, 2h-insulin, fasting plasma glucose, 2h-glucose, HbA_{1c}) were significantly associated with incident type 2 diabetes in multivariable-adjusted models (separate models for each glycemic marker). However, using a backward selection procedure with all glycemic markers in the model, only fasting blood glucose and 2h-glucose levels remained significantly associated with incident type 2 diabetes. Thus, among all glycemic and insulin traits, both glucose measures displayed the strongest associations with new-onset type 2 diabetes. The clinical diagnosis of type 2 diabetes is based on blood glucose levels or glycosylation of HbA_{1c} and is not based on insulin levels; this could, in part, explain the stronger association of glucose traits (as compared with insulin traits) with incident type 2 diabetes.

Strengths and limitations

Strengths of our investigation include the large sample size, the community-based design including cross-sectional and longitudinal components, the comprehensive phenotyping of our sample with respect to potential correlates and confounders and the availability of repeated measurements of fasting plasma insulin over

21 years. Moreover, the availability of other glycemic markers (including 2h-insulin, fasting plasma glucose, 2h-glucose, and HbA_{1c}) allowed us to compare comprehensively and conjointly model all glycemic markers in relation to incident type 2 diabetes. Compared with other longitudinal studies with repeated insulin measurements,^{10–12} our sample covered a broader age range (19–83 years), included slightly more women than men, and focused on relatively healthy non-smoking individuals free of prevalent diabetes, CVD, dyslipidemia, hypertension, and abdominal obesity, allowing analyses unconfounded by these conditions.

The following limitations of our study merit consideration. Insulin was measured using different assays across examination cycles. To account for the variability introduced by different assays, we standardized insulin levels within each exam. An additional limitation is that 2h-insulin levels were available only at examination cycle 5 of the Offspring cohort. We might have underestimated the incidence of type 2 diabetes since ascertainment of incident diabetes did not include the use of HbA_{1c} and of postload glucose. Finally, our analyses were conducted in white individuals of European ancestry, focusing on healthy individuals (as defined above).

Therefore, our findings cannot be generalized to other ethnicities. It is noteworthy that the relative contributions of adiposity, insulin resistance and β -cell function to the risk of diabetes may be different between Europeans and East Asians.³¹ East Asians, for example, tend to be less obese but more insulin resistant as compared with Europeans.^{31–32} Therefore, follow-up studies in East Asians and other ethnicities are warranted, and the comparison of these studies with our data may be critical.

CONCLUSION

Fasting and 2h-insulin levels share common but also have distinct clinical correlates. The consistent associations of lipid traits with fasting and 2h-insulin levels underscore the relevance of insulin levels not only for glucose homeostasis but also for lipid and lipoprotein metabolism. Furthermore, insulin levels (and other glycemic markers such as fasting glucose, 2h-glucose, and HbA_{1c}) may be markers of future diabetes risk.

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