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Relationships between urinary inositol excretions and whole-body glucose tolerance and skeletal muscle insulin receptor phosphorylation

April J. Stull[a,1], John P. Thyfault[b], Mark D. Haub[c], Richard E. Ostlund Jr [d], Wayne W. Campbell[a,*]

[a]*Department of Foods and Nutrition and Center on Aging and the Life Course, Purdue University, West, Lafayette, IN 47907, USA*
[b]*Departments of Nutritional Sciences and Internal Medicine and Harry S Truman VA Hospital, University of Missouri, Columbia, MO 65211, USA*
[c]*Department of Human Nutrition and the Center on Aging, Kansas State University, Manhattan, KS 66506, USA*
[d]*Department of Medicine, Washington University School of Medicine, St Louis, MO 63110, USA*

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Abstract

This study assessed the relationships of urinary D-*chiro*-inositol and *myo*-inositol excretions to indices of whole-body glucose tolerance and total content and tyrosine phosphorylation of the insulin receptor (activation) in skeletal muscle of older nondiabetic subjects. Fifteen adults (age, 65 ± 8 years; body mass index, 27.9 ± 3.3 kg/m^2 [mean ± SD]) completed duplicate assessments of oral (75-g oral glucose tolerance test [OGTT]) and intravenous (300 mg/kg body weight intravenous glucose tolerance test) glucose tolerance challenges and 24-hour urinary D-*chiro*-inositol and *myo*-inositol excretions. Skeletal muscle (vastus lateralis) biopsies were obtained at minute 60 of the OGTTs. Subjects with higher urinary D-*chiro*-inositol excretion had higher insulin ($\rho = 0.51$, $P \leq .05$) and C-peptide ($\rho = 0.56$, $P \leq .05$) area under the curves, and lower insulin sensitivity index ($\rho = -0.60$, $P \leq .05$) during the intravenous glucose tolerance test. The urinary *myo*- to D-*chiro*-inositol ratio was also inversely related to insulin area under the curve ($\rho = -0.59$, $P \leq .05$). Urinary D-*chiro*-inositol ($\rho = -0.60$, $P \leq .05$) and *myo*-inositol ($\rho = -0.60$, $P \leq .05$) were inversely related to tyrosine phosphorylation of the insulin receptor (phosphotyrosine 1162/1163), but not total content of the insulin receptor during the OGTT. The apparent relationships were modestly weakened when adjustments were made for sex. These findings support previous research linking higher urinary D-*chiro*-inositol excretion with a progressive decline in whole-body glucose tolerance. This is the first report to link higher urinary D-*chiro*-inositol excretion to a blunted activation of skeletal muscle insulin receptor signaling in older nondiabetic subjects.

1. Introduction

Peripheral insulin resistance is a component of the metabolic syndrome and the first step in the progression toward overt type 2 diabetes mellitus [1]. Researchers have related insulin resistance to a decrease in insulin receptor binding due to a reduction in the number of insulin receptors, in addition to defects in the postreceptor insulin signaling cascade [2]. A defect in the postreceptor insulin signaling can potentially lead to impairment of glycosyl-phosphatidyl-inositol (GPI)/inositol phosphoglycan (IPG) pathway [3]. Insulin stimulates the hydrolysis of GPI to IPG, which contains various compounds such as *myo*-inositol, *chiro*-inositol (L- and D-*chiro*), glucosamine, galacosamine, and other residues [3]. *myo*-Inositol is the most abundant inositol that is synthesized from glucose [4]. *myo*-Inositol is converted to *chiro*-inositol (conversion determined by the ratio of *myo*-inositol to *chiro*-inositol) in healthy rats [5]. There is evidence that, in diabetic rats, there is minimal or no conversion of *myo*-inositol to *chiro*-inositol [6,7]. This altered ratio may be due to a defect in the epimerase-type enzyme that catalyzes this reaction [6].

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* Corresponding author. Department of Foods and Nutrition, Purdue University, West Lafayette, IN 47907-2059. Tel.: +1 765 494 8236; fax: +1 765 494 0674.
E-mail address: campbellw@purdue.edu (W.W. Campbell).

[1] During the time the research was conducted, Dr April J Stull was a doctoral student at Purdue University; and currently, she is a postdoctoral fellow at Pennington Biomedical Research Center, Division of Nutrition and Chronic Disease and Botanicals Research Center, 6400 Perkins Rd., Baton Rouge, LA 70808.

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The IPG that contains the D-*chiro*-inositol has been suggested to exert biological effects as a potential second messenger or mediator of insulin action [8,9] by dephosphorylating (activating) glycogen synthase and pyruvate dehydrogenase, which are critical rate-limiting steps of nonoxidative and oxidative glucose disposal [10,11], respectively. In humans with impaired tolerance or type 2 diabetes mellitus, D-*chiro*-inositol content is low or undetectable in the muscle [12]; and IPG-D-*chiro*-inositol is also less active or inactive [13]. This could possibly decrease the activation of glycogen synthase and pyruvate dehydrogenase and negatively impact the insulin-stimulated GPI/IPG pathway.

Research findings document that subjects with type 2 diabetes mellitus also have altered urinary D-*chiro*-inositol excretion. In patients with uncontrolled type 1 and 2 diabetes mellitus, renal clearance and 24-hour urinary excretion of D-*chiro*-inositol were greater than those in healthy subjects [14]. The urinary loss of *myo*-inositol also increased with diabetes, but the urinary loss of D-*chiro*-inositol was disproportionately greater than the loss of urinary *myo*-inositol [14]. Similar results were found in patients with polycystic ovary syndrome who were insulin resistant when compared with healthy women [15]. Urinary D-*chiro*-inositol excretion increases with poor glycemic control, hyperinsulinemia, and diabetes [14,15]. Obesity does not seem to influence urinary D-*chiro*-inositol excretion [12,14,15]. Researchers suggest that urinary D-*chiro*-inositol excretion was inversely associated with insulin sensitivity [15], with the possibility that urinary D-*chiro*-inositol excretion and the *myo*- to D-*chiro*-inositol ratio are predictors of insulin resistance [16-19].

The primary purpose of this study was to assess the relationships of urinary D-*chiro*-inositol and *myo*-inositol to indices of glucose tolerance (glucose, insulin, C-peptide), insulin sensitivity, and skeletal muscle insulin receptor total content (independent of phosphorylation) and phosphorylation of specific tyrosine sites (1162/1163) necessary to activate the insulin receptor in older nondiabetic individuals. We hypothesized that there would be a positive association between urinary inositol excretions and glucose, insulin, and C-peptide area under the curve (AUC). Furthermore, there would be a negative association between urinary inositol excretions and insulin sensitivity and total content and tyrosine phosphorylation of the insulin receptor.

2. Materials and methods

2.1. Subjects

Sixteen subjects (6 men and 10 women) were recruited from the Little Rock and central Arkansas region to participate in this study. One woman discontinued participation in the study for reasons unrelated to the protocol. The inclusion criteria included the following: (1) aged at least 50 years, (2) body mass index (BMI) 21 to 35 kg/m^2, (3) no drug use known to alter carbohydrate metabolism, (4) no previous or current diagnosis of type 1 or type 2 diabetes mellitus or any other acute or chronic disease, (5) less than 40 minutes of physical exercise 4 or more days a week or the initiation of a structured exercise program in the past 3 months, (6) body weight stability (<2 kg change in the past 3 months), (7) no impaired renal function (serum creatinine concentration <150 μmol/L), and (8) postmenopausal (for women) for at least 2 years. A prestudy evaluation included a physical examination, resting electrocardiogram, 75-g oral glucose tolerance test (OGTT), routine blood and urine chemistries, and subjects signing a consent form. This information was not used for baseline testing during the study. Monetary compensation was given to each subject for participating. The study protocol, consent form, and recruitment flyers were approved by the Human Research Advisory Committee, University of Arkansas for Medical Sciences, Little Rock, AR.

2.2. Study design

The data presented in this report were obtained from subjects who participated in a randomized, placebo-controlled, double-blinded intervention study designed to assess the effects of pinitol (INZITOL, CAS #10284-63-6, 3-*O*-methyl-1,2,4 *cis*-3,5,6 *trans*-hexahydroxycyclohexane, 96% purity based on high-performance liquid chromatography analysis; Humanetics, Chanhassen, MN) supplementation on whole-body glucose tolerance, insulin sensitivity, and insulin action in skeletal muscle. Testing and evaluations were conducted within 3-day periods before (baseline) and at the end (post) of a 6-week intervention, during which time each subject was provided a controlled energy and macronutrient-defined diet. As described previously [20], there were no differences at baseline or post between the placebo (n = 8) and pinitol (n = 7) groups; and no significant changes over time occurred for any of the primary outcomes, except for an approximately 12% decrease in whole-body insulin sensitivity during the OGTT. Furthermore, there were no group-dependent differences in responses from baseline to post for any outcomes except fasting plasma C-peptide concentration during the OGTT. Because the treatment (placebo vs pinitol) and time (baseline vs poststudy) did not statistically influence the results, for the current investigation, the data from the placebo and pinitol groups were combined (N = 15) and the baseline and post results for each subject were averaged (pooled).

2.3. Testing procedures

At baseline and post, OGTTs and intravenous glucose tolerance tests (IVGTTs) were performed on consecutive days, with the subject residing at a General Clinical Research Center overnight between tests. Subjects were in a 12-hour fasting state before starting the OGTT and IVGTT. A catheter was inserted into an antecubital vein, a blood sample was drawn, and the subject consumed a sugar-free beverage

with nothing (placebo) or with 1000 mg pinitol (only for pinitol group at post testing) dissolved into it. The subject then consumed a 75-g glucose solution (OGTT) within 5 minutes or was administered 300 mg glucose per kilogram of body weight by intravenous injection of precisely portioned quantities of a 50% (wt/vol) glucose solution over a 60-second period (IVGTT). During the OGTT, blood was drawn at 30, 60, 90, 120, 150, and 180 minutes. During the IVGTT, blood was taken at 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 25, 30, 40, 50, 60, 70, 90, 100, 120, 140, 160, and 180 minutes. All blood samples were collected into heparinized tubes and centrifuged at 3000 rpm; and aliquots of plasma were stored at −20°C until analyzed for plasma glucose, insulin, and C-peptide concentrations.

2.4. Muscle biopsy

During the OGTT, at minute 50, a small portion of each subject's dominant leg was anesthetized with 4 mL of a 1% lidocaine solution. After the 60-minute blood sample was drawn, a sample of muscle from the vastus lateralis was obtained by the percutaneous needle biopsy technique (a 6-mm Bergstrom biopsy needle; Microsurgical Instruments, Lake Forest, IL) with applied suction [21]. The extracted muscle tissue was quickly blotted to remove blood, fat, or connective tissue. The muscle tissue was then frozen and stored in liquid nitrogen until analyzed for total content and tyrosine phosphorylation of the insulin receptor. The muscle samples were obtained in an oral glucose–stimulated hyperglycemic and hyperinsulinemic state that corresponded with maximum plasma insulin concentration [22].

2.5. Body composition and 24-hour urine collections

At baseline and post, in the morning after a 12-hour fast and urine void, the percentage of body fat and the fat-free mass (FFM) were estimated from body density using a whole-body plethysmographer (BOD POD; Life Measurement, Concord, CA) and the 2-compartment model equation of Siri [23]. Timed 24-hour urine collections were made during day 2 of dietary control. The urine was collected into a disposable 4-L polyethylene container, and 10 mL of 1.0 normal acetic acid was added as a preservative. Specific gravity was measured, and an aliquot was placed in polypropylene tubes and stored at −20°C until analyzed for inositols.

2.6. Analytical methods and calculations

During the OGTT and IVGTT, plasma glucose concentrations were analyzed using an oxidase method standard for a Beckman glucose analyzer (model 6517; Beckman Instruments, Fullerton, CA). Plasma insulin and C-peptide concentrations were analyzed using double antibody radioimmunologic procedures as described by Engdahl et al [24]. The integrated AUC for plasma glucose, insulin, and C-peptide was calculated using the trapezoidal method [25]. For the OGTT, hepatic and whole-body (hepatic plus peripheral) insulin sensitivities were estimated using the formulas described by Matsuda and Defronzo [26]; and 24-hour urinary *myo*-inositol and D-*chiro*-inositol were prepared and analyzed using mass spectrometry as previously described [20]. The *myo*- to D-*chiro*-inositol ratio was calculated from the measured amounts of urinary *myo*-inositol and D-*chiro*-inositol excretions. The muscle samples taken during the OGTT were homogenized in a buffer containing protease and phosphatase inhibitors, measured for protein with a bicinchoninic acid kit, and then analyzed for total insulin receptor content and insulin receptor phosphotyrosine pYpY1162/1163 activation using enzyme-linked immunosorbent assay kits from Biosource International

Table 1
Subject characteristics and OGTT and IVGTT parameters

Parameters	All (n = 15)	Men (n = 6)	Women (n = 9)
Age (y)	65 ± 8	67 ± 10*	64 ± 8
Height (cm)	170.5 ± 10.9	178.2 ± 10.5*	165.4 ± 8.1
Body weight (kg)	81.2 ± 10.5	83.4 ± 15.4	79.7 ± 6.3
BMI (kg/m^2)	27.9 ± 3.3	26.1 ± 2.5	29.2 ± 3.4
Body density (g/mL)	1.01 ± 0.02	1.03 ± 0.01*	0.99 ± 0.01
Body fat (%)	39.1 ± 9.8	29.3 ± 5.2*	45.6 ± 5.6
Fat mass (kg)	31.8 ± 8.8	24.8 ± 7.5*	36.5 ± 6.3
FFM (kg)	49.3 ± 10.1	58.5 ± 9.5*	43.2 ± 4.0
Hemoglobin A_{1c} (%)	5.4 ± 0.4	5.2 ± 0.4	5.5 ± 0.4
OGTT			
Glucose, fasting (mmol/L)	5.4 ± 0.5	5.6 ± 0.5	5.3 ± 0.5
Insulin, fasting (pmol/L)	73 ± 30	84 ± 36	65 ± 24
C-peptide, fasting (pmol/L)	591 ± 263	643 ± 208	556 ± 301
Glucose, AUC (mmol/[L 180 min])	494 ± 172	498 ± 227	492 ± 140
Insulin, AUC (nmol/[L 180 min])	46 ± 19	52 ± 25	42 ± 13
C-peptide, AUC (nmol/[L 180 min])	259 ± 79	273 ± 103	250 ± 64
Hepatic insulin sensitivity index	0.48 ± 0.17	0.39 ± 0.11	0.53 ± 0.18
Whole-body insulin sensitivity index	3.97 ± 1.30	3.41 ± 1.15	4.34 ± 1.32
IVGTT			
Glucose, fasting (mmol/L)	5.3 ± 0.5	5.4 ± 0.6	5.2 ± 0.5
Insulin, fasting (pmol/L)	62 ± 24	72 ± 30	55 ± 17
C-peptide, fasting (pmol/[L 180 min])	596 ± 223	583 ± 241	583 ± 241
Glucose, AUC (mmol/[L 180 min])	361 ± 63	358 ± 93	363 ± 38
Insulin, AUC (nmol/[L 180 min])	10 ± 5	13 ± 6	9 ± 3
C-peptide, AUC (nmol/[L 180 min])	76 ± 30	92 ± 31	65 ± 26
Insulin sensitivity (pmol/[L min])	7.48 ± 4.42	8.64 ± 4.17	8.64 ± 4.17
Acute insulin response to glucose (nmol/L)	13.66 ± 8.37	11.83 ± 8.54	11.83 ± 8.54
Glucose effectiveness (/min^{-1})	0.013 ± 0.006	0.013 ± 0.006	0.013 ± 0.006

Mean ± SD.
* $P \leq .05$ (men vs women).

Table 2
Urinary inositol excretions and skeletal muscle insulin receptor total content and phosphorylation

	All (n = 15)	Men (n = 6)	Women (n = 9)
Urine			
myo-Inositol (μmol/d)	181 ± 101 (21, 164, 394)	206 ± 102 (104, 175, 394)	164 ± 103 (21, 149, 317)
myo-Inositol (μmol/[kg FFM d])	3.75 ± 2.23 (0.46, 3.11, 7.53)	3.49 ± 1.58 (1.97, 3.10, 6.57)	3.93 ± 2.65 (0.46, 3.13, 7.53)
D-*chiro*-Inositol (μmol/d)	45 ± 26 (1, 45, 95)	60 ± 24 (36, 53, 95)	35 ± 22 (1, 44, 59)
D-*chiro*-Inositol (μmol/[kg FFM d])	0.90 ± 0.46 (0.03, 0.88, 1.45)	1.01 ± 0.28 (0.69, 0.98, 1.40)	0.83 ± 0.55 (0.03, 0.88, 1.49)
myo- to D-*chiro* ratio	5.43 ± 4.18 (2.50, 4.24, 17.50)	3.45 ± 0.99 (2.50, 3.21, 4.68)	6.74 ± 5.00 (2.76, 4.42, 17.52)
Muscle insulin receptor content (ng/μg protein)[a]			
Total	0.42 ± 0.12	0.36 ± 0.07	0.47 ± 0.12
Phosphotyrosine pY1162/1163	0.20 ± 0.07	0.16 ± 0.04	0.24 ± 0.08

Mean ± SD (low, median, high); n = 15.
[a] n = 14.

(Camarillo, CA). For the IVGTT, insulin sensitivity, acute insulin response to glucose, and glucose effectiveness were estimated using the MINMOD Millennium computer program (version 5.0, 2001) [27].

2.7. Statistical analyses

The data are reported as mean ± SD. The correlations between variables were established using the Spearman ρ nonparametric ranked correlation test. The Spearman partial rank-order correlation was used to control for potential confounders such as BMI and sex. A 2-sample *t* test was used to assess the differences between men and women and various parameters, such as subject characteristics, indices of glucose tolerance, and insulin sensitivity during OGTT and IVGTT, urinary inositol excretion, and total content and tyrosine phosphorylation of the insulin receptor. Statistical significance was assigned if P was less than or equal to .05. All data processing and calculations were performed using Statistical Analyses Systems Software (version 9.1; SAS Institute, Cary, NC).

3. Results

3.1. Subject characteristics

The subject characteristics, indices of glucose tolerance, and insulin sensitivity of the men and women who participated in this study are presented in Table 1. The men and women did not differ in their BMI, hemoglobin A_{1c}, indices of glucose tolerance, and insulin sensitivity. Age, height, body density, and FFM were higher and percentage of body fat and fat mass were lower in men compared with women ($P \leq .05$). This older population was studied because they were at risk for developing type 2 diabetes mellitus. Most of the participants studied were classified as having impaired fasting glucose and/or impaired glucose tolerance. The 15 subjects were classified as follows: fasting glucose—5 men and 6 women were normal (<5.6 mmol/L), 1 man and 3 women were impaired (5.6-6.9 mmol/L); 2-hour glucose during OGTT—2 men and 2 women were normal (<7.8 mmol/L), 3 men and 7 women were impaired (7.8-11.0 mmol/L), and 1 man was diabetic (>11.1 mmol/L). The subjects' urinary inositol excretions and skeletal muscle insulin receptor content and phosphorylation are listed in Table 2.

3.2. Urinary D-chiro-inositol excretion

The 24-hour urinary D-*chiro*-inositol excretion (in micromoles per day) was inversely related to insulin sensitivity and positively related to insulin and C-peptide AUC (Table 3), but not related to glucose AUC, during the IVGTT. These observations were not observed during the OGTT. When adjusted for BMI, the apparent relationship with urinary D-*chiro*-inositol excretion (in micromoles per day) remained significant for C-peptide AUC ($\rho = 0.59$, $P = .03$) and insulin sensitivity ($\rho = -0.58$, $P = .04$), but not for insulin AUC ($\rho =$

Table 3
Correlation between urinary inositol excretions and indices of glucose tolerance, insulin sensitivity, and tyrosine phosphorylation of the insulin receptor

Urinary inositols	Insulin AUC (mmol/[L 180 min])	C-peptide AUC (mmol/[L 180 min])	Insulin sensitivity index (mmoL/[L 180 min])	Phosphotyrosine T1162/1163 insulin receptor[a]
myo (μmol/d)	0.22	0.32	-0.38	-0.60*
myo (μmol/[kg FFM d])	0.15	0.22	-0.29	-0.42
D-*chiro* (μmol/d)	0.51*	0.56*	-0.60*	-0.60*
D-*chiro* (μmol/[kg FFM d])	0.34	0.52*	-0.42	-0.51
myo to D-*chiro* ratio	-0.59*	-0.36	0.41	0.03

Data were assessed using Spearman ρ nonparametric ranked correlation; n = 15.
* ρ, $P \leq .05$.
[a] n = 14.

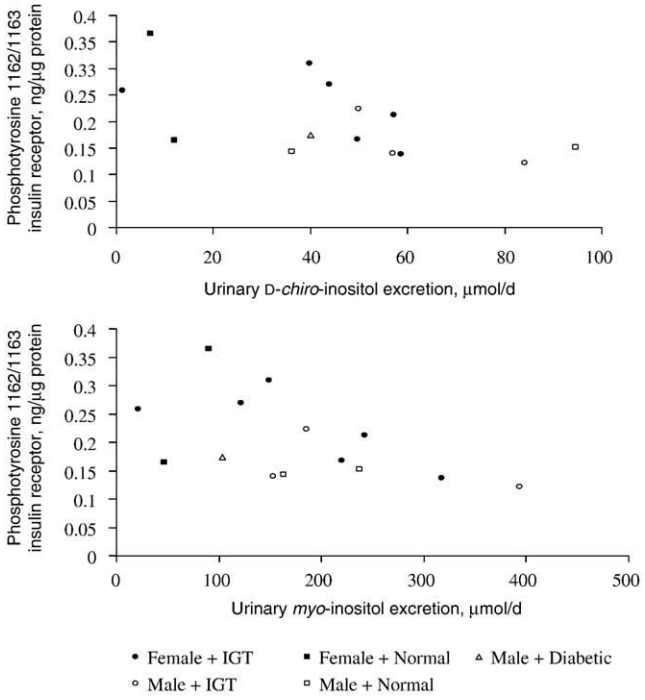

Fig. 1. Correlations between urinary D-*chiro*-inositol and *myo*-inositol excretions and tyrosine phosphorylation of the insulin receptor; n = 14.

0.45, P = .12). The relationships between urinary D-*chiro*-inositol excretion (in micromoles per day) and insulin AUC (ρ = 0.42, P = .15), C-peptide AUC (ρ = 0.53, P = .06), and insulin sensitivity (ρ = −0.51, P = .08) were not significant after adjusting for sex. However, when urinary D-*chiro*-inositol excretion was expressed as micromoles per kilogram FFM per day, urinary D-*chiro*-inositol excretion was positivity correlated with C-peptide AUC (Table 3) and remained significant after adjusting for BMI (ρ = 0.62, P = .02) and sex (ρ = 0.61, P = .03).

Urinary D-*chiro*-inositol excretion (in micromoles per day) was negatively correlated with the insulin receptor phosphotyrosine 1162/1163 (Table 3 and Fig. 1) during OGTT, but not correlated to the total insulin receptor content. When adjusted for BMI, the relationships between urinary D-*chiro*-inositol excretion and the insulin receptor phosphotyrosine 1162/1163 remained significant (ρ = −0.62, P = .03) and showed a trend when adjusted for sex (ρ = −0.50, P = .08).

3.3. Urinary myo-inositol excretion

Urinary *myo*-inositol excretion (in micromoles per day) was not correlated to indices of glucose tolerance and insulin sensitivity (Table 3). Urinary *myo*-inositol excretion was negatively correlated to the insulin receptor phosphotyrosine 1162/1163 (Table 3 and Fig. 1) during OGTT, but was not correlated to total insulin receptor content. When corrected for BMI, the relationship with the insulin receptor phosphotyrosine 1162/1163 remained significant for urinary *myo*-inositol excretion (ρ = −0.61, P = .03), but was not maintained when adjusted for sex (ρ = −0.52, P = .08). When *myo*-inositol was expressed as micromoles per kilogram FFM per day, the correlations were not significant after adjusting for BMI (ρ = −0.43, P = .14) and sex (ρ = −0.42, P = .17).

3.4. Urinary myo- to D-chiro-inositol ratio

Urinary *myo*- to D-*chiro*-inositol ratio was negatively correlated to insulin AUC during the IVGTT (Table 3) and was not maintained when adjusted for BMI (ρ = −0.52, P = .07) and sex (ρ = −0.21, P = .51). There were no statistically significant associations between urinary *myo*- to D-*chiro*-inositol ratio and glucose AUC, C-peptide AUC, and insulin sensitivity. Urinary *myo*- to D-*chiro*-inositol ratio was not correlated to the total content and tyrosine phosphorylation of the insulin receptor.

4. Discussion

The current study documents that higher urinary D-*chiro*-inositol is related to indices of glucose tolerance and insulin sensitivity. Campbell et al [22] reported that 24-hour urinary excretions of *myo*-inositol and D-*chiro*-inositol were positively related to plasma C-peptide AUC (indirect indicator of insulin secretion) during an OGTT in a group of older overweight subjects. Furthermore, there was an inverse correlation between *myo*- to D-*chiro*-inositol ratio and C-peptide AUC [22]. Ostlund et al [14] reported that 24-hour urinary D-*chiro*-inositol excretion was positively correlated with fasting plasma glucose, glycated hemoglobin, and urinary glucose in patients with diabetes [14]. Baillargeon et al [15] showed that women who secreted greater amounts of urinary D-*chiro*-inositol also had decreased insulin sensitivity (inverse relationship). The present and previous human studies [14,15,22] show that subjects with higher urinary D-*chiro*-inositol excretion had greater insulin secretion (indicated by insulin and C-peptide AUC) and lower insulin sensitivity. The current study findings were in a selection of older nondiabetic subjects who, as a whole (group mean), had normal insulin sensitivity. However, when individuals were observed separately, there were various degrees of insulin sensitivity.

The relationships between urinary D-*chiro*-inositol excretion and insulin sensitivity, insulin AUC, C-peptide AUC, and the insulin receptor phosphotyrosine 1162/1163 were apparent, just not maintained when adjusted for sex. In addition, the relationship between urinary *myo*-inositol excretion and the insulin receptor phosphotyrosine 1162/1163 was not maintained when adjusted for sex. Campbell et al [22] also found that the correlation between urinary inositol excretions and C-peptide AUC was strongly influenced by sex. When compared with women, men had a greater excretion of urinary D-*chiro*-inositol and *myo*-inositol and higher C-peptide AUC measured during an

OGTT [22]. However, a sex effect was not evaluated. This could likely reflect the small sample size. It is evident that the men and women in the current study had differences in FFM. When D-*chiro*-inositol was expressed in micromoles per kilogram FFM per day, the relationships between urinary D-*chiro*-inositol and C-peptide AUC remained even after adjustment for sex. Limited data have shown that men and women have differences in peripheral insulin sensitivity and insulin receptor binding [28]. Older women had higher peripheral insulin sensitivity and higher insulin receptor binding than men [28]. In opposition to the study findings, Ostlund et al [14] found no sex-related differences in urinary D-*chiro*-inositol and *myo*-inositol excretions. However, the study was conducted in younger individuals [14].

Because of the lack of previous research on the relationship between urinary D-*chiro*-inositol excretion and insulin signaling, we started our evaluation at the initial steps of the insulin signaling pathway with measurements of total insulin receptor content and activation of the insulin receptor at specific sites (1162/1163). Previous research has documented that, depending on an individual's insulin-resistant state, urinary inositol excretion and tyrosine phosphorylation of the insulin receptor can be affected. In people with type 2 diabetes mellitus and polycystic ovary syndrome, urinary D-*chiro*-inositol excretions were elevated [14,15] and tyrosine phosphorylation of the insulin receptor were reduced [29]. The present study found an inverse correlation between urinary inositol excretions and the insulin receptor phosphotyrosine 1162/1163 before adjustments were made for BMI and sex. The autophosphorylation of the insulin receptor is obligatory for insulin sensitivity because it initiates the propagation of the insulin signaling cascade, finally culminating in the translocation of glucose transporters (glucose transporter 4) and activation of glucose transport. It has been proposed that activation of the insulin receptor can lead to activation of large G proteins [30]. Afterward, the G proteins can activate phospholipase C or D, which hydrolyzes GPI at the cell surface and releases IPG extracellularly [30]. The IPG containing D-*CHIRO*-inositol must reenter the cell through the *myo*-/D-*chiro*-inositol transporter [31] to exert biological activities as an insulin second messenger [8,9]. One of the biological effects is activation of glycogen synthase. In skeletal muscle samples from people with type 2 diabetes mellitus, IPG *chiro*-inositol was reduced or undetectable, whereas IPG *myo*-inositol remained the same [12,13]. After administration of insulin, the *chiro*-inositol concentrations remained undetected; and *myo*-inositol concentrations increased then decreased after 15 minutes [12]. We would speculate that, if people with type 2 diabetes mellitus have reduced phosphorylation of the tyrosine insulin receptor, then the GPI/IPG pathway would be altered, subsequently reducing IPG D-*chiro*-inositol activation of glycogen synthase.

It is generally recognized that urinary D-*chiro*-inositol excretion is altered or abnormal with worsening glucose control [14,15,22]. The proposed mechanisms for elevated urinary D-*chiro*-inositol excretion include reduced renal threshold for D-*chiro*-inositol [15], abnormal tissue/cellular uptake of D-*chiro*-inositol [31], and/or abnormal intracellular processing of D-*chiro*-inositol (*myo*-inositol conversion to D-*chiro*-inositol) [7]. The current study supports previous studies [14,15,22] that found elevated urinary D-*chiro*-inositol excretion associated with increased insulin and C-peptide AUC and decreased insulin sensitivity. Caution is warranted not to overinterpret the present findings because this was a small observational study with results apparently influenced by the sex of the subjects and mechanisms that were not evaluated.

In conclusion, the present study in nondiabetic older subjects supports previous research linking higher urinary D-*chiro*-inositol excretion with a decline in whole-body glucose tolerance and extends them to include a blunted activation of tyrosine phosphorylation of the insulin receptor in skeletal muscle. The current study was not designed to evaluate sex differences, but the potential that sex influenced these relationships underscores that this issue needs to be further explored in larger groups of men and women who have a wide range of insulin sensitivities.

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