Circulating Levels of Phthalate Metabolites Are Associated With Prevalent Diabetes in the Elderly

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OBJECTIVE—Phthalates are ubiquitous industrial high-volume chemicals known as ligands to peroxisome proliferator–activated receptors (PPARs). Because PPAR-γ agonists modulate insulin sensitivity and are used to treat type 2 diabetes, we investigated whether circulating levels of phthalate metabolites are related to prevalent type 2 diabetes.

RESEARCH DESIGN AND METHODS—A total of 1,016 subjects, aged 70 years, were investigated in the Prospective Investigation of the Vasculature in Uppsala Seniors Study. Four phthalate metabolites were detected in almost all participant sera by an API 4000 liquid chromatograph/tandem mass spectrometer. Type 2 diabetes was defined as the use of pharmacological hypoglycemic agents or a fasting plasma glucose >7.0 mmol/L.

RESULTS—A total of 114 subjects were shown to have diabetes. Following adjustment for sex, BMI, serum cholesterol and triglycerides, educational level, and smoking and exercise habits, high levels of the phthalate metabolites monomethyl phthalate (MMP) ($P < 0.01$), monoisobutyl phthalate (MiBP) ($P < 0.05$), and monoethyl phthalate (MEP) ($P < 0.05$), but not mono(2-ethylhexyl) phthalate, were associated with an increased prevalence of diabetes. Using the fasting proinsulin–to–insulin ratio as a marker of insulin secretion and the homeostasis model assessment-insulin resistance index as a marker of insulin resistance, MiBP was mainly related to poor insulin secretion, whereas MEP and MMP mainly were related to insulin resistance.

CONCLUSIONS—The findings in this cross-sectional study showed that several phthalate metabolites are related to diabetes prevalence, as well as to markers of insulin secretion and resistance. These findings support the view that these commonly used chemicals might influence major factors that are regulating glucose metabolism in humans at the level of exposure of phthalate metabolites seen in the general elderly population.

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Phthalates (phthalate diesters) are a large group of ubiquitous, industrial high-volume chemicals that are commonly used as plasticizers in, for example, polyvinylchloride plastics to make plastic products more flexible. Therefore, phthalates are found in numerous household products, such as food packaging, furniture, and toys, and in medical devices, such as tubing and intravenous bags. Certain plastics may contain up to 40–50% phthalate by weight. In addition, phthalates are used in personal care products, such as cosmetics, and also in pharmaceuticals. Because phthalates are additives and, as such, not covalently bound to the plastic, they can easily leach and transfer to air and food. As a consequence thereof, humans are exposed to phthalates through inhalation, ingestion, and dermal exposure, and exposure is unavoidable because of the abundance of plastic in our society. Phthalates are rapidly degraded into the respective phthalate monoesters in phase I reactions catalyzed by lipases and esterases. The respective monoesters are eliminated in the urine as glucuronide conjugates or are further metabolized, and it is in fact the monoester metabolites that have been claimed to be responsible for adverse health effects (1–8).

Although they have relatively short half-lives in humans, phthalates have been associated with a number of health problems, including increased risk for adverse reproductive development, obesity, asthma, atherosclerosis, and allergies (9–12).

It has been known for several years that phthalates can bind to members of the nuclear peroxisome proliferator–activated receptors (PPARs) (13–19). These receptors are known to be involved in adipose tissue and lipid homeostasis, and the natural ligands mainly are fatty acids. Pharmaceutical compounds have been developed as agonists for PPAR-α and -γ available on the market, such as fibrates and glitazones, are known to influence fat distribution and change lipid status (20,21). Furthermore, PPAR-γ antagonists are known to influence glucose homeostasis via reduction of insulin resistance (22) and are used for the treatment of type 2 diabetes (20).

Because it has been reported that phthalate levels in humans are associated with obesity (10,23), a well-known effect of PPAR-γ receptor activation, and because obesity is an important risk factor for diabetes development (24), we hypothesized that high levels of phthalates in humans also might be associated with diabetes. A recent small study performed in Mexico supports this hypothesis (25). To further evaluate this hypothesis, we used data from the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) Study, in which we have measured circulating levels of phthalate metabolites in almost 1,000 elderly individuals. As a secondary objective, we also investigated if the phthalate metabolite levels were associated with markers of insulin secretion and resistance, two major features involved in the regulation of glucose

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metabolism and independent risk factors for type 2 diabetes development.

RESEARCH DESIGN AND METHODS

—Eligible for the PIVUS study were all subjects aged 70 years living in the community of Uppsala, Sweden. The subjects were randomly chosen from the register of community living. A total of 1,016 subjects participated during 2001–2004, giving a participation rate of 50.1%. The study was approved by the ethics committee of Uppsala University.

All subjects were investigated in the morning after an overnight fast. No medication or smoking was allowed after midnight. The subjects were asked to answer a questionnaire about their medical history, educational level, exercise habits, smoking habits, and regular medication. Educational level was divided into three groups (<9, 9–12, and >12 years). Exercise habits were divided into four groups (less than two times per week of light exercise [no sweat], two or more times per week of light exercise, one to two times per week of heavy exercise [sweat], and more than two times per week of heavy exercise). Venous blood samples were collected and stored at −70°C until analysis. Lipid variables and fasting plasma blood glucose were measured using standard laboratory techniques. Basic characteristics of subjects are given in Table 1.

Diabetes was defined as a history of diabetes or a fasting glucose value >7.0 mmol/L. In the total sample, 119 subjects showed diabetes. Of those, 88 had a history of diabetes. In that group, the mean duration of diabetes was 8.9 years (SD 7.7). Only four subjects reported a diabetes duration >20 years.

Phthalate analysis

Human serum (0.5 mL) was analyzed for levels of 10 phthalate metabolites using an isotope liquid chromatograph/tandem mass spectrometer (API 4000LC-MS/MS) at ALS Environmental Canada (http://www.alsglobal.com/environmental.aspx) following the general procedures presented by the Centers for Disease Control and Prevention as previously described in detail (26). In brief, quality control of the analysis was maintained by analyzing a method blank (calf serum) and two spiked calf serum samples (20 ng/mL, all analytes) along with every 17 samples. The limit of detection (LOD) was 0.2 ng/mL. Four of 10 phthalate metabolites, namely mono(2-ethylhexyl) phthalate (MEHP), monoethyl phthalate (MEP), monoisobutyl

Table 1—*Baseline characteristics in the investigated sample*

	All subjects		Women		Men		Diabetes		Nondiabetes	
	n	Means (SD)	*n*	Means (SD)	*n*	Means (SD)	*n*	Means (SD)	*n*	Means (SD)
Proportion of female subjects (%)	1,016	52					119	42	897	51
Height (cm)	1,016	169 (9.1)	509	162.1 (5.6)	507	175.8 (6.5)	119	170.4 (9.4)	897	168.8 (9.1)
Weight (kg)	1,016	77.3 (14.4)	509	71.2 (13.1)	507	83.5 (13)	119	84.5 (16.2)	897	76.4 (13.9)
Waist circumference (cm)	1,004	91.2 (11.6)	503	87.6 (11.6)	501	94.7 (10.4)	116	97.5 (12.1)	888	90.3 (11.3)
Fasting plasma glucose (mmol/L)	1,013	5.3 (1.6)	508	5.2 (1.5)	505	5.5 (1.7)	116	8.4 (3.1)	897	4.9 (0.5)
Systolic blood pressure (mmHg)	1,012	149.6 (22.7)	506	153.3 (22.6)	506	146 (22.2)	119	154.7 (23.7)	893	149 (22.5)
Diastolic blood pressure (mmHg)	1,012	78.7 (10.2)	506	78.0 (10.1)	506	79.3 (10.3)	119	79.7 (11.6)	893	78.5 (10.0)
HDL cholesterol (mmol/L)	1,013	1.5 (0.4)	507	1.7 (0.4)	506	1.4 (0.4)	119	1.36 (0.4)	894	1.5 (0.4)
LDL cholesterol (mmol/L)	1,011	3.4 (0.9)	506	3.5 (0.9)	505	3.2 (0.9)	119	2.9 (0.9)	892	3.4 (0.9)
Serum triglycerides (mmol/L)	1,013	1.3 (0.6)	508	1.3 (0.6)	505	1.3 (0.6)	119	1.5 (0.8)	894	1.3 (0.6)
BMI (kg/m^2)	1,016	27.0 (4.3)	509	27.1 (4.9)	507	27.0 (3.7)	119	29.1 (5.2)	897	26.8 (4.1)
Fasting plasma insulin (mU/L)	1,007	9.2 (7.1)	503	8.8 (6.1)	504	9.6 (7.9)	118	15.4 (13.7)	889	8.4 (5.1)
HOMA-IR (mU/L × mmol/L)	1,004	2.4 (3.6)	502	2.2 (2.9)	502	2.6 (4.2)	115	6.7 (9.1)	889	1.9 (1.2)
Fasting plasma proinsulin (pmol/L)	1,002	10.8 (10.7)	499	9.4 (8.9)	503	12.3 (12.1)	117	24.9 (20.3)	885	9.0 (6.8)
Current smoking (%)	1,015	11	509	11	506	10	119	11	896	11
Antihypertensive treatment (%)	1,007	31	502	31	505	31	119	60	888	28
Statin use (%)	1,016	15	509	13	507	16	119	29	897	13
MEHP (ng/mL) [median (25th and 75th percentile)]	1,003	4.5 (2.0–15.5)	502	4.7 (2.0–14.5)	501	4.3 (2.1–17.4)	117	4.9 (2.6–15.2)	886	4.5 (2.0–15.8)
MEP (ng/mL) [median (25th and 75th percentile)]	1,003	11.6 (7.2–17.5)	502	11.6 (7.2–16.8)	501	11.6 (7.2–18.5)	117	12.2 (8.9–19.5)	886	11.4 (7.0–17)
MiBP (ng/mL) [median (25th and 75th percentile)]	1,003	13.5 (9.3–29.3)	502	13.4 (9.5–24.5)	501	13.5 (9.1–33.3)	117	16.2 (10.4–76.3)	886	13.3 (9.2–24.5)
MMP (ng/mL) [median (25th and 75th percentile)]	1,003	1.5 (0.8–3.1)	502	1.5 (0.9–3.0)	501	1.5 (0.8–3.2)	117	1.7 (1.0–4.1)	886	1.5 (0.8–3.0)

Continuous variables are given as means (SD) or median (25th and 75th percentiles). Proportions (%) are given for smoking, antihypertensive treatment, and statin use.

phthalate (MiBP), and monomethyl phthalate (MMP), were detectable in all but 5–12 subjects (at least 96% of subjects). The fact that some subjects showed undetectable levels rules out a general contamination of these compounds. Only the four metabolites with detectable levels were used in the statistical analysis. For the rest of the metabolites, 31–100% of the observations were below the LOD. The measured serum concentrations of MEHP, MEP, MiBP, and MMP are given in Table 1. The analysis of the phthalates took place 5–8 years following the collection of the samples.

Insulin and proinsulin measurements

At the laboratory of the Department of Public Health and Caring Sciences/Geriatrics, University Hospital, Uppsala, plasma proinsulin and insulin concentrations were determined using the Proinsulin ELISA and the Insulin ELISA immunoassays (Mercodia, Uppsala, Sweden) on a Bio-Rad Coda automated EIA analyzer (Bio-Rad Laboratories, Hercules, CA).

Calculations of insulin secretion and resistance

The ratio of fasting proinsulin to insulin was used as an index of insulin secretion, since this ratio increases with failing β-cell function (27,28). The homeostasis model assessment-insulin resistance (HOMA-IR), as an index of insulin resistance, was calculated as fasting insulin × glucose/22.5 (29).

Statistics

All four phthalate metabolites, as well as the indices of insulin secretion and resistance, were skewed toward high levels but were normally distributed following ln-transformation.

Relationships between phthalate metabolites and prevalent diabetes were evaluated by logistic regression models, first using the phthalate metabolites as continuous variables and thereafter following division of the phthalate metabolites into quintiles. For the continuous analysis, two steps of adjustments were used: *1*) adjustment for sex only and 2) multiple adjustments for sex, serum cholesterol and triglycerides, BMI, smoking and exercise habits, and educational levels. In the quintile analysis, *P* values are given for linear trend analysis as well as for quadratic trend. In the quintile analysis, only multiple-adjusted *P* values are given.

A similar approach was used when relating phthalate metabolites to either the proinsulin-to-insulin ratio or to HOMA-IR index, except that linear regression was used. In this analysis, only nondiabetic subjects were included. General additive models were used (30) to visualize the relationship between some of the phthalate metabolites and prevalent diabetes in a continuous fashion in Supplementary Fig. 1. These models were adjusted for the same covariates as the multiple-adjusted models described above.

RESULTS—Mean values or proportions of the established risk factors are shown in Table 1. Relationships between the four evaluated phthalate metabolites are given in Supplementary Table 1. MMP was significantly related to the other phthalate metabolites (although inversely compared with MEHP). MiBP was significantly related to MEHP but not significantly so compared with MEP. MEP and MEHP were not significantly related.

Phthalates versus diabetes

When the four phthalate metabolites were related to prevalent diabetes in logistic regression models using continuous variables, both MEP and MiBP were significantly related to diabetes following adjustment for sex only. Following multiple adjustments, however, only MiBP levels were significantly related to diabetes (odds ratio [OR] 1.30 [95% CI 1.10–1.55], $P = 0.0025$; for details see Table 2). In Supplementary Table 2, details on different levels of adjustments are presented.

No significant interactions between the four phthalate metabolites and sex, obesity, smoking, and education levels were detected when interaction terms between the phthalate metabolites and these four confounders were introduced in the models. A sensitivity analysis for these four confounders is presented in Supplementary Table 3. Of note is that the OR for MiBP is higher in men than women (1.48 [95% CI 1.19–1.85] in men and 1.08 [0.80–1.46] in women). However, the interaction term between MiBP and sex regarding prevalent diabetes is not significant ($P = 0.11$).

In quintile analysis, MEP and MiBP, as well as MMP, showed a significantly elevated prevalence of diabetes in the highest quintile compared with the lowest (OR 2.00–2.50), and the linear trend tests were significant. Furthermore, for MEP the quadratic trend test was significant. The highest OR was seen in the third quintile (OR 2.87 [95% CI 1.37–6.03]; for additional details see Table 3).

Phthalates versus the proinsulin-to-insulin ratio

MEHP and MiBP were significantly related to a high proinsulin-to-insulin ratio, both following sex adjustment only and following multiple adjustments, respectively. MEP was weakly related to the proinsulin-to-insulin ratio in an inverse way in linear regression models using continuous variables (Table 4). In quintile analysis, the quadratic trend test for MIBP was significant, being consistent with the finding that the highest proinsulin-to-insulin ratio was seen in the third quintile (for details see Supplementary Table 4).

Phthalates versus the HOMA-IR index

MEP and MMP were significantly related to a high HOMA-IR index, both following sex adjustment only and following multiple adjustments, respectively (Table 4). In quintile analysis, the quadratic trend test for MEP was significant, being consistent with the finding that most of the effect on HOMA-IR was seen in the third quintile (for details see Supplementary Table 5).

In the analysis of HOMA-IR and the proinsulin-to-insulin ratio, no diabetic subjects were included because the diabetic state or pharmacological treatment for diabetes may affect the proinsulin and insulin measurements.

Table 2—*Relationships between four phthalate metabolites and prevalent diabetes*

	Sex adjusted		Multiple adjusted	
	OR (95% CI)	*P*	OR (95% CI)	*P*
MEHP	1.05 (0.92–1.21)	0.461	0.97 (0.84–1.13)	0.729
MEP	1.30 (1.00–1.69)	0.049	1.28 (0.97–1.7)	0.089
MiBP	1.25 (1.07–1.46)	0.006	1.30 (1.10–1.55)	0.003
MMP	1.12 (0.95–1.33)	0.174	1.21 (1.00–1.46)	0.052

The phthalate metabolites are used as ln-transformed continuous variables in the analysis. The first models are just sex adjusted, whereas the multiple-adjusted models used sex, serum cholesterol and triglycerides, BMI, smoking and exercise habits, and educational levels as confounders.

Table 3—*Relationships between four phthalate metabolites and prevalent diabetes*

	Quintile 1		Quintile 2		Quintile 3		Quintile 4		Quintile 5		*P* value linear trend	*P* value quadratic
	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*		
MEHP	1.00	—	1.73 (0.87–3.44)	0.117	1.61 (0.81–3.2)	0.175	1.2 (0.59–2.44)	0.620	1.24 (0.61–2.51)	0.550	0.965	0.176
MEP	1.00	—	2.25 (1.06–4.79)	0.035	2.87 (1.37–6.03)	0.005	2.44 (1.14–5.21)	0.021	2.27 (1.08–4.81)	0.031	0.061	0.024
MiBP	1.00	—	1.19 (0.59–2.38)	0.6217	0.84 (0.41–1.76)	0.653	1.37 (0.7–2.66)	0.355	2.00 (1.03–3.88)	0.040	0.038	0.183
MMP	1.00	—	1.71 (0.83–3.48)	0.1429	1.65 (0.8–3.42)	0.174	1.6 (0.79–3.24)	0.189	2.54 (1.25–5.13)	0.010	0.026	0.982

The phthalate metabolites are divided into quintiles in the analysis, and the first quintile is used as the referent. The *P* values for linear trend and quadratic trend are adjusted for sex, serum cholesterol and triglycerides, BMI, smoking and exercise habits, and educational levels.

Table 4—*Linear regression analysis of relationships between four phthalate metabolites and the proinsulin-to-insulin ratio or the HOMA-IR index*

	Sex adjusted		Multiple adjusted	
	β (95% CI)	*P*	β (95% CI)	*P*
Proinsulin-to-insulin ratio				
MEHP	0.05 (0.027–0.073)	<0.001	0.046 (0.022–0.069)	<0.001
MEP	−0.051 (−0.097 to −0.004)	0.032	−0.05 (−0.097 to −0.002)	0.040
MiBP	0.057 (0.028–0.085)	<0.001	0.06 (0.03–0.089)	<0.001
MMP	−0.006 (−0.034 to 0.023)	0.703	−0.005 (−0.035 to 0.025)	0.733
HOMA-IR index				
MEHP	−0.005 (−0.031 to 0.02)	0.688	−0.012 (−0.035 to 0.011)	0.317
MEP	0.090 (0.039–0.142)	0.001	0.069 (0.023–0.116)	0.004
MiBP	0.030 (−0.002 to 0.062)	0.063	0.014 (−0.015 to 0.043)	0.338
MMP	0.071 (0.039–0.102)	<0.001	0.047 (0.017–0.076)	0.002

The phthalate metabolites are used as ln-transformed continuous variables in the analysis. The first models are just sex adjusted, whereas the multiple-adjusted models used sex, serum cholesterol and triglycerides, BMI, smoking and exercise habits, and educational levels as confounders. Only nondiabetic subjects were included in the analysis.

CONCLUSIONS—The current study showed that some of the measured circulating phthalate metabolites, MEP, MMP, and MiBP, were associated with prevalent diabetes in the present cross-sectional study. Furthermore, MiBP levels were inversely related to a marker of poor insulin secretion, whereas MMP and MEP levels were related to insulin resistance, two major factors involved in regulation of an impaired glucose metabolism and independent risk factors for type 2 diabetes development.

Comparison with the literature

In a recent cross-sectional study of Mexican women, self-reported diagnosis of diabetes was related to several phthalate metabolites (25). However, neither MEP nor MiBP was found to be significantly related to diabetes in the Mexican study, and MMP was not evaluated. The current study has the advantage that three times as many diabetic patients were included. Furthermore, we used the combination of self-reported diabetes and measurements of fasting glucose and/or use of pharmacological treatment of diabetes to define prevalent diabetes. The addition of fasting glucose measurements resulted in the addition of another 31 diabetic case subjects to the 85 subjects that reported a history of diabetes. Thus, if self-reported diagnosis only and no fasting glucose measurements had been used to define cases, 26% of the diabetes cases would have been misclassified and observations made would have been diluted.

In the sensitivity analysis in the current study, a higher OR for MiBP regarding diabetes was found in men compared with women (1.48 vs. 1.08). However, the interaction term between MiBP and sex regarding diabetes was not significant, so future studies have to determine whether this observed relationship between MiBP and diabetes really differs between men and women.

In an evaluation of male participants in the National Health and Nutrition Examination Survey 1999–2002, high levels of MBP, monobenzyl phthalate (MBzP), and MEP were associated with insulin resistance (23). Thus, the finding of a relationship between MEP and a high HOMA-IR index is consistent between the studies. MBP and MBzP were measured in the PIVUS study, but because only a small fraction of the participants showed detectable levels in the circulation, we were not able to perform any meaningful evaluation of these phthalate metabolites. To the best of our knowledge, no other study has investigated whether phthalate metabolites are related to markers of insulin secretion.

Measurement of phthalates and sources of exposure

Because the parent phthalates are so abundant, it is a hopeless task to measure those compounds without major contamination. Therefore, usually their metabolites, not abundant in the environment, are measured in the circulation or in the urine (31). The majority of previous studies on human phthalate exposure is compromised on

urine testing. Measurements in the urine have the advantage that higher levels are found and thereby more metabolites could be properly detected. Thus, a limitation of the current study is that only four phthalate metabolites could be investigated in detail with regard to diabetes.

In the current study, MiBP, MMP, MEP, and MEHP were detectable in almost all analyzed serum samples. The common feature of the metabolites MEP, MiBP, and MMP is that all these three metabolites are derived from degradation of associated phthalate parent compounds (diethyl phthalate, di-isobutyl phthalate, and dimethyl phthalate, respectively) used as solvents/carriers of fragrances used in personal care products. Di-2-ethyl phthalate, with the metabolite MEHP, is on the other hand mainly used as plasticizer to make plastic compounds more flexible.

In a Danish study, the correlations between levels of 13 metabolites in different matrices (urine, semen, and serum) were examined in 60 young men. Both MEP and MiBP levels were correlated in serum and urine, indicating that serum levels could be used as biomarkers of human exposure (1). In the Danish study, MiBP, MEHP, MBzP, mono-(2-ethyl-5-oxohexyl) phthalate, and mono-(2-ethyl-5-hydroxyhexyl) phthalate were found in lower levels and in fewer samples, but the LOD was higher than in the current study. Frederiksen et al. (1) found higher levels and a detection rate of monoisononyl phthalate, whereas mono-n-octyl phthalate was not detectable in any of their samples. For these metabolites, their LOD was comparable with the current study.

Sources of exposure of these compounds and comparisons with the levels in other studies are described in more detail in our previous recent publication by Olsén et al. (26) and at http://www.atsdr.cdc.gov/.

Possible mechanisms

Because phthalate metabolites are known ligands to PPARs (13–19), receptors known to influence glucose homeostasis, impairments in PPAR-signaling pathways are most likely to contribute to the actions of phthalates on glucose metabolism and diabetes development. PPAR activation has been shown to be involved in different steps in glucose homeostasis, such as influence on insulin resistance (22) and on insulin secretion (32), influence on circulating levels of lipids (21), and altering the amount of visceral and subcutaneous fat (21,33). Additional experimental studies on phthalates have to be performed to elucidate the exact actions whereby phthalates could influence these PPAR-mediated actions.

Limitations

This study was performed in a sample of elderly white subjects. Thus, we cannot extrapolate these findings to other ethnicities and age-groups. The study also was conducted as a cross-sectional study, and, as such, a risk of reverse causality always exists. Thus, the present data have to be confirmed in prospective studies. Some pharmaceuticals contain phthalates. Whether this is an issue for patients with diabetes taking medication is not known, and details on certain brands of antidiabetes drugs are not collected in this study.

In the current study, we used serum measurements of phthalate metabolites. It is more common to use urinary measurements. The advantage of urinary measurements is that usually higher levels are found compared with serum and thereby more phthalate metabolites could be quantified above the lower detection limit. Therefore, we can only report associations regarding four metabolites, although in fact 10 metabolites were evaluated. Serum levels also might change more rapidly than urinary levels and therefore repeated measurements would be desirable for a more precise measure of exposure.

In conclusion, the findings in this cross-sectional study showed that several phthalate metabolites are related to diabetes prevalence, as well as to markers of insulin secretion and resistance, and support the view that these commonly used chemicals might influence glucose metabolism in humans at the level of exposure seen in the general elderly population.

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P.M.L. conceived of the project and contributed to the critical revision of the manuscript for important intellectual content. B.Z. was responsible for laboratory analyses of insulin and proinsulin measurements and calculations and contributed to the critical revision of the manuscript for important intellectual content. L.L. performed data analysis and contributed to the critical revision of the manuscript for important intellectual content. L.L. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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