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The Effects of Inositol Metabolism in Pregnant Women on Offspring in the North and South of China

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Background: Inositol is an essential nutrient for cell growth, survival and embryonic development. Myo-inositol is the predominant form in natural. To investigate the correlation between inositol metabolism and embryonic development, we assessed the metabolic characteristics of myo-inositol, phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) and phosphatidylinositol 3,4,5-trisphosphate ($PI(3,4,5)P_3$) of pregnant women in the North China (Yangquan and Weihai) and South China (Nanchang and Haikou) China.

Material/Methods: All data were collected by face-to-face interview during pregnant women health visits using a questionnaire. Plasma levels of myo-inositol, $PI(4,5)P_2$ and $PI(3,4,5)P_3$ from 89 randomly collected pregnant women were detected by gas chromatography-mass spectrometry and enzyme linked immunosorbent assay.

Results: A total of 400 pregnant women were included in this survey. The plasma levels of myo-inositol and $PI(4,5)P_2$ in the North China group of pregnant women were significantly higher than that in the South China group ($P<0.01$). The birth weight of fetuses in the North China group was heavier than that in the South China group ($P<0.01$). The birth length of fetuses in Yangquan was the longest among the 4 cities ($P<0.01$). The incidence rate of birth defects was 3.05% in the North China group, and 0.0% in the South China group. In bivariate linear correlation analysis, the body weight correlated with myo-inositol (r=0.5044, $P<0.0001$), $PI(4,5)P_2$ (r=0.5950, $P<0.0001$) and $PI(3,4,5)P_3$ (r=0.4710, $P<0.0001$), the body length was correlated with $PI(4,5)P_2$ (r=0.3114, $P=0.0035$) and $PI(3,4,5)P_3$ (r=0.2638, $P<0.0130$).

Conclusions: The plasma levels of myo-inositol and $PI(4,5)P_2$ in pregnant women had significant difference between the North and the South of China, which might be correlated with fetal development and birth defects.

MeSH Keywords: **Body Weight • Congenital Abnormalities • Fetal Development • Pregnant Women**

Abbreviations: **$PI(4,5)P_2$** – phosphatidylinositol 4,5-bisphosphate; **$PI(3,4,5)P_3$** – phosphatidylinositol 3,4,5-trisphosphate; **IP3** – inositol 1,4,5-trisphosphate; **DAG** – diacylglycerol; **PKC** – protein kinase C; **NTDs** – neural tube defects; **h** – hour; **min** – minute; **BMI** – body mass index; **PI3K** – phosphoinositide 3-kinase

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Background

Inositol, also called cyclohexanehexol, is an essential and indispensable nutrient for human and animals to maintain normal physiological functions. It is involved in various biological processes such as growth regulation, membrane formation, fertilization, hormone secretion, and neurotransmitter signal transduction [1–4]. Studies have showed that the absence of inositol seriously affected the hatching blastocysts during the development of hamster embryos [5]. Moreover, as an osmotic substance, inositol has an important role in maintaining the balance of intracellular osmotic pressure through sodium/inositol transporter [6,7]. Increased levels of inositol resulted in high osmotic pressure, a dangerous environment for cells [7]. Therefore, abnormal inositol levels may lead to developmental disorders.

Inositol is produced by glucose *in vivo* or uptake from diets. In eukaryotic cells, a large number of phosphatidyl inositol phosphate derivatives were produced by the inositol metabolism. Among them, phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) was catalyzed into inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG) [8,9]. Both function as second messengers. IP3 entered the cytoplasm and activated IP3 receptors on the smooth endoplasmic reticulum, which opened calcium channels [3,10]. DAG remained on the cell membrane and activated protein kinase C (PKC)-mediated signal cascade [11]. Myo-inositol was the predominant form in natural. It has been found that myo-inositol metabolic disorder is associated with the damage of PKC signaling pathway [12]. Furthermore, PI3K-induced phosphorylation of $PI(4,5)P_2$ promoted the formation of phosphatidylinositol 3,4,5-trisphosphate ($PI(3,4,5)P_3$), leading to the activation of Akt pathway, which was required for cell proliferation, cell growth, cell survival and metabolism [13,14] (Supplementary Figure 1).

Inositol was closely related to fetal/pediatric developmental diseases, such as neural tube defects (NTDs) [15–17], neonatal respiratory distress syndrome [18,19], retinopathy of prematurity [20], et al. It has been reported that supplemental inositol could reduce the incidence of NTDs in folate-resistant curly tail mice [21,22]. We have observed significantly lower myo-inositol concentrations in pregnant women with NTDs, compared to normal pregnant women [23,24]. There were significant differences between the North China group and the South China Group for birth defects [25,26]. The correlation between inositol metabolism and the occurrence of birth defects, and the effects of inositol metabolism on offspring in pregnant women, in the North and South of China remain incompletely understood. In this study, the plasma levels of myo-inositol, $PI(3,4)P_2$ and $PI(3,4,5)P_3$ of pregnant women from the North of China (Yangquan and Weihai) and the South of China (Nanchang and Haikou) were investigated, and the correlation between inositol metabolism and birth defects was evaluated.

Material and Methods

Data collection

According to different geographical characteristics, the cities of Yangquan and Weihai (the North of China), and Nanchang and Haikou (the South of China) were randomly selected for investigation. One maternity and childcare hospital, and/or family planning service agencies were chosen in each city, from September to November in 2018, for a total of 400 pregnant women. Face to face questionnaire survey was performed. We also followed up fetal birth outcomes. 89 pregnant women were randomly selected from the 4 cities for the detection of myo-inositol, $PI(4,5)P_2$ and $PI(3,4,5)P_3$. The study was approved by Ethics Committee of the Capital Institute of Pediatrics (No. SHERLL2017022).

Detection of plasma myo-inositol levels

Myo-Inositol levels were measured using previously established method [23]. The experimental procedures were briefly described as following. Plasma sample (30 µL) was added into the 10 mL anhydrous ethanol, and dried by the rotary evaporator, at 70°C. Then 5 mL TMCS/HMDS/N, N-DMF (1: 2: 8, v/v/v) was added and dried at 70°C for 1 hour, then 5 mL hexanes and 10 mL saturated NaCl solution were added, vortexed for 1 minute and centrifuged at 6000 rotations per minute (rpm) for 5 minutes at room temperature. The supernatant was dried under nitrogen at 40°C. The residues were resuspended with 1 mL hexanes and then injected into the gas chromatography-mass spectrometry system [Agilent 7890A gas chromatography equipment combined with 5975C mass spectrometer (Agilent Technologies)].

Analysis of plasma $PI(4,5)P_2$ and $PI(3,4,5)P_3$ levels

The plasma levels of $PI(4,5)P_2$ and $PI(3,4,5)P_3$ of pregnant women were evaluated by the $PI(4,5)P_2$ Mass ELISA Kit (Echelon, K-4500) and $PI(3,4,5)P_3$ Mass ELISA Kit (Echelon, K-2500s) according to the manufacturer's instruction. The samples, controls (Blank and No Lipid controls), and standards were run in triplicate. Standard solution, PBS 0.25% PS (PBS-T 3% PS for $PI(3,4,5)P_3$ and samples were added to the designated incubation plate, and 60 µL/well of diluted $PI(4,5)P_2$ or $PI(3,4,5)P_3$ detector were added. The incubation plate was sealed and incubated on a plate shaker, at room temperature for 1 hour. 100 µL mixtures per well of the incubation plate was transferred to the corresponding well in the detection plate. After 1-hour incubation, the solution was discarded, and the wells washed for 3 times with 200 µL/well PBS-T. 100 µL of diluted secondary detector was added to each well. Plate sealed and incubated on shaker at room temperature for 1 hour. After washing, 100 µL of TMB solution was added to each well for

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30 minutes in the dark. Then 50 µL of 1 N H_2SO_4 stop solution was added to each well. Absorbance was read at 450 nm on a plate reader.

Data analysis

All data were analyzed using SPSS version 20.0. The one-way ANOVA and Student's *t*-test was used to examine differences in the levels of myo-inositol, PI(4,5)P_2 and PI(3,4,5)P_3. Chi-square statistics and Fisher's exact test were used to evaluate differences among proportions. Pearson's test was used to assess the correlation between two parameters. A *P*-value of ≤0.05 was considered statistically significant.

Results

General Characteristics of the Participants

We recruited 400 pregnant women in the North and South of China. The association between inositol metabolism and fetal development was investigated. The general characteristics of the participants were shown in Table 1. There were 28 pregnant women (7.0%) older than the age of 35 years. The moderate physical labor was 79 women (19.75%). The degree of education in university and higher was 24.75%. There were 237 women who were pregnant with their first child; 25.0% of pregnant women had adverse history of pregnancy (such as spontaneous miscarriage, induced abortion and stillbirth). There were 242 pregnant women who planned for pregnancy in advance, which accounted for 60.50% of the total study population. The prevalence of folic acid supplementation was 98% during the periconceptional period. There were significant differences in education degree and children numbers of family among 4 cities investigated (*P*<0.05).

We randomly selected 89 pregnant women from the North and South of China for the detection of myo-inositol, PI(4,5)P_2 and PI(3,4,5)P_3. There were no significant differences in age, body mass index (BMI), gestational week, education, folate supplement, and labor among different cities in the North and South of China (Table 2).

General characteristics of the birth outcomes

The birth outcomes of 343 pregnant women are reported: 57 pregnant women were lost to follow-up. Of the 343 pregnant women, there was no significant difference in gender or gestational age among 4 cities in the North and South of China. The birth weight of fetuses in Yangquan was heavier than that in Haikou (*P*<0.01), and the birth weight in Weihai was heavier than that in Nanchang and Haikou (*P*<0.01). The birth length of fetuses in Yangquan was the longest among the 4 cities (*P*<0.01). None of the fetuses had suffered from metabolic diseases. There was 1 infant with congenital heart disease and 1 with left foot syndactyly in Yangquan, 3 infants with patent foramen ovale and 1 infant with ventricular septal defect and patent foramen ovale in Weihai. The incidence rate of birth defects was 2.06% in Yangquan and 4.00% in Weihai. The incidence of birth defects was 3.05% in the North of China and 0.0% in the South of China. No NTDs were found among the 343 pregnant women (Table 3).

For the 89 tested samples, the birth weight of fetuses in Weihai was heavier than that in Nanchang and Haikou (*P*<0.01), the birth length of fetuses in Yangquan was longer than that in Weihai and Nanchang (*P*<0.01). Meanwhile, the birth weight and body length of fetuses in the South of China were lower than that in the North of China (*P*<0.01). The incidence rate of birth defects was 5.00% in Weihai, 2.08% in the North of China, and 0.00% in the South of China (Table 4).

Plasma myo-inositol levels of pregnant women in different areas

Inositol, more precisely myo-inositol, plays crucial role as the structural basis for a number of secondary messengers in eukaryotic cells, is associated with clinical diseases [27,28]. The plasma myo-inositol levels of pregnant women in different cities were detected by gas chromatography-mass spectrometry. The myo-inositol levels of pregnant women in the South of China were lower than that in the North of China (*P*<0.01). The myo-inositol levels of pregnant women in Weihai were the highest (*P*<0.01), and that in Hainan were the lowest among the 4 cities (*P*<0.01) (Figure 1A, 1B). In bivariate linear correlation analysis, plasma myo-inositol levels correlated with body weight in North of China (r=0.4116, *P*=0.0037), South of China (r=0.4201, *P*=0.0062), Yangquan (r=0.7061, *P*<0.0001), Nanchang (r=0.6863, *P*=0.0008), and total areas (r=0.5044, *P*<0.0001) (Figure 1C). The plasma myo-inositol levels correlated with body length in Yangquan (r=0.5339, *P*=0.0034), Nanchang (r=0.4942, *P*=0.0268) and Haikou (r=0.4713, *P*=0.0310, Figure 1D).

Plasma PI(4,5)P_2 levels of pregnant women in different areas

PI(4,5)P_2 is a key mediator of major signaling pathways that influence diverse cellular functions [29]. The plasma PI(4,5)P_2 levels of pregnant women in the South of China were lower than that in the North of China (*P*<0.01). The lowest levels of PI(4,5)P_2 were detected in pregnant women from Haikou (*P*<0.01), and that in Nanchang were lower compared to Weihai (*P*<0.01) (Figure 2A, 2B). In bivariate linear correlation analysis, plasma PI(4,5)P_2 levels correlated with body weight in the North of China (r=0.5840, *P*<0.0001), the South of

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Table 1. General characteristics of the study participants.

Program	Yangquan	Weihai	Nanchang	Haikou	Total (%)	*P*	North	South	Total (%)	*P*
Age group										
≤35	91	90	96	95	372 (93.00)	0.262	181	191	372 (93.00)	0.050
>35	9	10	4	5	28 (7.00)		19	9	28 (7.00)	
Area										
Suburb	17	23	25	28	93 (23.25)	0.304	40	53	93 (23.25)	0.124
Urban	83	77	75	72	307 (76.75)		160	147	307 (76.75)	
Labor intensity										
Light physical labor	78	86	74	83	321 (80.25)	0.148	164	157	321 (80.25)	0.379
Moderate physical labor	22	14	26	17	79 (19.75)		36	43	79 (19.75)	
Education										
Less than high school	12	26	41	19	98 (24.50)	0.000	38	60	98 (24.50)	0.014
High school/technical secondary school	26	18	27	25	96 (24.00)		44	52	96 (24.00)	
College	39	25	16	27	107 (26.75)		64	43	107 (26.75)	
University and above	23	31	16	29	99 (24.75)		54	45	99 (24.75)	
Number of children										
0	69	58	47	63	237 (59.25)	0.013	127	110	237 (59.25)	0.084
≥1	31	42	53	37	163 (40.75)		73	90	163 (40.75)	
History of pregnancy										
No	86	70	73	71	300 (75.00)	0.206	156	144	300 (75.00)	0.240
Spontaneous miscarriage	4	8	7	7	26 (6.50)		12	14	26 (6.50)	
Induced abortion	7	19	18	21	65 (16.25)		26	39	65 (16.25)	
Stillbirth	3	3	2	1	9 (2.25)		6	3	9 (2.25)	
Planning for pregnancy										
No	35	45	40	38	158 (39.50)	0.528	80	78	158 (39.50)	0.838
Yes	65	55	60	62	242 (60.50)		120	122	242 (60.50)	
Folate supplement										
Yes	97	99	96	100	392 (98.00)	0.191	196	196	392 (98.00)	1.000
No	3	1	4	0	8 (2.00)		4	4	8 (2.00)	
Total	**100**	**100**	**100**	**100**	**400**		**200**	**200**	**400**	

China (r=0.3746, *P*=0.0158), Yangquan (r=0.6580, *P*=0.0001), Nanchang (r=0.6107, *P*=0.0042) and total areas (r=0.5950, *P*<0.0001, Figure 2C); and the PI(4,5)P_2 levels correlated with body length in Yangquan (r=0.4632, *P*=0.0131), and total areas (r=0.3114, *P*=0.0035, Figure 2D).

Plasma PI(3,4,5)P_3 levels of pregnant women at different areas

PI(3,4,5)P_3 was the product of the class I phosphoinositide 3-kinase (PI3K) phosphorylation of PI(4,5)P_2 [30]. The PI(3,4,5)P_3 levels of pregnant women in Weihai were higher than that

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Table 2. Characteristics of 89 randomly selected study participants for the detection inositol metabolism.

Program	Yangquan	Weihai	Nanchang	Haikou	Total (%)	*P*	North	South	Total (%)	*P*
Age group										
≤35	28	19	19	21	87 (97.75)	0.349	47	40	87 (97.75)	0.712
>35	0	1	1	0	2 (2.25)		1	1	2 (2.25)	
BMI										
<18.5	0	1	1	0	2 (2.25)	0.147	1	1	2 (2.25)	0.059
18.5–24	7	3	7	10	27 (30.34)		10	17	27 (30.34)	
>24	21	16	12	11	60 (67.41)		37	23	60 (67.41)	
Gestational week										
≤12	3	2	3	3	11 (12.36)	0.592	5	6	11 (12.36)	0.798
13–27	9	2	6	4	21 (23.60)		11	10	21 (23.60)	
≥28	16	16	11	14	57 (64.04)		32	25	57 (64.04)	
Education										
University and above	5	6	2	6	19 (21.35)	0.223	11	8	19 (21.35)	0.888
College	11	4	4	7	26 (29.21)		15	11	26 (29.21)	
High school/technical secondary school	7	2	5	5	19 (21.35)		9	10	19 (21.35)	
Less than high school	5	8	9	3	25 (28.09)		13	12	25 (28.09)	
Folate supplement										
No	0	0	1	0	1 (1.12)	0.449	0	1	1 (1.12)	0.461
Yes	28	20	19	21	88 (98.88)		48	40	88 (98.88)	
Labor										
Light physical labor	27	17	17	20	81 (91.01)	0.362	44	37	81 (91.01)	1.000
Moderate physical labor	1	3	3	1	8 (8.99)		4	4	8 (8.99)	
Total	**28**	**20**	**20**	**21**	**89**		**48**	**41**	**89**	

Table 3. The birth outcomes of the study participants in different areas ($\bar{\chi}$±SD).

Area	Yangquan	Weihai	Nanchang	Haikou	North	South
Gender n (%)						
Male	47 (48.45)	54 (54.00)	34 (52.31)	42 (51.85)	101 (51.27)	76 (52.05)
Female	50 (51.55)	46 (46.00)	31 (47.69)	39 (48.15)	96 (48.73)	70 (47.95)
Gestational age (days)	277.65±7.79	274.58±11.39	278.18±7.00	275.04±9.08	276.03±9.90	276.44±8.34
Birth weight (kg)	3.48±0.44	3.59±0.50	3.34±0.37$^{\#}$	3.22±0.45$^{\#,*}$	3.54±0.47	3.27±0.42$^{\&}$
Birth length (cm)	50.62±1.66	49.79±1.23*	49.26±1.87*	49.46±1.68*	50.20±1.51	49.37±1.76$^{\&}$
Birth defects n (%)	2 (2.06)	4 (4.00)	0 (0.00)	0 (0.00)	6 (3.05)	0 (0.00)

* *P*<0.01 versus Yangquan; $^{\#}$ *P*<0.01 versus Weihai; $^{\&}$ *P*<0.01 versus North of China.

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Table 4. The birth outcomes in 89 randomly selected study participants for the detection inositol metabolism ($\bar{\chi}$±SD).

Area	Yangquan	Weihai	Nanchang	Haikou	North	South
Gender n (%)						
Male	13 (46.43)	11 (55.00)	8 (40.00)	9 (42.86)	24 (50.00)	17 (41.46)
Female	15 (53.57)	9 (45.00)	12 (60.00)	12 (57.14)	24 (50.00)	24 (58.54)
Gestational age (days)	278.07±8.45	273.45±12.28	277.8±6.32	273.67±7.01	276.15±10.36	275.68±6.92
Birth weight (kg)	3.50±0.55	3.71±0.43	3.26±0.27#	3.24±0.25#	3.59±0.51	3.25±0.25&
Birth length (cm)	50.79±2.10	49.40±1.31*	48.85±1.69*	49.81±1.17	50.21±1.92	49.34±1.51&
Birth defects n (%)	0 (0.00)	1 (5.00)	0 (0.00)	0 (0.00)	1 (2.08)	0 (0.00)

# P<0.01 versus Weihai; * P<0.01 versus Yangquan; & P<0.01 versus North of China.

in Yangquan and Nanchang (P<0.05, Figure 3A, 3B). In bivariate linear correlation analysis, plasma PI(3,4,5)P_3 levels correlated with the body weight in the North of China (r=0.5499, P<0.0001), the South of China (r=0.3419, P=0.0287), Yangquan (r=0.6155, P=0.0005), Haikou (r=0.7042, P=0.0004), and total areas (r=0.4710, P<0.0001, Figure 3C), and the PI(3,4,5)P_3 levels correlated with body length in the South of China (r=0.4776, P=0.0016), Yangquan (r=0.4635, P=0.0130), Nanchang (r=0.4620, P=0.0403), Haikou (r=0.4799, P=0.0277), and total areas (r=0.2638, P=0.0130, Figure 3D).

A 3D scatter plot based on myo-inositol, PI(4,5)P_2, and PI(3,4,5)P_3 levels were produced to visualize the distribution, as shown in Figure 4. The levels of myo-inositol, PI(4,5)P_2, and PI(3,4,5)P_3 in distribution were different in the 4 cities selected.

Discussion

Myo-inositol could regulate a variety of signal pathways that involved in cell survival and cell differentiation [1,31], and has been implicated in congenital malformation, fetal growth insufficient and children's growth and development [32–34]. Here we investigated the basic situation of pregnant women in the North and South of China through questionnaires. We followed up the birth outcomes of fetuses, including gender, gestational age, birth length, birth weight, birth defects, and metabolic diseases. The levels of myo-inositol, PI(4,5)P_2 and PI(3,4,5)P_3 were measured in randomly selected pregnant women. The relationship among myo-inositol metabolism levels in pregnant women, fetal development and birth defects was evaluated.

According to different geographical characteristics, 2 North cities (Yangquan and Weihai) and 2 South cities (Nanchang and Haikou) were randomly selected in this study. We recruited 400 pregnant women recruited from the 4 cities. There were no significant differences in general characteristics of study participants, except education degree and children numbers of family. The difference in children numbers of family might be due to the implementation of the second child policy in China. In recent years, the incidence rate of birth defects was about 5.6% in China, and congenital heart disease was the most common disease among birth defects and far exceed of NTDs according to the official report on prevention of birth defects in China published in 2012 (*http://www.gov.cn/gzdt/2012-09/12/content_2223371.htm*). In our study, there was 1 infant with congenital heart disease and 1 infant with left foot congenital syndactyly in Yangquan, 3 infants with patent foramen ovale and 1 infant with ventricular septal defect and patent foramen ovale in Weihai. No NTDs were observed. The incidence rate of birth defects was 3.05% in the North of China and 0.0% in the South of China in this study. These results were consistent with the aforementioned official report. Although there were differences in the birth weight and birth length in different areas, they all met the Chinese neonatal critical field.

NTDs were a complex genetic disease [35] and closely related to folate deficiency. The periconceptional supplement of folic acid could prevent 50% to 70% of NTDs [36,37], however, more than 30% of NTDs could not be prevented by folic acid. Inositol has been reported to be involved in NTDs. Studies have shown that the lack of inositol in pregnant women was an important risk factor for NTDs [38]. Serum inositol level in the NTD groups was significantly lower than that in the healthy control group [15,39]. We also found that plasma inositol and its metabolic intermediates in NTD pregnant women were significantly decreased in Shanxi province, which was a high incidence area of NTDs [23,24]. Animal studies have shown that lack of inositol during mouse embryonic development process could increase the susceptibility of NTDs [40]. Inositol supplementation (0.08 mg/day) could reduce the incidence of NTDs from 20.4% to 9.5%, in offspring of pregnant rat with diabetes mellitus [41]. In this study, we examined the levels of myo-inositol in pregnant women from different cities. The results

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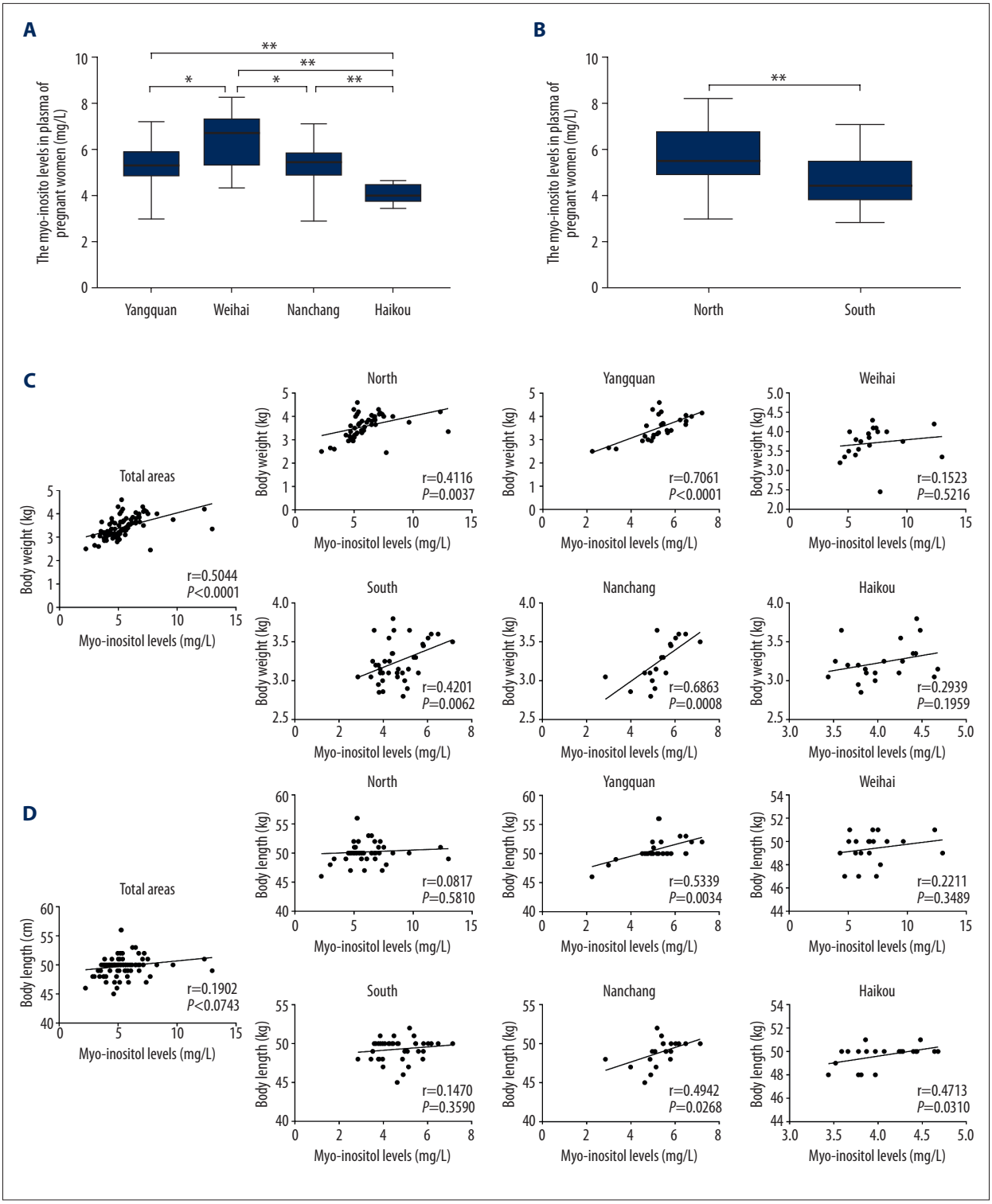

Figure 1. Plasma myo-inositol levels of pregnant women in different areas. (**A, B**) The plasma myo-inositol levels of pregnant women in different areas. (**C**) Bivariate linear correlation analysis between birth weight and myo-inositol levels in different areas. (**D**) Bivariate linear correlation analysis between birth length and myo-inositol levels in different areas. * $P<0.01$, ** $P<0.0001$.

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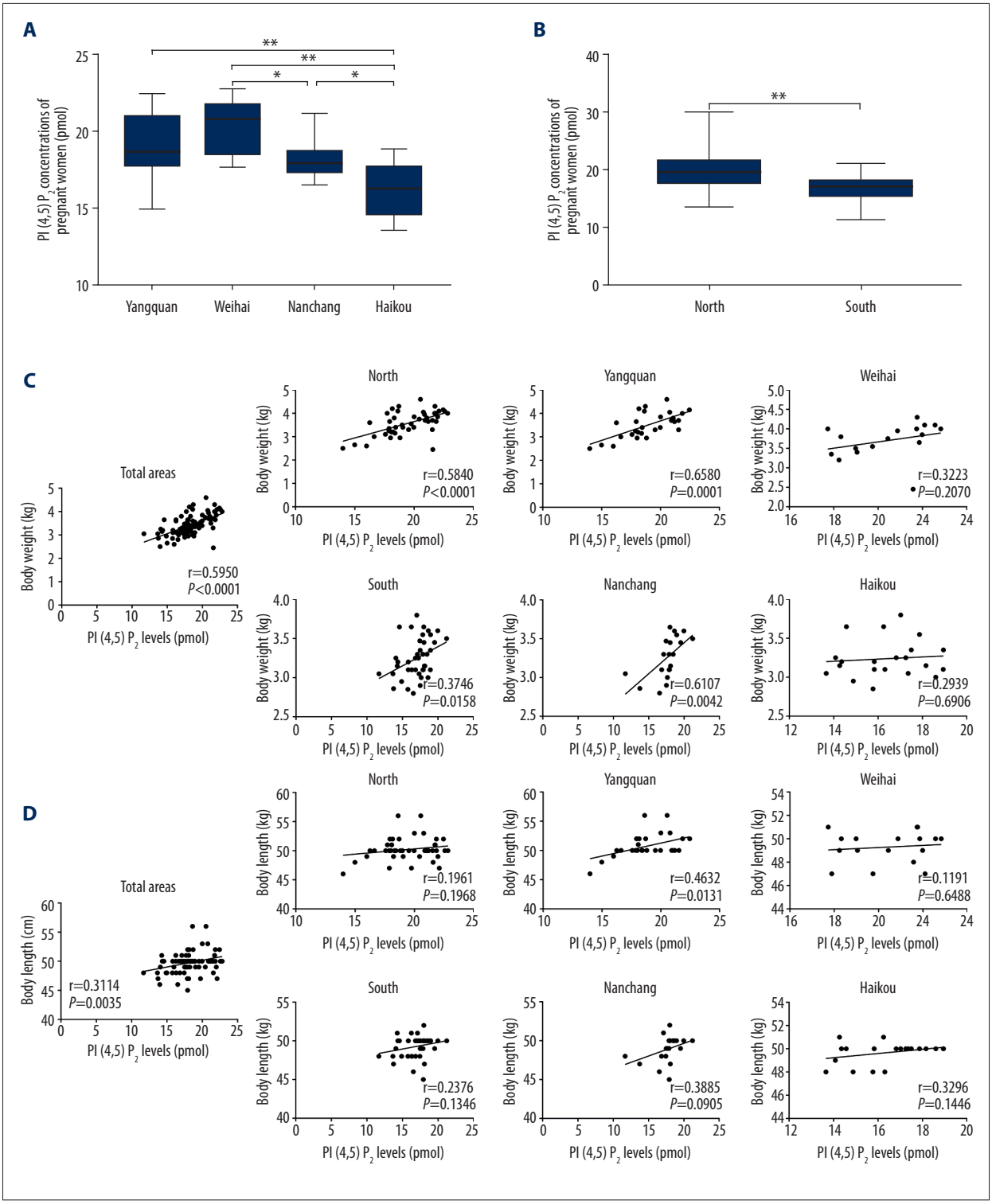

Figure 2. Plasma PI(4,5)P_2 levels of pregnant women in different areas. (**A, B**) The plasma PI(4,5)P_2 levels of pregnant women in different areas. (**C**) Bivariate linear correlation analysis between birth weight and PI(4,5)P_2 levels in different areas. (**D**) Bivariate linear correlation analysis between birth length and PI(4,5)P_2 levels in different areas. * $P<0.01$, ** $P<0.0001$.

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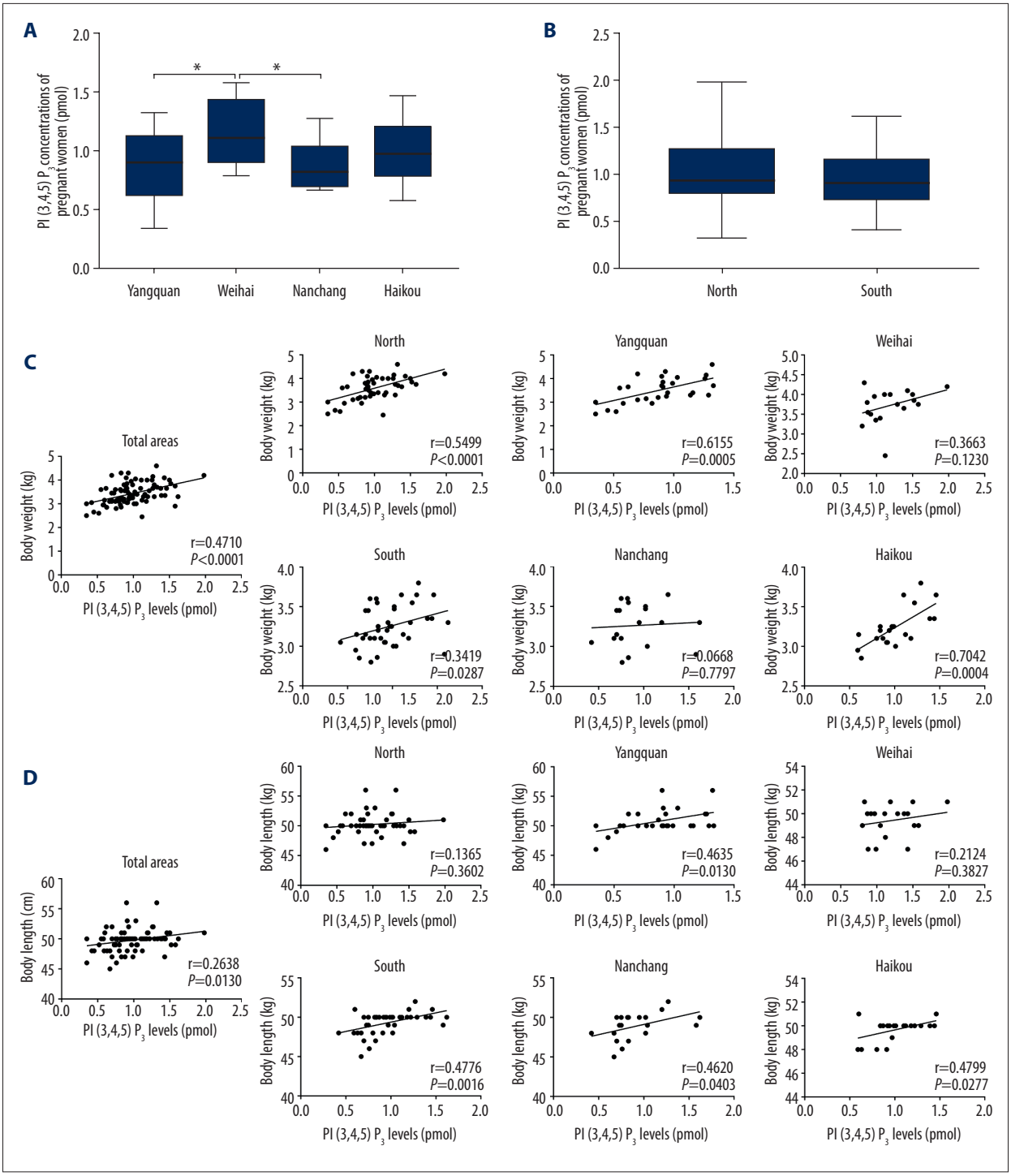

Figure 3. Plasma PI(3,4,5)P_3 levels of pregnant women in different areas. (**A, B**) The plasma PI(3,4,5)P_3 levels of pregnant women in different areas. (**C**) Bivariate linear correlation analysis between birth weight and PI(3,4,5)P_3 levels in different areas. (**D**) Bivariate linear correlation analysis between birth length and PI(3,4,5)P_3 levels in different areas.* $P<0.01$.

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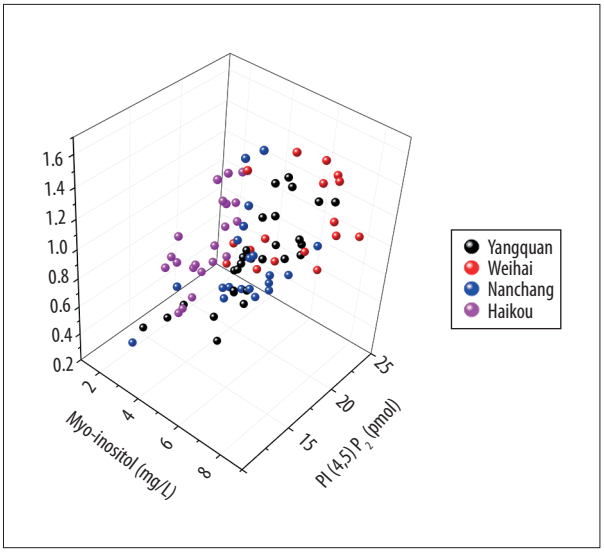

Figure 4. 3D distribution analysis based on myo-inositol, $PI(4,5)P_2$, and $PI(3,4,5)P_3$ levels.

showed that myo-inositol levels of pregnant women had significant difference in different cities, and that levels in the North of China were higher than that in the South of China. However, the relationship between the differences in myo-inositol levels and susceptibility of NTDs was not observed in the 4 different areas. It might due to the limited size of samples in the study.

In eukaryotic cells, many second messengers were formed on the basis of inositol structure, such as IP3, $PI(4,5)P_2$, $PI(3,4,5)P_3$, etc. [42]. $PI(4,5)P_2$ could be catalyzed by phosphatase C to form 2 second messenger molecules, i.e., IP3 and DAG [8], and could be further phosphorylated by PI3K to form another second messenger $PI(3,4,5)P_3$, which activates the PI3K/AKT signaling pathway [30,43]. The PI3K/AKT pathway could affect cell proliferation, survival and apoptosis [13,14]. $PI(4,5)P_2$ and $PI(3,4,5)P_3$ were not only key biological molecules with biological effects, but also as important products in inositol metabolism pathway [9]. In this study, $PI(4,5)P_2$ and $PI(3,4,5)P_3$ levels of pregnant women in different cities were measured. These results showed that the levels of these 2 inositol metabolites in pregnant women had significant differences in different cities, and the $PI(4,5)P_2$ levels in the North of China were higher than that in the South of China. To determine whether different levels of myo-inositol, $PI(4,5)P_2$ and $PI(3,4,5)P_3$ in pregnant women were associated with fetal development, we used Pearson's test to access the correlation among myo-inositol, $PI(4,5)P_2$, $PI(3,4,5)P_3$, body weight, and body length. The results showed that body weight or body length of fetuses correlated with myo-inositol, $PI(4,5)P_2$ and $PI(3,4,5)P_3$. Further studies are required to identify which of the inositol metabolite (myo-inositol, $PI(4,5)P_2$ and $PI(3,4,5)P_3$) is directly involved in regulation of fetal development.

There were some limitations in this study. The sample size was relatively small, and only 4 cities were selected to study in the North and South of China. The mechanism of the inositol metabolite (myo-inositol, $PI(4,5)P_2$ and $PI(3,4,5)P_3$) on the regulation of fetal development needed to be clarified. Further large, well designed studies are required to assess the association between inositol metabolism and fetal development and birth defects.

Conclusions

In this study, we found that the plasma levels of myo-inositol, $PI(4,5)P_2$ and $PI(3,4,5)P_3$ in pregnant women were different in the North and South of China. The fetal body weight or body length correlated with myo-inositol, $PI(4,5)P_2$ and $PI(3,4,5)P_3$. These results might be correlated with fetal development and birth defects. Larger studies would be required to confirm these findings.

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Conflict of interest

None.

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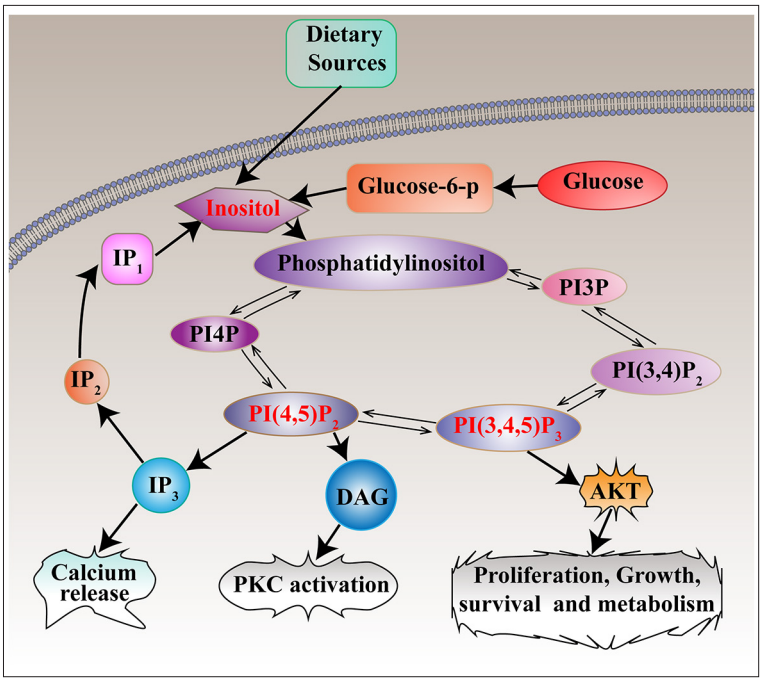
Supplementary Data



Supplementary Figure 1. The pathway of inositol metabolism.

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