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## Urinary arsenic and measures of insulin sensitivity and $\beta$ -cell function in non-diabetic Amish adults

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### Abstract

**Background**—Substantial evidence supports an association between arsenic and diabetes at high exposure levels, but results are mixed at low exposure levels. The etiology of diabetes involves insulin resistance and  $\beta$ -cell dysfunction. However, only a few epidemiologic studies have examined measures of insulin resistance and  $\beta$ -cell function in relation to arsenic exposure and no studies have tested for associations with the oral glucose tolerance test (OGTT). We examined the association between urinary total arsenic and OGTT-based markers of insulin sensitivity and  $\beta$ -cell function.

**Methods**—We studied 221 non-diabetic adults (mean age=52.5 years) from the Amish Family Diabetes Study. We computed OGTT-based validated measures of insulin sensitivity and  $\beta$ -cell function. Generalized estimating equations accounting for sibship were used to estimate associations.

**Results**—After adjusting for age, sex, waist-to-hip ratio and urinary creatinine, an interquartile range increase in urinary total arsenic (6.24  $\mu\text{g/L}$ ) was significantly, inversely associated with two insulin sensitivity measures (Stumvoll metabolic clearance rate= $-0.23 \text{ mg}/(\text{kg}\times\text{min})$ , (95% CI:  $-0.38, -0.089$ ),  $P=0.0015$ ; Stumvoll insulin sensitivity index= $-0.0029 \mu\text{mol}/(\text{kg}\times\text{min}\times\text{pM})$ , (95% CI:  $-0.0047, -0.0011$ ),  $P=0.0015$ ). Urinary total arsenic was also significantly associated with higher fasting glucose levels (0.57 mg/dL (95% CI: 0.06, 1.09) per interquartile range increase,  $P=0.029$ ). No significant associations were found between urinary total arsenic and  $\beta$ -cell function measures.

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**Conclusions**—This preliminary study found that urinary total arsenic was associated with insulin sensitivity, but not  $\beta$ -cell function measures, suggesting that low level arsenic exposure may influence diabetes risk through impairing insulin sensitivity.

### Keywords

Arsenic;  $\beta$ -cell function; insulin sensitivity; oral glucose tolerance test

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## INTRODUCTION

Arsenic is the top hazard that poses the most important potential threat to human health including diabetes on the priority list of the U.S. Agency for Toxic Substances and Disease Registry [1]. The main sources of arsenic are contaminated drinking water and food [2,3]. Potential biological mechanisms by which arsenic influences diabetes include high affinity of arsenic with sulfhydryl groups in insulin, insulin receptor, and glucose transporters; increased oxidative stress which can lead to formation of amyloid in pancreatic islet cells, causing  $\beta$ -cell dysfunction; interference with gene expression involving signal transduction and gene transcription related to insulin pathways [nuclear factor- $\kappa$ B (NF- $\kappa$ B), tumor necrosis factor  $\alpha$  (TNF $\alpha$ ), IL-6, PPAR $\gamma$ ], leading to insulin resistance [4–6]. Substantial evidence supports an association between arsenic and diabetes at high exposure levels, but results are mixed at low exposure levels [7,8].

The etiology of diabetes involves insulin resistance and  $\beta$ -cell dysfunction [9,10]. However, only a few epidemiologic studies have examined measures of insulin resistance and  $\beta$ -cell function in relation to arsenic exposure and such studies utilized indices derived from fasting glucose and insulin [11,12]. No studies have tested for associations with the oral glucose tolerance test (OGTT). We examined the association between urinary total arsenic and the OGTT-based markers of insulin sensitivity and  $\beta$ -cell function.

## MATERIALS AND METHODS

### Study Population

This is a preliminary study of the association between urinary arsenic and glucose homeostasis measures, conducted in the Amish Family Diabetes Study (AFDS), a genetic epidemiology study of type 2 diabetes in the Old Order Amish living in Lancaster, Pennsylvania [13]. In total, the AFDS included 953 subjects aged 18 years from 45 multigenerational families recruited between 1995 and 1998. Detailed participant recruitment procedures of the AFDS can be found elsewhere [13]. All participants gave written informed consent and underwent a detailed clinical examination at the Amish Research Clinic. Participants were instructed to fast for 12 hours before their appointment and to bring a first morning void urine sample.

This preliminary study was based on 221 AFDS participants with normal (n=164) or impaired (n=57) glucose tolerance. Subjects were sampled from nondiabetic individuals who had undergone a two hour OGTT (n=823 subjects: 668 with normal; 155 impaired glucose tolerance) and who had sufficient volumes (7 mL) of stored urines remaining in our

biorepository for the heavy metal assay. The mean ( $\pm$ SD) age of this sample ( $52.9 \pm 13.2$  years) was slightly higher than that of the full AFDS ( $49.2 \pm 17.0$  years).

### Outcome Assessment

After acquisition of a fasting blood sample, a 75-g OGTT was administered. Blood samples were then drawn for determination of glucose and insulin values at 30-min intervals for 3-h. Glucose and insulin concentrations were assayed with a Beckman glucose analyzer (Beckman Coulter, Fullerton, CA) and radioimmunoassay (Linco, St. Louis, MO), respectively.

Using OGTT results, we computed three validated measures of insulin sensitivity (Stumvoll estimated metabolic clearance rate (Stumvoll MCR) [14], Stumvoll insulin sensitivity index (Stumvoll ISI) [14], and Matsuda index [15]) and three validated measures of  $\beta$ -cell function (Stumvoll insulin secretion, phase-1 and phase-2 [14]; and insulinogenic index [16] (see detailed formula in Table 1)). As secondary measures, we also computed one fasting-state-based index of insulin sensitivity (homeostatic model assessment-insulin resistance (HOMA-IR)) and one fasting-state-based index of  $\beta$ -cell function (HOMA-% $\beta$ ) [17]. Details of each measure including mathematical formula and clinical significances are provided in Table 1.

### Arsenic Assessment

Urinary total arsenic concentrations were determined using inductively coupled plasma mass spectrometry by the University of Michigan Environmental Health Sciences Core Center's Trace Metals Laboratory. All arsenic concentrations among participants were above the limit of detection (0.1  $\mu$ g/L). We conducted quality control procedures including analysis of urine-based reference materials before, during and after every analytical run, and use of calibration standards, procedural blanks, duplicate samples, and spiked samples. The coefficient of variation was 5%. Urinary creatinine was measured in the AFDS and used to adjust for urine dilution.

### Data analysis

To account for correlations among participants in the same sibship, we used generalized estimating equations with an exchangeable correlation structure where pair-wise correlations between participants from the same sibship were equal, to estimate differences for an interquartile range (IQR) increase in urinary arsenic (6.24  $\mu$ g/L). All models were adjusted for age and sex (Model 1); and further adjusted for waist-to-hip ratio and BMI (Model 2). In Model 2 for Stumvoll MCR and Stumvoll ISI, however, BMI was not included because BMI is used in the formula to calculate each of these indices. Both models were also adjusted for urinary creatinine to account for urine dilution [18]. However, adjustment for urinary creatinine may introduce bias if creatinine production is influenced by diabetes and/or arsenic [8]. We, therefore, report regression results for both models without urinary creatinine adjustment as well. Two-sided  $P < 0.05$  was considered statistically significant.

## RESULTS

The mean (SD) age was 52.9 (13.2) years and 115 participants (52%) were female (Table 2). The means (SDs) of insulin sensitivity and  $\beta$ -cell function measures were 7.8 (2.6) mg/(kg $\times$ min) for Stumvoll MCR; 0.091 (0.032)  $\mu$ mol/(kg $\times$ min $\times$ pM) for Stumvoll ISI; 4.9 (2.2) for Matsuda index; 2.6 (1.8) for HOMA-IR; 153.9 (124.6) for HOMA-% $\beta$ ; 960.9 (461.6) pM for Stumvoll insulin secretion phase-1; 265.1 (104.4) pM for Stumvoll insulin secretion phase-2; and 89.7 (81.9) for insulinogenic index. The median urinary total arsenic concentration was 5.5  $\mu$ g/L (IQR: 3.1–9.4) (Table 3). The creatinine-adjusted median concentration was 6.1  $\mu$ g/g (IQR: 4.1–10.2). Participants aged 60 and older had higher concentrations (both crude and creatinine-adjusted) than younger participants. Men had higher crude total arsenic concentrations than women (6.4 vs. 4.2  $\mu$ g/L) but lower creatinine-adjusted total arsenic concentrations (5.4 vs. 7.0  $\mu$ g/g). Participants with impaired glucose tolerance had higher crude and creatinine adjusted urinary total arsenic concentrations than those with normal glucose tolerance.

Urinary total arsenic was significantly and inversely associated with all insulin sensitivity indices with adjustment for age, sex and urinary creatinine (Model 1, Table 4). After further adjusting for adiposity (Model 2), associations remained significant for two of the three OGTT-based insulin sensitivity measures; an IQR increase in urinary total arsenic (6.24  $\mu$ g/L) was significantly, inversely associated with Stumvoll MCR ( $-0.23$  mg/(kg $\times$ min), 95% confidence interval (CI):  $-0.38$ ,  $-0.09$ ;  $P=0.0015$ ) and Stumvoll ISI ( $-0.0029$   $\mu$ mol/(kg $\times$ min $\times$ pM), 95% CI:  $-0.0047$ ,  $-0.0011$ ;  $P=0.0015$ ). Urinary total arsenic was also significantly associated with higher glucose levels (0.57 (95% CI: 0.06, 1.09) mg/dL per IQR increase;  $P=0.029$ ). No significant associations were found between urinary arsenic and measures of  $\beta$ -cell function. The results remained unchanged in the models without urinary creatinine adjustment (Table 4).

## DISCUSSION

This is the first epidemiologic study to examine arsenic exposure and OGTT-based measures of insulin sensitivity and  $\beta$ -cell function. In this preliminary study of non-diabetic Amish adults, urinary total arsenic was inversely associated with OGTT-based insulin sensitivity measures. Notably, these associations were stronger and remained statistically significant following covariate adjustment compared to the widely used index of insulin resistance, HOMA-IR, which is based on fasting measures of insulin and glucose. Possibly, the previous mixed results [11,12,19–21] may be partly due to low sensitivity of HOMA-IR. The OGTT-based insulin sensitivity measures, such as Stumvoll MCR, showed better correlations with the hyperinsulinemic-euglycemic clamp-based insulin sensitivity than the fasting indices, such as HOMA-IR [22]. We did not observe significant associations of urinary total arsenic with any measures of  $\beta$ -cell function.

To our knowledge, only two human studies have examined arsenic exposure and measures of both insulin sensitivity and  $\beta$ -cell function [12,19]. In 72 Mexican subjects with mean urinary total arsenic concentrations of 133.4 (SD=67)  $\mu$ g/L for non-diabetic (n=32) and 100.9 (SD=65.2)  $\mu$ g/L for type 2 diabetic subjects (n=40), urinary total arsenic was inversely

associated with HOMA2-% $\beta$ , but was not significantly associated with HOMA2-IR [12]. A national survey conducted in Korea (n=3,602; median urinary total arsenic concentrations=117.7  $\mu\text{g/g}$  creatinine) also reported a significant inverse association of urinary total arsenic with HOMA2-% $\beta$ , but no significant association with HOMA2-IR [19]. Their arsenic exposure levels were much higher than those found in our study (5.4  $\mu\text{g/L}$ ), which is comparable to that found in non-Hispanic white, non-fish eating, never smoker adults from NHANES 2003–2008 data (5.7  $\mu\text{g/L}$  (IQR: 3.2–11.0), unpublished data). A recent National Toxicology Program (NTP) workshop review suggested that the arsenic effects on  $\beta$ -cell function are concentration dependent [8]: low concentrations (in the submicromolar range) may lead to impaired glucose-stimulated insulin secretion through adaptive cellular responses to arsenic-induced oxidative stress, whereas high concentrations may lead to apoptosis or necrosis via irreversible oxidative damage to  $\beta$ -cells. The NTP workshop review also suggested low concentrations may inhibit insulin signaling and insulin-dependent glucose uptake by adipocytes or skeletal muscle cells. Although it is unclear why our findings are inconsistent with the previous ones, low-level arsenic exposure found in the present study may result in insulin resistance through inhibition of insulin signaling and insulin-dependent glucose uptake [8,23,24]. More epidemiologic studies with a wide range of exposure levels and measures of insulin sensitivity and  $\beta$ -cells function are warranted to investigate concentration-dependent mechanisms.

Non-significant associations between urinary total arsenic and fasting state-based measures seem to be due to confounding by adiposity. The age- and sex-adjusted association between urinary total arsenic and HOMA-IR was statistically significant (Model 1, Table 4), but the effect estimate was substantially attenuated after adjustment for adiposity (BMI and waist-to-hip ratio) (Model 2, Table 4). In this population, urinary total arsenic concentrations were modestly correlated with BMI and waist-to-hip ratio (Pearson correlation coefficient for both measures=0.17). Adiposity is a well-known risk factor for insulin resistance and diabetes [25]. Given that drinking water and diet are major environmental sources of arsenic exposure [2] and more food and water consumption is expected in obese individuals, adiposity may play a role as a positive confounder and therefore, reduced effect estimates are expected with adiposity adjustment. However, previous literature has reported an inverse association between BMI and arsenic biomarkers [26–28]. This is because obese individuals are more likely to consume more methyl donors, such as methionine, folic acid and vitamin B12, that facilitate arsenic methylation, resulting in faster arsenic excretion [26]. Gruber et al. found an inverse association between toenail arsenic and dietary fat intake, suggesting dietary fat may inhibit arsenic absorption [29]. Different population characteristics including dietary habits, life-style, and genetic variations may explain the inconsistency observed in our population, but our study is limited to fully understand plausible links between adiposity and arsenic metabolism and excretion because of the lack of arsenic species data. Future studies of the role of adiposity in arsenic metabolism and in the arsenic-diabetes association in the Amish population are needed.

There are several limitations. We did not measure arsenic species. Total urinary arsenic reflects all arsenic species including inorganic forms of arsenic and their methylated metabolites and the organic forms. A National Toxicology Program workshop recommended arsenic speciation analysis because it is assumed that the inorganic arsenic and methylated

metabolites, but not the organic forms, may be associated with type 2 diabetes [8]. It is also important to consider the organic forms of arsenic (e.g., arsenobetaine), a less-toxic species of arsenic found in seafood, in data analysis. However, it is unresolved whether the organic species of arsenic should be adjusted as a covariate or subtracted from total arsenic concentrations [30–32]. Given that exposure to the organic forms of arsenic occurs through fish consumption and fish is not a common component of the Amish diet, the contribution of the organic forms to the urinary total arsenic concentrations might be minimal in our study. Although the study participants are not diabetic subjects, they have a family history of diabetes given the study design of AFDS, thus they may be at higher risk of diabetes than those without a family history. Our study was conducted in a cross-sectional setting that raises concerns of the validity of causal inferences between urinary arsenic and insulin sensitivity.

This preliminary study suggests several future directions. Given that arsenic metabolism, such as arsenic methylation efficiency, has been associated with diabetes in several studies including prospective evidence with incident diabetes [33], it will be important to evaluate the associations between arsenic metabolism and OGTT-based measures of insulin sensitivity and  $\beta$ -cell function. Future studies will also need to evaluate potential sources of arsenic exposure in this population of the Amish. Although the exposure level found in this preliminary study was low, given that all of the Amish use well-water for drinking and they adhere to traditional life-style and dietary habits, it will be important to identify main sources of arsenic (especially inorganic arsenic) in this community.

In conclusion, this preliminary study using OGTT-based measures of insulin sensitivity and  $\beta$ -cell function suggests that low-level arsenic exposure may influence diabetes risk through impairing insulin sensitivity rather than insulin secretion through pancreatic  $\beta$ -cells.

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**Table 1**  
The meaning and equation of each fasting state measure and OGTT-derived index

Index	Definition/Clinical significance	Equation	Reference
<i>Measures of insulin sensitivity</i>			
Stumvoll metabolic clearance rate	Correlates with the metabolic clearance rate (MCR) derived from the hyperinsulinemic euglycemic clamp which measures the rate of glucose uptake into tissues (primarily muscle and adipose tissue). MCR from clamp studies is calculated as the average glucose infusion rate divided by the average plasma glucose concentration during the last hour of a hyperinsulinemic euglycemic clamp. Higher levels indicate greater insulin sensitivity.	$18.8 - 0.271 \times \text{BMI} - 0.0052 \times I_{120} - 0.27 \times G_{90}$	14
Stumvoll insulin sensitivity index	Correlates with the insulin sensitivity index (ISI) derived from the hyperinsulinemic euglycemic clamp and represents the amount of glucose metabolized per unit of plasma insulin. ISI from clamp studies is calculated as the metabolic clearance rate divided by the mean insulin concentration during the same period of the clamp. Higher levels indicate greater insulin sensitivity.	$0.226 - 0.0032 \times \text{BMI} - 0.0000645 \times I_{120} - 0.0037 \times G_{90}$	14
Matsuda index	Correlates with the rate of whole-body glucose uptake into tissues (primarily muscle and adipose tissue) during the hyperinsulinemic euglycemic clamp. Higher levels indicate greater insulin sensitivity.	$10000 / \sqrt{G_0 \times I_0 \times G_{\text{mean}} \times I_{\text{mean}}}$ (glucose in "mg/dL," and insulin in "μU/mL")	15
<i>Measures of β-cell function</i>			
Stumvoll insulin secretion, phase 1	Correlates with first phase insulin secretion (rise in insulin levels during the 10 minutes immediately after starting the glucose infusion which rapidly raises glucose levels) during a hyperglycemic clamp. Higher levels indicate greater insulin secretion capacity.	$1283 + 1.829 \times I_{30} - 138.7 \times G_{30} + 3.772 \times I_0$	14
Stumvoll insulin secretion, phase 2	Correlates with second phase insulin secretion, the steady state insulin levels during the last hour of the hyperglycemic clamp. Higher levels indicate greater insulin secretion capacity.	$287 + 0.4164 \times I_{30} - 26.07 \times G_{30} + 0.9226 \times I_0$	14
Insulinogenic index	Measure of early-phase insulin secretion. For a given rise in plasma glucose during the first 30 minutes of an OGTT, a larger index indicates greater insulin secretion	$(I_{30} - I_0) / (G_{30} - G_0)$	16
<i>Fasting-state measures</i>			
HOMA-IR	Insulin resistance index calculated from fasting glucose and insulin based on a physiologic model of the glucose and insulin relationship <i>in vivo</i> (Homeostasis Model). Lower levels indicate greater insulin sensitivity.	$(G_0 \times I_0) / 22.5$ . (insulin in μU/mL)	17
HOMA-%β	Beta-cell function index calculated from the Homeostasis Model. It is expressed as a percent of normal β cell function.	$(20 \times I_0) / (G_0 - 3.5) \%$ .	17

Notes: G<sub>0</sub>, fasting glucose; G<sub>30</sub>/G<sub>90</sub>, glucose 30 and 90 min after the administration of 75g glucose; G<sub>mean</sub>, mean glucose during oral glucose tolerance test (OGTT); I<sub>0</sub>, fasting insulin; I<sub>30</sub>/I<sub>120</sub>, insulin 30 and 120 min after the administration of 75g glucose; I<sub>mean</sub>, mean insulin during OGTT. Body mass index (BMI) in kg/m<sup>2</sup>, Insulin in pmol/L and glucose in mmol/L unless stated otherwise.

Table 2

Population characteristics (N=221).

	Mean $\pm$ SD or otherwise specified
Age (years)	52.9 $\pm$ 13.2
Female, N (%)	115 (52)
BMI (kg/m <sup>2</sup> )	27.5 $\pm$ 5.0
Waist-to-hip ratio	0.87 $\pm$ 0.066
Fasting glucose (mg/dL)	91.4 $\pm$ 8.0
Fasting insulin* ( $\mu$ U/mL)	11.4 $\pm$ 8.3
Glucose-120 min (mg/dL)	119.4 $\pm$ 31.8
Stumvoll MCR <sup>‡</sup> (mg/(kg $\times$ min))	7.8 $\pm$ 2.6
Stumvoll ISI <sup>‡</sup> ( $\mu$ mol/(kg $\times$ min $\times$ pM))	0.091 $\pm$ 0.032
Matsuda index*	4.9 $\pm$ 2.2
Stumvoll insulin secretion, phase 1 <sup>‡</sup> (pM)	960.9 $\pm$ 461.6
Stumvoll insulin secretion, phase 2 <sup>‡</sup> (pM)	265.1 $\pm$ 104.4
Insulinogenic index <sup>‡</sup>	89.7 $\pm$ 81.9
HOMA-IR*	2.6 $\pm$ 1.8
HOMA-% $\beta$ *	153.9 $\pm$ 124.6

\* N=219.

<sup>‡</sup> N=215.<sup>‡</sup> N=216.

**Table 3**

Distributions (median and interquartile range (Q1 and Q3)) of urinary total arsenic by covariates.

	N	Creatinine unadjusted (µg/L)	Creatinine adjusted (µg/g)
All	221	5.45 (3.14, 9.37)	6.12 (4.10, 10.15)
Age (yrs)			
20–39	32	4.83 (2.38, 6.34)	4.60 (3.70, 9.26)
40–59	122	4.95 (2.45, 8.80)	5.70 (3.88, 9.24)
60	67	7.48 (4.59, 11.37)	7.44 (5.25, 13.16)
Sex			
Male	106	6.43 (4.72, 10.08)	5.42 (3.95, 9.31)
Female	115	4.17 (2.17, 8.47)	7.02 (4.45, 11.99)
BMI (kg/m <sup>2</sup> )			
<25	72	6.03 (2.90, 8.64)	6.06 (4.37, 10.59)
25–29	88	5.09 (3.02, 9.00)	6.06 (4.00, 8.25)
30	61	5.83 (3.42, 11.15)	6.48 (3.79, 12.94)
High waist to hip ratio <sup>a</sup>			
No	119	4.96 (2.33, 8.34)	6.00 (3.87, 9.31)
Yes	102	6.06 (3.94, 10.94)	6.60 (4.24, 11.80)
OGTT			
Normal	164	5.11 (2.55, 8.75)	5.71 (3.90, 9.59)
Impaired	57	6.32 (4.58, 11.24)	7.08 (4.81, 11.99)

<sup>a</sup>High waist to hip ratio was defined as waist to hip ratio 0.9 for men and 0.85 for women [34].

**Table 4**

Differences in OGTT-based insulin measures per interquartile range (6.24 µg/L) increase in urinary total arsenic (n=221).

	With creatinine adjustment		Without creatinine adjustment	
	Model 1*	Model 2 <sup>†</sup>	Model 1	Model 2
Fasting glucose (mg/dL)	0.82 (0.30, 1.34) <sup>‡</sup>	0.57 (0.059, 1.09) <sup>§</sup>	0.81 (0.29, 1.32) <sup>‡</sup>	0.54 (0.030, 1.05) <sup>§</sup>
Glucose (120 min) (mg/dL)	1.12 (-0.33, 2.57)	0.74 (-0.77, 2.25)	1.63 (0.27, 3.00) <sup>§</sup>	1.17 (-0.26, 2.59)
<i>Measures of insulin sensitivity</i>				
Stumvoll MCR (mg/(kg×min))	-0.27 (-0.42, -0.11) <sup>‡</sup>	-0.23 (-0.38, -0.089) <sup>‡</sup>	-0.30 (-0.45, -0.15) <sup>‡</sup>	-0.27 (-0.41, -0.13) <sup>‡</sup>
Stumvoll ISI (µmol/(kg×min×pM))	-0.0033 (-0.0052, -0.0014) <sup>‡</sup>	-0.0029 (-0.0047, -0.0011) <sup>‡</sup>	-0.0037 (-0.0055, -0.0019) <sup>‡</sup>	-0.0034 (-0.0051, -0.0017) <sup>‡</sup>
Matsuda index (percent difference)	-3.8 (-6.2, -1.3) <sup>‡</sup>	-1.5 (-4.3, 1.2)	-4.3 (-6.6, -1.9) <sup>‡</sup>	-1.8 (-4.5, 0.95)
<i>Measures of β-cell function</i>				
Stumvoll insulin secretion, phase 1 (pM)	19.4 (-22.5, 61.3)	4.8 (-37.1, 46.8)	24.9 (-14.5, 64.2)	7.9 (-32.2, 47.9)
Stumvoll insulin secretion, phase 2 (pM)	4.9 (-4.5, 14.3)	1.5 (-7.9, 10.9)	6.3 (-2.5, 15.1)	2.3 (-6.6, 11.3)
Insulinogenic index (percent difference)	0.51 (-6.7, 8.3)	-1.8 (-8.8, 6.0)	0.81 (-6.1, 8.2)	-1.8 (-8.7, 5.6)
<i>Fasting-state measures</i>				
HOMA-IR (percent difference)	3.1 (0.79, 5.4) <sup>‡</sup>	0.72 (-1.8, 3.3)	3.7 (1.4, 6.1) <sup>‡</sup>	1.1 (-1.5, 3.7)
HOMA-%β (percent difference)	-0.64 (-3.2, 2.0)	-1.8 (-4.6, 1.1)	-0.10 (-2.7, 2.5)	-1.4 (-4.2, 1.4)

\* Model 1 adjusted for age and sex in generalized estimating equations accounting for sibship.

<sup>†</sup> Model 2 additionally adjusted for waist-to-hip ratio and BMI. BMI was not adjusted when modeling Stumvoll MCR and Stumvoll ISI because BMI is used to calculate those indices. For both model 1 and 2, results when urine creatinine was included as a covariate and when it was not are presented separately.

<sup>‡</sup> p-value <0.01,

<sup>§</sup> 0.01 p-value <0.05.