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ABSTRACT

The contribution of different biological pathways to the development of type 2 diabetes was quantified in a case-cohort design based on circulating blood biomarkers from participants aged 35–65 years in the EPIC–Potsdam Study. The analytic sample included 613 participants with incident diabetes and 1965 participants without diabetes. The proportion that each biomarker contributed to the risk of diabetes was quantified using effect decomposition method. Summarized risk of each biomarker was estimated by an index based on quintiles of gamma-glutamyltransferase (GGT), HDL-cholesterol, hs-CRP, and adiponectin. Cox proportional hazard regression was used to estimate relative risks adjusted for age, sex, body mass index, waist-circumference, education, sport activity, cycling, occupational activity, and smoking, alcohol intake, and consumptions of red meat and whole grain bread. Adiponectin explained a total of 32.1% (CI= 16.8, 49.1) of the risk related to index. For the other biomarkers the corresponding proportions were 23.5% (CI= 10.1, 37.8%) by HDL-cholesterol, 21.5% (CI= 11.5, 32.8%) by GGT, and 15.5% (CI= 4.44, 27.3%) by hs-CRP. The results support the hypothesis that the different biological pathways reflected by GGT, HDL-cholesterol, CRP and adiponectin independent from each other contribute to the risk of type 2 diabetes. Of these pathways the highest contribution was observed for adiponectin which contributed one third to the risk and that equal proportion was contributed by GGT and HDL-cholesterol, although the contribution of inflammation was lower.

Keywords: Adiponectin/blood; Biological markers/blood; Diabetes; Liver enzymes; Inflammation; Prospective studies

INTRODUCTION

Over the past years, a number of circulating blood biomarkers have been found to be associated with the risk of type 2 diabetes [1]. Most of these markers reflect different metabolic pathways that may be involved in the pathogenesis of type 2 diabetes, including biomarkers of reduced insulin sensitivity (such as adiponectin), hepatic fat accumulation (such as gamma-glutamyltransferase, GGT, or alanine-aminotransferase, ALT), dyslipidemia (high density lipoprotein cholesterol, HDL-C, or triglycerides), and inflammation (such as C-reactive protein, CRP). Several studies have suggested that these biomarkers are associated with diabetes risk independently of conventional risk factors such as age, family history, waist circumference, and nutritional variables [1]; however, most studies on the association between biomarkers and risk of type 2 diabetes, have either focused on estimating the strength of the association of single biomarkers with the risk of diabetes (usually quantified as relative risks) [2, 3], or on the improvement of the prediction of diabetes by biomarkers [4-6]. Limited quantitative information is currently available about to what extent these biomarkers of different metabolic pathways relative to each other contribute to risk of type 2 diabetes.

The approach in the present study was among a given set of biomarkers of different metabolic pathways to estimate the relative contribution of each biomarker to risk of type 2 diabetes. Such information is important to put the relative relevance of biomarkers of different metabolic pathways into perspective, which is from a clinical and public health aspect informative when it comes to the decision about the development of measures to affect biomarkers of pathways that are meant to reduce diabetes risk. Chen et al. introduced a procedure for the estimation of the proportion of treatment effects by surrogate markers, which previously has been used primarily in analyses of intervention data [7], and can also be used in cohort studies [8]. In order to quantify the relative contribution of biomarkers of

different metabolic pathways to the risk of type 2 diabetes, we applied effect decomposition procedure to multiple-covariate adjusted in data from the EPIC-Potsdam cohort study.

MATERIALS AND METHODS

The EPIC-Potsdam study is part of the multicenter European Prospective Investigation into Cancer and Nutrition (EPIC) [9, 10]. In Potsdam, Germany, 27,548 subjects (16,644 women and 10,904 men) were recruited from the general population between 1994 and 1998 [11]. The preferred age range at baseline was 35 – 65 years in women and 40 – 65 years in men. The baseline examination included anthropometric measurements, a personal health interview, a health questionnaire and blood sampling. Informed consent was obtained from participants; approval was given by the Ethics Committee of the medical association of the State of Brandenburg, Germany.

Peripheral venous citrate-blood samples were drawn at baseline. The blood samples were centrifuged at 1,000*g* for 10 min at 4°C. Plasma was then removed and stored in aliquots in freezers at 80°C until assays of the markers of interest were performed. A health interview and a questionnaire inquiring information on educational attainment, smoking and alcohol consumption habits, occupational activity level, and leisure time physical activity which was assessed as sport activities and cycling both calculated as the average time spent per week during the 12 months before the baseline recruitment. Usual food intake including whole grain bread and red meat consumptions of the preceding year was assessed by a self administered 148-item food-frequency questionnaire. High reproducibility 6-month apart was observed for meat and coffee and moderate reproducibility for bread consumption as well as high agreement with repeated 24h dietary recalls were observed [12]. The prevalence of diabetes at baseline was evaluated by a physician using information on self-reported medical diagnoses, medication records, and ongoing nutritional therapy. Uncertainties regarding a

proper diagnosis were clarified with the participant or treating physician. Waist circumference was measured midway between the lower rib margin and the superior anterior iliac spine to the nearest 0.5 cm with a non-stretching tape applied horizontally, the proper use of which was controlled with a mirror [13]. Weight and height were measured without shoes on in light clothing and body mass index was calculated as kg/m^2.

Follow-up questionnaires were sent out every 2–3 years to identify incident cases of type 2 diabetes. Complete questionnaires of follow-up round 1 were returned from 96%, of follow-up round 2 from 96%, follow-up round 3 from 92%. We also included all questionnaires of the 4th follow-up round sent out until 31 January 2005, of which 90% were returned by 31 August 2005. Potential cases of incident diabetes were identified via self-reports of a diabetes diagnosis, diabetes-relevant medication or dietary therapy for diabetes. All cases identified during the follow-up rounds were verified by questionnaires mailed to the diagnosing physician, inquiring the date and type of diagnosis, diagnostic tests, and treatment of diabetes. Only subjects with a diagnosis of type 2 diabetes that was confirmed by a physician (International Statistical Classification of Diseases, 10th Revision, E11) and a diagnosis date after the baseline examination were considered as incident cases of type 2 diabetes.

The present study was conducted in a prospective case cohort design [14]. During an average follow-up time of 7 years, a total of 801 incident cases with a mean time interval between baseline and diabetes diagnosis of 4 years were identified among those participants of the cohort with available blood samples and data on covariates and without a prior history of diabetes. Of these, 613 remained for analyses after exclusion of 92 observations due to dilution or missing biomarker value, and further 96 were excluded due to plasma glucose values within the diabetic range (random glucose 200 mg/dl or more, or fasting glucose 126 mg/dl or more) or implausible low (<50 mg/dl). A random sub-cohort of 2, 500 individuals

was selected from all participants of the EPIC-Potsdam study population. Of these, 2, 322 had data available on blood samples and data on covariates and had no history of diabetes at the baseline as well as had verified information of potential diabetes occurrence during follow-up. Finally, 2, 021 participants remained for the analyses after applying similar exclusion criteria (n = 152) due to dilution or missing biomarker value (n = 122) and further exclusion due to implausible glucose values (n = 27). In agreement with the case-cohort design [14], 557 of the incident cases in the full cohort were identified as external cases and 56 in the random sub-cohort.

Plasma levels of ALT and GGT, HDL-C, triglycerides, and hs-CRP were determined using the automatic ADVIA 1650 analyzer (Siemens Medical Solutions, Erlangen, Germany). Total adiponectin concentration was measured by ELISA (Linco Research, St Charles, MI, USA) [15]. Red blood cell levels of glycaeted haemoglobin (HbA1c) were measured using the HPLC procedure according to the manufacturer's instructions (Tosoh, Stuttgart, Germany). All assay procedures were performed as described by the manufacturer. For each biomarker approximate sex-specific quintiles were derived using values for participants from the sub-cohort. The determined plasma biomarker concentrations were corrected for dilution due to citrate.

The correlation between the biomarkers was studied with age- and sex-adjusted partial Spearman correlation coefficients in the random sub-cohort comprising the analytical sample of 2, 021 participants. The relative risks of developing type 2 diabetes with 95% confidence intervals (CI) between quintiles of biomarkers were calculated using Cox's proportional hazards regression, modified for the case-cohort design according to the Prentice method [14]. Age at baseline in 1-y categories was entered as stratum variable. Age was used as the primary time-dependent variable in all models: entry time was defined as the subject's age at

recruitment and exit time as the date of diagnosis, death, or returns of the last follow-up questionnaire (whichever came first). Analyses were adjusted for baseline information including sex, body mass index (continuous), waist circumference (continuous), education (in or no training, vocational training, technical school, or technical college or university degree), occupational activity (light, moderate, or heavy), sports activity (0, 0.1– 4, or 4 or more h/week), cycling (0, 0.1–2.4, 2.5– 4.9, or 5 or more h/week), smoking (never, past, or current ≤20 cigarettes/day or current >20 cigarettes/day), alcohol intake (0, 0.1–5, 5.1–10, 10.1–20, 20.1– 40, or over 40 g/day) and consumptions of red meat, whole grain bread and coffee (continuous). Tests of linear trends in relative risks across quintiles of the biomarker levels were carried out based on Wald chi-square statistics by including the variables in the model as continuous variables. The proportion of different potential pathways contributing to the development of type 2 diabetes was estimated by using the procedure introduced by Chen et al. [7]. We considered in our analysis the following 4 metabolic pathways (represented by the biomarkers in parenthesis): insulin sensitivity (adiponectin), hepatic fat accumulation (GGT, and ALT); dyslipidemia (HDL-C and triglycerides), and inflammation (hs-CRP). To quantify the relative proportion that each biomarker contributed to the risk of diabetes we first constructed an index score based on quintiles of biomarker levels for each metabolic pathway. In cases where two or more biomarkers were available to reflect a common metabolic pathway the biomarker that showed the strongest association with risk of type 2 diabetes was chosen (based on the relative risk in the highest versus lowest quintile). Thus, GGT was chosen as a marker of hepatic fat accumulation, and HDL-C as a marker for dyslipidemia. For GGT, and hs-CRP, quintile scores based on the sex-specific distribution of the biomarker level in the sub-cohort were assigned, such that each score ranged from 1 for the lowest quintile to 5 for the highest quintile. For adiponectin and HDL-C, the scores were based on reverse order of the quintiles such that a score of 1 was assigned for the highest quintile and a score of 5 was assigned for the lowest quintile. The component scores were then summed up

to obtain an overall index score based on quintiles of biomarkers ranging from 4 to 20. We then examined the association of the index score (in quintiles) with risk of type 2 diabetes adjusted for the individual biomarkers (all in quintiles) and estimated the proportion of the association explained by each biomarker using effect decomposition [7]. Under this procedure, the regression coefficients were multiplied with a coefficient estimated as a product of covariance matrix of the regression coefficients of the covariates and covariance vector between quintiles of index score and the covariates. In the effect decomposition analysis, the index score as well as the biomarkers were included as quintiles but treated as a continuous variable. The corresponding 95% confidence interval (CI) was calculated based on Fieller's theorem [16]. In additional analyses, we created an index that also included HbA1c.

RESULTS

Subjects who developed diabetes during follow-up were older and more likely to be men compared with those who remained free of diabetes (Table 1). In unadjusted analyses, subjects who developed diabetes were more likely to be current- or former smokers at baseline and less likely to have university degree education than subjects who did not develop diabetes. They also had a higher body mass index and a higher waist circumference. The unadjusted mean plasma concentrations of HbA1c, GGT, ALT, triglycerides and hs-CRP were generally higher in cases than in non-cases, whereas HDL-C and adiponectin concentrations were lower among incident cases than non-cases.

All biomarkers were modestly positively correlated with HbA1c (Table 2). Of the liver specific markers, the age- and sex adjusted correlation between GGT and ALT was 0.53. Both, GGT and ALT levels were moderately correlated with triglycerides and hs-CRP. HDL-C showed moderate negative correlation with triglycerides and positive correlation with adiponectin.

All biomarkers were either directly or inversely associated with the risk of type 2 diabetes after adjustment for age, sex, body mass index, waist-circumference, education, sport activity, cycling, occupational activity, and smoking, alcohol intake, and consumptions of red meat, whole grain bread and coffee (Table 3). The strongest association was observed for HbA1c with a relative risk (RR) between extreme quintiles of 14.3 (95% confidence interval (CI) = 8.17, 25.0; *P* for linear trend (*P*) <0.001).

In order to explore whether the biomarkers were independently from each other associated with the risk of type 2 diabetes, we included selected biomarkers reflecting different metabolic pathways into one model in addition to the other covariates. The strength of the associations between the biomarker levels and risk of type 2 diabetes was attenuated for some markers but remained significant (Table 4). In this model, relative risks between the extreme quintiles were 2.76 (CI= 1.65, 4.60; *P*<0.001) for GGT and 2.40 (CI= 1.41, 4.07; *P*=0.01) for hs-CRP. Strong inverse associations were observed for HDL-C (RR between extreme quintiles: 0.35 (CI= 0.21, 0.56; *P*<0.01)) and adiponectin concentrations (RR between extreme quintiles: 0.26 (CI= 0.16, 0.40; *P*<0.001)). In further analyses we also included HbA1c in the model with the other biomarkers. In that model the corresponding relative risks between the extreme quintiles were 13.2 (CI= 7.41, 23.4; *P*<0.001) for HbA1c, 1.97 (CI= 1.16, 3.35; P<0.001) for GGT, 0.31 (CI= 0.19, 0.51; P<0.001) for HDL-cholesterol, 2.17 (CI= 1.20, 3.92; P=0.01) for hs-CRP and 0.28 (CI=0.17, 0.44; P<0.001) for adiponectin.
In order to quantify to what extent the different metabolic pathways contributed to risk of type 2 diabetes, we first calculated an index for each participant, based on quintiles of GGT, HDL-C, hs-CRP and adiponectin (see methods section for details). This index was positively correlated with plasma levels of GGT (r= 0.52), and hs-CRP (r= 0.59), and inversely correlated with HDL-C (r= -0.52) and adiponectin (r= -0.60). The relative risk between the

extreme quintiles of biomarker score was 8.81 (CI= 4.67, 16.6) in model including body mass index, waist circumference, sex, sport, biking, education, smoking, alcohol consumption, consumptions of red meat, whole grain bread and coffee. After further entering adiponectin, GGT, HDL-C and hs-CRP into the model the corresponding relative risk was 1.52 (CI= 0.40, 5.74). In the second step, the contribution of the components of this biomarker index was evaluated by calculating the proportion of the association that was explained by each biomarker (Table 5). All biomarkers explained significant proportions of the association between the index and risk of type 2 diabetes. Adiponectin accounted for the greatest proportion (32.1%; CI= 16.8, 49.1%), whereas the contributions of the other biomarkers were 21.5% (CI= 11.5, 32.8%) for GGT, 23.5% (CI= 10.1, 37.8%) for HDL-cholesterol, 15.5% (CI= 4.44, 27.3%) for hs-CRP. Replacement of GGT by ALT in the biomarker index did not significantly alter the proportion contributed by a pathway related to liver function, since the corresponding proportion contributed by ALT was 21.8% (CI= 11.7, 31.9%). Furthermore, exclusion of cases diagnosed within the first 2 years of follow-up did not materially change the results (data not shown). In additional analyses we further adjusted for HbA1c levels to account for baseline glucose profile. This further adjustment for HbA1c levels had only marginal effect on the results in general, but the proportion observed for hs-CRP did not remain significant. Thus, the relative proportional contribution to diabetes risk after adjustment for HbA1c were 38.2% (CI= 21.2, 57.6%) for adiponectin, 23.8% (CI= 7.44, 41.2%) for HDL-C 21.9%, (CI= 10.6, 34.6%) for GGT, and 11.8% (CI= -1.42, 25.5%) for hs-CRP. In a further step, we repeated our analysis with a biomarker index that included HbA1c. In that model, HbA1c accounted for 31.7% (CI= 24.9, 40.0%) of the association, whereas the contributions accounted for by adiponectin, HDL-C, GGT and hs-CRP were 24.9% (CI= 14.5, 36.4%), 20.5% (CI=11.0, 30.9%), 16.5% (CI= 8.31, 25.5%), 11.1% (CI= 1.30, 21.3%), respectively.

DISCUSSION

The results of the present study on the relative contribution of different pathway to diabetes risk suggest that of the biomarkers considered in our analysis variation in adiponectin levels accounted for the largest part, i.e. one third of the diabetes risk. While variations in HDL-C and GGT, accounted for equal smaller proportions, relatively lower contribution was found for hs-CRP.

In humans, adiponectin is one of the proteins secreted by the adipose tissue and that acts as a hormone with potential anti-inflammatory and insulin sensitizing properties [17, 18]. Several mechanisms through which adiponectin may decrease the risk of type 2 diabetes have been suggested, including suppression of hepatic gluconeogenesis, stimulation of fatty acid oxidation in the liver, stimulation of fatty acid oxidation and glucose uptake in skeletal muscle, and stimulation of insulin secretion [17, 18]. Adiponectin was found to be inversely associated with diabetes risk in a previous report from EPIC-Potsdam [15] and several other studies [3]. The liver enzymes GGT and ALT have been proposed as markers of accumulation of hepatic fat, and the accumulation of fat in the liver increases gluconeogenesis and decrease the glycogen storage in the liver [19]. GGT is also a non-specific marker of oxidative stress, which may additionally contribute to the development of type 2 diabetes [20]. Low HDL-cholesterol levels are one component of the metabolic syndrome. An inverse relationship between HDL-C and HbA1c levels has been described in type 2 diabetic patients [21, 22], and poor glycemic control is shown to be an independent risk factor for low HDL-C in type 2 diabetes [23]. Finally, low-grade chronic inflammation has also been suggested to be involved in the pathway to diabetes [24-34].

Previous studies have shown that circulating HbA1c levels are associated with the conversion of pre-diabetic phase into clinical diabetes [35-38]. Further, a strong association between

baseline HbA1c and incidence of type 2 diabetes was also suggested in a large-scale prospective study among middle aged women [39]. This is in line with our finding of a strong association between HbA1c and risk of type 2 diabetes. However, this finding is not unexpected, given that type 2 diabetes is characterized by insulin resistance and beta cell failure with associated abnormalities in glucose metabolism. Thus, HbA1c as a measure of long term blood glucose levels can be considered a rather downstream marker of onset of disease rather than a metabolic pathway to type 2 diabetes. We therefore excluded HbA1c levels from the main analysis. Interestingly, though, the relative contributions of the biomarkers were not substantially different when in sensitivity analyses we further adjusted for HbA1c levels to account for potentially different blood glucose profiles of the participants at baseline.

Some limitations of this study need consideration. All potential cases in our study were verified by a physician. Although we considered only clinically apparent type 2 diabetes and did not screen our study population during follow-up, we excluded participants with plasma glucose values within the diabetic range at baseline. Furthermore, exclusion of case subjects diagnosed within the first 2 years of follow-up did not alter the result. Thus, it is unlikely that prevalent but undiagnosed cases of diabetes have influenced the results. The use of self-report may have reduced sensitivity to detect incident diabetes cases, and thereby led to underestimation of diabetes incidence in our study. However, we aimed to increase sensitivity by also including questions on diabetes medication and dietary therapy to detect potential incident cases. Further, the validity of relative risk estimates primarily depends on specificity rather than sensitivity. Since only medically verified diagnoses were included in the analyses, any potential bias in the relative risk estimates is likely to be negligible. Also, in the present study the self-reported incidence rate of diabetes was approximately in the same level as reported over all ages based on the diabetes register of the former German Democratic

Republic (GDR) [40]. A single assessment of a biochemical indicator, as conducted in our study, may be susceptible to short-term variation, which would bias the results toward the null [41]. Most biomarkers used in our study are rather stable over time and have shown high reliability [42-44]. Nevertheless, we can not rule out the possibility that additional sources of measurement variability (related to blood processing, or long-term storage) may have introduced random measurement error. In addition, although we adjusted for a number of established risk factors of diabetes, because of the observational nature of our study we can not rule out the possibility of residual confounding. One further limitation of our study was that only about one third of the participants provided fasting blood samples, and therefore we used HbA1c instead of glucose. It is possible that using glucose may have resulted differently. However, we expect the difference in the contribution to the diabetes risk to be marginal given that the correlation between fasting glucose and HbA1c is high. Another potential limitation is that the used approach to estimate the relative contribution of each biomarker to risk of type 2 diabetes does not provide information on their performance in risk prediction. Studies on risk prediction models have shown that measurement of blood levels of glucose and HbA1c, HDL-C, triglycerides, GGT and ALT modestly improves the prediction of diabetes beyond life style and clinical variables [4-6].

Caution is needed when interpreting the results of the analyses on proportion of the association explained. First, the proportion estimates, decomposed from the total effect by adjusting for other biomarkers, may be biased if there is unmeasured confounding between the biomarkers and the outcome [45-47]. In the present study, we included a large variety of known risk factors as well as of biomarkers, thereby minimizing unmeasured confounding. Second, the estimate for total effect contributed by the pathways reflected by these biomarkers may depend on the method used in score computation. In the present study, the total effect of each biomarker was described by summing up the quintile distributions of the

biomarkers. This method depends on the distribution of the biomarker concentration in the underlying population. However, since our analysis is based on a population based study we expect that the distribution of biomarker concentration should be generalizable to other populations with similar characteristics. Nevertheless, our method of summing up the biomarker score implies that each biomarker is given equal weight, and results therefore need to be interpreted cautiously. Third, some of the used biomarkers may not be perfect markers of the relevant metabolic pathways. For example, in our study adiponectin was used to reflect insulin sensitivity. Although, it is well established that adiponectin is one of the major regulators of insulin sensitivity [17, 18, 48], the role of adiponectin in insulin resistance pathway is not completely clear [49].

The results of the present study give further support to the hypothesis that different biological pathways such as insulin resistance, hepatic fat accumulation, dyslipidemia, and inflammation independent from each other contribute to the risk of type 2 diabetes. Of the biomarkers of different biological pathways studied here, the highest relative contribution to risk of type 2 diabetes was observed for adiponectin (about one third), followed by GGT and HDL-cholesterol, whereas the contribution was lower for CRP. These results support the hypothesis that among these biomarkers, measures that aim to modify adiponectin levels are expected to have the largest impact on risk of type 2 diabetes.

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Table 1. Mean values and percentages of personal characteristics at baseline among incident cases and non-cases

	Non-cases (N=1965)		Cases (N= 613)	
	Mean	SD	Mean	SD
Age (years)[a]	49.8	9.00	55.0	7.47
Sex (% men) [b]	36.9		59.2	
Body mass index (kg/m^2)	25.8	4.16	30.4	4.49
Waist circumference (cm)	84.8	12.4	100	12.0
Sport activities (h/week)	1.03	1.79	0.67	1.51
Bicycling (h/week)	1.85	2.90	1.48	2.86
Education (%)				
In or no training	3.4		4.9	
Vocational training	33.9		40.3	
Technical school	24.6		24.5	
University degree	38.1		30.3	
Occupational activity (%)				
Light physical work	59.9		53.8	

Moderate physical work	33.8		35.2	
Heavy physical work	6.3		10.9	
Current- or ex smoker (%)	51.7		64.9	
Alcohol consumption (%)				
0.0 g (ethanol)/d	2.9		3.6	
0.1-5.0 g/d	35.1		33.3	
5.1-10.0 g/d	20.0		19.6	
10.1-20.0 g/d	18.4		17.6	
20.1-40.0 g/d	15.6		13.1	
>40g/d	8.0		12.9	
Red meat consumption (g/day)	41.8	28.7	50.5	34.2
Whole grain bread consumption (g/day)	46.4	54.0	37.5	46.1
Coffee consumption (cups/day)	2.81	2.12	2.67	2.11
HbA1c (%)	6.43	0.55	7.20	0.94
Gamma-glutamyltransferase (U/L)	27.6	71.7	48.1	63.3
Alanine-aminotransferase (U/L)	22.0	14.9	34.3	23.2

HDL -cholesterol (mmol/L)	1.37	0.36	1.13	0.28
Triglycerides (mmol/L)	1.37	0.95	2.24	1.62
hs-CRP (mg/L)	1.79	3.22	3.45	4.88
Adiponectin (ng/ml)	8.18	3.96	5.65	2.64

Table 2. Partial Spearman correlation coefficients (adjusted for age and sex) between different biomarkers in the random sub-cohort (N= 2021)

	HbA1c	Gamma-glutamyltransferase	Alanine-aminotransferase	HDL-cholesterol	Triglycerides	hs-CRP
Gamma-glutamyltransferase	0.10*					
Alanine-aminotransferase	0.08*	0.53*				
HDL-cholesterol	-0.09*	0.01	-0.03			
Triglycerides	0.11*	0.29*	0.27*	-0.25*		
hs-CRP	0.15*	0.29*	0.17*	-0.08*	0.24*	
Adiponectin	-0.08*	-0.15*	-0.12*	0.34*	-0.33*	-0.16*

*P<0.001, **P<0.05

Table 3. Adjusted relative risks (95% CI) of type 2 diabetes across quintiles of plasma concentrations of individual biomarker

	Quintiles of plasma concentration					
	I (referent)	II	III	IV	V	P-for trend
Gamma-glutamyltransferase (U/L)						
Quintiles in men [a]	11.0 (<14.3)	17.6 (14.3 – <22.0)	26.4 (22.0 – <30.8)	39.6 (30.8 – <51.7)	74.8 (≥51.7)	
Quintiles in women [a]	5.50 (<7.70)	8.80 (7.70 – <11.0)	12.1 (11.0 – <14.3)	16.5 (14.3 – <23.1)	35.2 (≥23.1)	
Relative risk, model A [b]	1	1.90 (1.12, 3.20)	2.82 (1.69, 4.70)	5.80 (3.56, 9.45)	7.65 (4.71, 12.4)	<0.001
Relative risk, model B [c]	1	1.57 (0.90, 2.74)	1.98 (1.17, 3.37)	3.60 (2.17, 5.99)	4.00 (2.42, 6.62)	<0.001
Alanine-aminotransferase (U/L)						
Quintiles in men	14.3 (<16.5)	18.7 (16.5 – <22)	24.2 (22.0 – <28.6)	31.9 (28.6 – <37.4)	49.5 (≥37.4)	
Quintiles in women	9.90 (< 12.1)	13.2 (12.1 – <14.3)	15.4 (14.3 – <17.6)	18.7 (17.6 – <22.0)	27.5 (≥22.0)	
Relative risk, model A	1	1.27 (0.78, 2.09)	2.20 (1.40, 3.46)	2.82 (1.80, 4.43)	5.85 (3.81, 8.97)	<0.001
Relative risk, model B	1	1.23 (0.74, 2.05)	1.65 (1.03, 2.65)	1.82 (1.14, 2.89)	3.01 (1.94, 4.67)	<0.001
HDL –cholesterol (mmol/L)						
Quintiles in men	0.86 (<0.97)	1.04 (0.97 – <1.12)	1.17 (1.12 – <1.24)	1.33 (1.24 – <1.44)	1.59 (≥1.44)	
Quintiles in women	1.01 (<1.16)	1.25 (1.16 – <1.35)	1.42 (1.35 – <1.52)	1.60 (1.52 – <1.73)	1.91 (≥1.73)	

Relative risk, model A	1	0.72 (0.55, 0.94)	0.42 (0.32, 0.57)	0.26 (0.19, 0.37)	0.14 (0.09, 0.21)	<0.001
Relative risk, model B	1	0.79 (0.59, 1.05)	0.56 (0.41, 0.77)	0.43 (0.29, 0.62)	0.26 (0.16, 0.40)	<0.001
Triglycerides (mmol/L)						
Quintiles in men	0.72 (<0.90)	1.08 (0.90 – <1.24)	1.47 (1.24 – <1.69)	1.99 (1.69 – <2.37)	3.19 (≥2.37)	
Quintiles in women	0.54 (<0.68)	0.78 (0.68 – <0.89)	0.99 (0.89 – <1.12)	1.29 (1.12 – <1.52)	2.03 (≥1.52)	
Relative risk, model A	1	1.27 (0.79, 2.05)	2.90 (1.88, 4.48)	3.18 (2.08, 4.87)	5.83 (3.87, 8.80)	<0.001
Relative risk, model B	1	0.95 (0.59, 1.55)	1.83 (1.17, 2.86)	1.75 (1.12, 2.73)	2.60 (1.68, 4.02)	<0.001
hs-CRP (mg/L)						
Quintiles in men	0.11 (<0.22)	0.22 (0.22 – <0.44)	0.55 (0.44 – <0.77)	1.16 (0.77 – <1.98)	3.85 (≥1.98)	
Quintiles in women	0.11 (<0.22)	0.33 (0.22 – <0.55)	0.77 (0.55 – <1.21)	1.76 (1.21 – <2.97)	5.56 (≥2.97)	
Relative risk, model A	1	2.05 (1.24, 3.40)	2.80 (1.71, 4.57)	3.98 (2.48, 6.38)	7.77 (4.89, 12.4)	<0.001
Relative risk, model B	1	1.90 (1.12, 3.22)	1.76 (1.03, 3.00)	2.10 (1.26, 3.49)	3.52 (2.12, 5.84)	<0.001
Adiponectin (µg/mL)						
Quintiles in men	3.07 (<3.86)	4.49 (3.86 – <5.16)	5.75 (5.16 – <6.37)	6.98 (6.37 – <8.12)	9.71 (≥8.12)	
Quintiles in women	4.74 (<5.96)	6.97 (5.96 – <7.76)	8.70 (7.76 – <9.64)	10.7 (9.64 – <12.2)	14.4 (≥12.2)	
Relative risk, model A	1	0.47 (0.35, 0.63)	0.30 (0.22, 0.41)	0.24 (0.18, 0.34)	0.11 (0.07, 0.16)	<0.001

Relative risk, model B	1	0.49 (0.36, 0.67)	0.36 (0.25, 0.50)	0.35 (0.25, 0.50)	0.18 (0.12, 0.28)	<0.001

[a]Values are Medians (quintile cut off points)

[b]Model A: Adjusted for: age, sex, education, sport activity (0, 01-4, or >4 h/week), cycling (0, 0.1-2.4, 2.5-4.9 or >4.9 h/week), occupational activity (light, moderate, heavy), smoking (never, past or current <20, current 20 or more cigarettes/day), alcohol intake (0, 0.1-5, 5.1-10, 10.1-20, 20.1-40 or >40g/day), consumptions of red meat, whole grain bread and coffee (continuous)

[c]Model B: Further adjusted for body mass index and waist-circumference

Table 4. Adjusted relative risks (95% CI) of type 2 diabetes across quintiles of individual biomarkers in a model including all biomarkers simultaneously

	Quintiles of plasma concentration					
	I (referent)	II	III	IV	V	P-for trend
Gamma-glutamyltransferase						
Model A[a]	1	1.30 (0.74, 2.26)	1.76 (1.04, 2.99)	3.46 (2.10, 5.71)	3.73 (2.25, 6.18)	<0.001
Model B[b]	1	1.25 (0.71, 2.19)	1.46 (0.85, 2.51)	2.69 (1.62, 4.46)	2.76 (1.65, 4.60)	<0.001
HDL-cholesterol						
Model A	1	0.81 (0.60, 1.09)	0.56 (0.40, 0.77)	0.37 (0.25, 0.55)	0.26 (0.17, 0.42)	<0.001
Model B	1	0.86 (0.64, 1.15)	0.63 (0.45, 0.89)	0.49 (0.32, 0.75)	0.35 (0.21, 0.56)	<0.001
hs-CRP						
Model A	1	1.49 (0.85, 2.60)	1.73 (1.01, 2.96)	2.20 (1.31, 3.69)	3.96 (2.39, 6.54)	<0.001
Model B	1	1.51 (0.86, 2.64)	1.29 (0.74, 2.26)	1.48 (0.86, 2.53)	2.40 (1.41, 4.07)	<0.001
Adiponectin						
Model A	1	0.50 (0.36, 0.68)	0.43 (0.30, 0.60)	0.40 (0.28, 0.58)	0.22 (0.14, 0.35)	<0.001
Model B	1	0.50 (0.36, 0.69)	0.43 (0.30, 0.62)	0.45 (0.31, 0.65)	0.26 (0.16, 0.40)	<0.001

[a]Model A: age, sex, education, sport activity (0, 01-4, or >4 h/week), cycling (0, 0.1-2.4, 2.5-4.9 or >4.9 h/week), occupational activity (light, moderate, heavy), smoking (never, past or current <20, current 20 or more cigarettes/day), alcohol intake (0, 0.1-5, 5.1-10, 10.1-20, 20.1-40 or >40g/day), consumptions of red meat, whole grain bread and coffee (continuous), plus the biomarkers presented in the table

[b]Model B: Further adjusted for body mass index and waist-circumference

Table 5. The estimated relative contribution (95% CI) of four biomarkers to risk of type 2 diabetes[a]

	Relative contribution to risk of type 2 diabetes (%)[a]	
	prop.	(95% CI)
Adiponectin	32.1	(16.8, 49.1)
GGT	21.5	(11.5, 32.8)
HDL-C	23.5	(10.1, 37.8)
hs-CRP	15.5	(4.44, 27.3)

[a]The relative contribution to the association between score based on quintiles of four biomarkers (biomarker score) and diabetes risk was estimated using effect decomposition method. The relative risk between the extreme quintiles of biomarker score was 8.81 (CI= 4.67, 16.6) in model including body mass index, waist circumference, sex, sport, biking, education, smoking, alcohol consumption, consumptions of red meat, whole grain bread and coffee, and after further entering adiponectin, GGT, HDL-C and hs-CRP in the model the corresponding relative risk was 1.52 (CI= 0.40, 5.74).