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Original Article

PI3K/Akt pathway expression in children with different obesity degrees and its relationship with glucolipid metabolism and insulin resistance

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Abstract: Objective: This study investigated and analyzed the expression of PI3K/Akt pathway in children with different degree of obesity and its connection with glucolipid metabolism and insulin resistance (IR). Methods: 157 children with simple obesity, who admitted to our hospital from March 2020 to September 2020, were enrolled as obesity group. These children were divided into mild-group (n=67), moderate-group (n=55) and severe-group (n=35) referring to their body mass index (BMI). Another 60 healthy children admitted to hospitalized were randomly chosen as control group. The expression of PI3K mRNA and Akt mRNA in peripheral blood mononuclear cells (PBMCs) of each group were detected by RT-PCR, and its connection with glucose and lipid metabolism, as well as IR was analyzed. Results: Each group of children had insignificant difference in FBG (Fasting blood glucose) level (*P>0.05*). The triglyceride (TG), total cholesterol (TC), Low-density lipoprotein (LDL), Fasting insulin (FINS) and Homeostasis model assessment insulin resistance (HOMA-IR) levels in each obesity group were substantially higher than those in control group (*P<0.05*), and these levels decreased remarkably with the increase of obesity severity (*P<0.05*). The high-density lipoprotein (HDL) level of children in each obesity group was notably lower than that of the control group (*P<0.05*), and the level decreased remarkably with the ascending degree of obesity (*P<0.05*). The levels of PI3K mRNA and Akt mRNA in PBMCs of children in each obesity group were obviously lower than those in control group (*P<0.05*), and these index levels decreased much with the increasing worsen of children's obesity degree (*P<0.05*). The relative expression of PI3K mRNA and Akt mRNA in children with simple obesity was negatively correlated with TG, TC, LDL, FINS and HOMA-IR (*P<0.05*), positively correlated with HDL (*P<0.05*), and was not associated with FBG level (P>0.05). Conclusion: The inhibition of PI3K/Akt signaling pathway in children with simple obesity is associated with the abnormal glucolipid metabolism and IR, which affects the occurrence and progression of obesity.

Keywords: Obesity, children, PI3K/Akt pathway, glucolipid metabolism, insulin resistance

Introduction

In recent years, the childhood obesity has greatly increased, and become a crucial epidemic disease affecting physical and mental health of children in our country [1]. Childhood simple obesity is a chronic disease of nutritional imbalance which is closely related to lifestyle. It will not only severely affect children's physical and mental health, but also influence the health status after adulthood, which constitutes as an important risk factor for diabetes, hypertension and cardiovascular diseases in adulthood [2, 3]. The increasing rate of overweight and obesity in children will inevitably lead to an increase of chronic cardiovascular diseases in both childhood and adulthood. Studies have shown that insulin resistance (IR) is an important risk factor and core link that causes obesity and related complications. However, its mechanism in obese children is still unclear. Lipid metabolism disorders are closely related to IR [4, 5]. Besides, studies have reported that the mechanism of IR is the block of signal transduction process after the insulin hormone binds to its receptor [6]. PI3K/

Akt signaling pathway is a key pathway involved in various life activities. It is involved in cell division, differentiation and apoptosis, and is closely related to IR-related diseases such as TypeII diabetes, cardiovascular disease, etc. [7, 8]. This study further analyzed the mechanism of simple obesity in children, explored the expression of PI3K/Akt pathway in children with different obesity degrees and its correlation with glucolipid metabolism and IR.

Materials and methods

Clinical materials

157 cases of children, who admitted from March 2020 to September 2020 with simple obesity, were enrolled into the obesity group. The children were divided into mild-group (20%-29% exceeding the standard value, n=67) and moderate-group (30%-39% exceeding the standard value, n=35) and severe-group (40%-59% exceeding the value, n=35) referring to their body mass index (BMI). Besides, another 60 healthy children admitted to hospitalized for checkup were randomly chosen as the control group. The Hospital Ethics Committee agreed to the study.

Inclusive and exclusive criteria

Inclusive criteria: (1) Children in obesity-group met the diagnostic criteria in *Growth Curve of Body Mass Index of Children and Adolescents Aged 0-18 Years in China* [9]; (2) Children aged 3 to 12 years; (3) Children in control-group had normal weight, and no simple obesity occurred; (4) The children were in good compliance, and could well cooperated with inspections; (5) The informed consents had been voluntarily signed by the children's family.

Exclusive criteria: (1) Children with cardiac insufficiency; (2) Children with hepatic or renal dysfunction; (3) Those combined with hereditary metabolic diseases; (4) Children with secondary obesity caused by other reasons; (5) Children with acute/chronic inflammatory disease or autoimmune disease; (6) Children with malignant tumors.

Detection of PI3K/Akt pathway expression

We collected the early morning fasting venous blood from two groups of children, added lymphocyte separation fluid, and separated peripheral blood mononuclear cells (PBMCs). Extracted the total RNA of PBMCs by Trizol, measured the purity and concentration of RNA, and then identified. Reverse transcribed the RNA into cDNA by reverse transcription kit, and stored at -20°C for later use. Using cDNA as template, we amplified PI3K, Akt and GAPDH fragments by RT-PCR, and detected each index three times; and using GAPDH as internal reference, we calculated the relative expression of target mRNA by $2^{-\Delta\Delta Ct}$. The primers were designed and synthesized by Sangon Biotech (Shanghai) Engineering Technology Co., Ltd. The forward and reverse primers of PI3K were 5'-CCAGAAGAAGGGACAGTGGTATG-3' and 5'-TCGTAGCCAATCAGGGAGGT-3'; those of Akt were 5'-ATGGACTTCCGGTCAGGTTCA-3' and 5'-GCCCTTGCCCAGTAGCTTCA-3'; and in GAPDH, the forward and reverse primers were 5'-ggcacagtCAagGCTGAGAATG-3' and 5'-atggtGGtGAagACGCCAGTA-3' respectively.

Detection of glucose and lipid metabolism and IR

We collected the early morning fasting venous blood from two groups of children, and stored at room temperature after heparin anticoagulation. Centrifugated the samples at 3000 r/min for 5 min, and stored the supernatant at -80°C for later use. The levels of triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) were detected by automatic biochemical analyzer (Beckman, USA). Fasting insulin (FINS) levels were determined by chemiluminescence immunoassay and Fasting blood glucose (FBG) was determined by glucose oxidase-peroxidase endpoint colorimetric assay. The Homeostasis model assessment insulin resistance (HOMA-IR) = (FBG×FINS)/22.5.

Statistical analysis

Data analysis of the research were conducted by software SPSS 22.0. The comparison of measurement data in two groups was conducted by *t*-test, and the comparison between multiple groups was by variance analysis; The comparison of enumeration data in two groups and multiple groups were conducted by χ^2 test and rank sum test respectively. The difference in correlation was referred as $P<0.05$. The graphic software used was GraphPad Prism 8.0.

Table 1. Comparison of clinical data between children in each group

Well	Number of cases	Gender		Age (Age, $\bar{x} \pm s$)	BMI (kg/m^2, $\bar{x} \pm s$)	Waist (cm, $\bar{x} \pm s$)	Hipline (cm, $\bar{x} \pm s$)
		Male	Female				
Control group	60	35	25	9.85±2.46	17.85±2.02	67.95±7.39	73.49±5.83
Mild-group	67	39	28	10.25±3.13	22.75±1.86^{a}	72.53±6.42^{a}	77.63±4.97^{a}
Moderate-group	55	36	19	9.38±3.20	25.03±1.82^{a,b}	76.44±8.30^{a,b}	82.38±7.53^{a,b}
Sever-group	35	24	11	9.75±2.97	26.79±1.74^{a,b,c}	81.27±5.69^{a,b,c}	87.52±8.45^{a,b,c}
F/χ^2	-	1.654		1.280	27.948	24.593	19.753
P	-	0.647		0.379	<0.001	<0.001	<0.001

Note: Compared with the control group, $^{a}P<0.05$; compared with the mild-group, $^{b}P<0.05$; compared with the moderate group, $^{c}P<0.05$.

Table 2. Comparison of glucolipid metabolism among different groups of children ($\bar{x} \pm s$)

Group	Number of cases	TG (mmol/L)	TC (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	FINS (μU/ml)	FBG (mmol/L)
Control group	60	0.55±0.15	3.54±1.05	2.47±0.69	1.96±0.42	7.19±1.64	5.06±0.93
Mild-group	67	0.68±0.19^{a}	4.19±0.97^{a}	2.04±0.52^{a}	2.64±0.53^{a}	8.62±1.10^{a}	5.25±1.02
Moderate-group	55	0.83±0.25^{a,b}	4.99±1.20^{a,b}	1.86±0.33^{a,b}	3.11±0.69^{a,b}	11.04±1.81^{a,b}	5.18±0.98
Sever-group	35	1.08±0.30^{a,b,c}	6.38±1.36^{a,b,c}	1.52±0.30^{a,b,c}	3.97±0.83^{a,b,c}	14.05±2.57^{a,b,c}	5.09±1.12
F	-	27.596	23.029	18.967	25.669	25.006	0.192
P	-	<0.001	<0.001	<0.001	<0.001	<0.001	0.793

Note: Compared with the control group, $^{a}P<0.05$; compared with the mild-group, $^{b}P<0.05$; compared with the moderate-group, $^{c}P<0.05$.

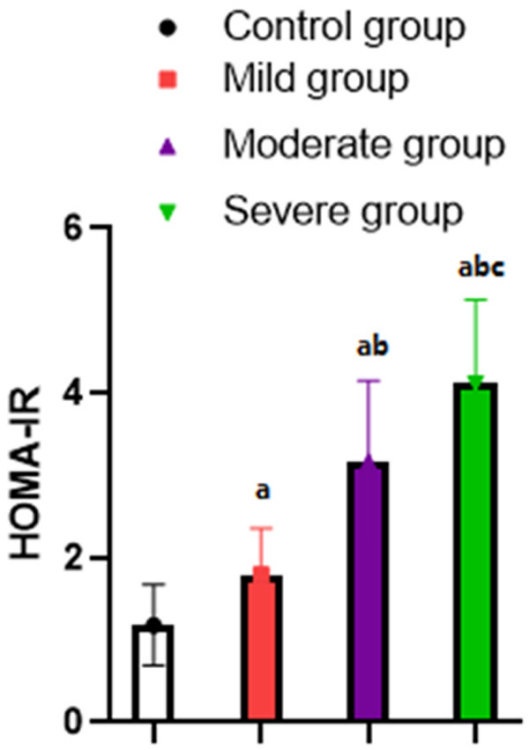

Figure 1. Comparison of IR in different groups of children. Note: Compared with the control group, *aP<0.05*; compared with the mild-group, *bP<0.05*; compared with the moderate-group, *cP<0.05*.

Results

Clinical materials

Each group of children had insignificant difference in gender or age (*P>0.05*). The differences in BMI, waist circumference and hip circumference of children in each group were statistically significant (*P<0.05*). The indexes of the children in obesity-group were critically higher than those in control-group (*P<0.05*), and the indexes had increased with the climbing severity of obesity degree (*P<0.05*), as shown in **Table 1**.

Comparison of glucolipid metabolism between groups

Each group of children had insignificant difference in FBG level (*P>0.05*). The levels of TG, TC, LDL and FINS in each obesity group were substantially higher than those in control group (*P<0.05*), the severe-group had significantly higher index degree than mild-and moderate-group (*P<0.05*), and the moderate-group had obviously higher degree than the mild-group (*P<0.05*). The HDL level of children in each obesity group was notably lower than that of the

Table 3. Comparison of relative expression levels of PI3K mRNA and Akt mRNA in peripheral blood mononuclear cells between groups

Group	Number of cases	PI3K mRNA	Akt mRNA
Control group	60	1.015±0.027	1.128±0.053
Mild-group	67	0.901±0.039[a]	0.896±0.045[a]
Moderate-group	55	0.821±0.064[a,b]	0.802±0.042[a,b]
Severe-group	35	0.762±0.053[a,b,c]	0.718±0.056[a,b,c]
F	-	29.302	37.603
P	-	<0.001	<0.001

Note: Compared with the control group, [a]*P<0.05*; compared with the mild-group, [b]*P<0.05*; compared with the moderate-group, [c]*P<0.05*.

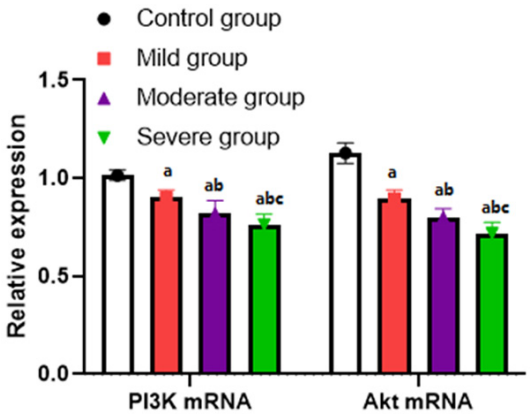

Figure 2. Comparison of relative expression levels of PI3K mRNA and Akt mRNA in peripheral blood mononuclear cells.

control group (*P<0.05*). In addition, the HDL level in severe-group was dramatically lower than that in moderate- and mild-group (*P<0.05*), and the index in moderate-group was obviously lower than in mild-group (*P<0.05*) (**Table 2**).

Comparison of each group's IR degree

The HOMA-IR of children in each obesity group was substantially higher than that of the control group (*P<0.05*), and the level increased with the uprising of obesity severity (*P<0.05*), as shown in **Figure 1**.

Comparison of PI3K/Akt pathway in PBMCs between each group

The levels of PI3K mRNA and Akt mRNA in PBMCs of children in each obesity group were obviously lower than those in control group (*P<0.05*). The severe-group had remarkably lower degree of PI3K mRNA and Akt mRNA than mild and moderate-groups (*P<0.05*), and the moderate-group had less PI3K mRNA and Akt mRNA degrees than the mild-group (*P<0.05*). (**Table 3**; **Figure 2**).

Correlation analysis

The relative expression levels of PI3K mRNA and AKT mRNA showed a remarkably negative-correlation with TG, TC, LDL, FINS, HOMA-IR (r=-0.486, -0.430, -0.503, -0.627, -0.795, *P<0.05*; R= -0.395, -0.311, -0.326, -0.411, -0.698, *P<0.05*), a positive-correlation with HDL (r=0.364, *P<0.05*; r=0.279, *P<0.05*), and non-correlation with FBG (r=-0.192, *P>0.05*; r=-0.021, *P>0.05*) (**Figures 3** and **4**).

Discussion

Obesity is a state of increased body fat capacity caused by the imbalance of energy metabolism, excessive energy intake or insufficient consumption, and is also a most significant risk factor for TypeII diabetes and cardiovascular disease. Obesity is often accompanied by various degrees of dyslipidemia, hyperinsulinemia, and insulin resistance (IR) [10, 11]. IR is a state in which the sensitivity of targeting organs to insulin action is reduced. In other words, it is a state where a normal dose of insulin produces a biological effect that is lower than its normal degree. Current studies have shown that the primary mechanism of IR is due to the obstruction in the transmission of the insulin signal binding to its receptor. PI3K/Akt is of the signaling pathways associated with IR [12, 13]. PI3K/Akt signaling pathway is a key pathway that involved in a variety of life activities of the body, including cell division, differentiation, apoptosis, etc. Modern researches have issued that this signaling pathway is closely related to IR-related diseases, such as TypeII diabetes mellitus, cardiovascular disease, obesity, etc. [14, 15]. Currently, the mechanism of childhood simple obesity has yet been completely elucidated. In order to figure out if PI3K/Akt signaling pathway is involved in the occurrence and progression of childhood simple obesity, this study explored the expressions of PI3K/Akt signaling pathway in children with different obesity degrees and its correlation with glucolipid metabolism and IR.

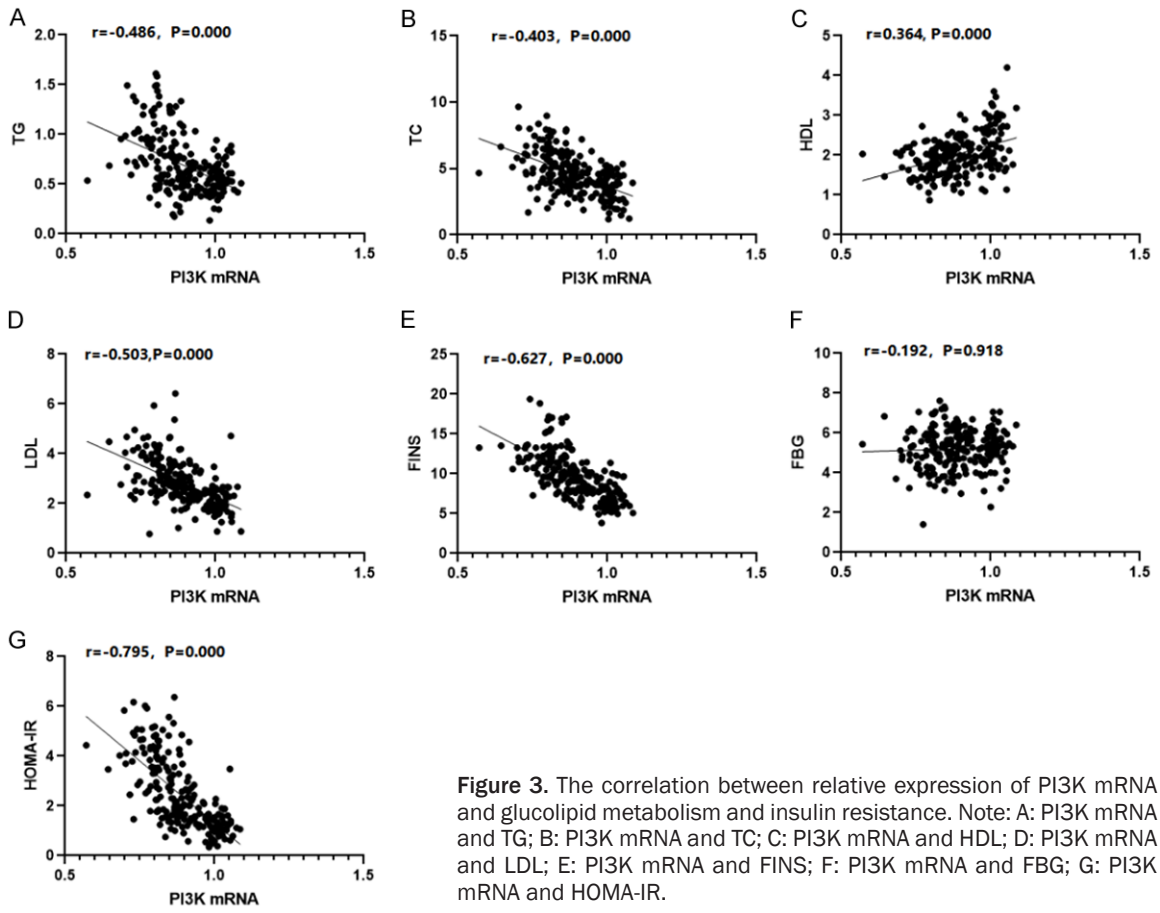

Figure 3. The correlation between relative expression of PI3K mRNA and glucolipid metabolism and insulin resistance. Note: A: PI3K mRNA and TG; B: PI3K mRNA and TC; C: PI3K mRNA and HDL; D: PI3K mRNA and LDL; E: PI3K mRNA and FINS; F: PI3K mRNA and FBG; G: PI3K mRNA and HOMA-IR.

The results of this study showed that the levels of BMI, waist circumference, hip circumference, TG, TC, LDL, FINS and HOMA-IR in each obesity group increased substantially than in control group, while the HDL was critically lower than that of the control group. This result is consistent with scholars' researches, and indicates that obese children have varying degrees of glucolipid metabolism disorders, which primarily manifested as hyperinsulinemia and IR. The high TG, TC and LDL degree and the low HDL in obese groups indicated the disordered lipid metabolism, low utilization rate of free fatty acids in body, and appearance of hypertriglyceridemia that caused by the accumulation of free fatty acids in the blood, which jointly contribute to obesity [18, 19]. HOMA-IR can comprehensively reflect the output of liver glycogen and the dynamic balance of insulin secretion, and can also be used as a common indicator to evaluate insulin homeostasis and fat metabolism. The higher level of insulin and the severed IR status would result in more fat accumulated in body; the consumption of sugar and fat, and the reduction of metabolic capacity in body will then lead to the obesity [20, 21].

The levels of PI3K mRNA and Akt mRNA in PBMCs of children in obesity groups were obviously lower than those in control group, and the levels had seen with an obvious decline with the aggravation of children's obesity. This suggested that the inhibition of PI3K/Akt signaling pathway exists in children with simple obese, and such inhibition of pathway is related to the severity of obesity. The activation of PI3K/Akt signaling pathway can help to treat the obesity by improving the glucolipid metabolism conditions, and this conclusion is consist with the scholars [22, 23]. In addition, we have analyzed the correlation between PI3K/Akt signaling pathway, glucolipid metabolism and IR in obese children in this study. According to the acquired results, the relative expression levels of PI3K mRNA and Akt mRNA in children with simple obesity were substantially and positively correlated with TG, TC, LDL, FINS and HOMA-IR while negatively correlated with HDL. This indicated

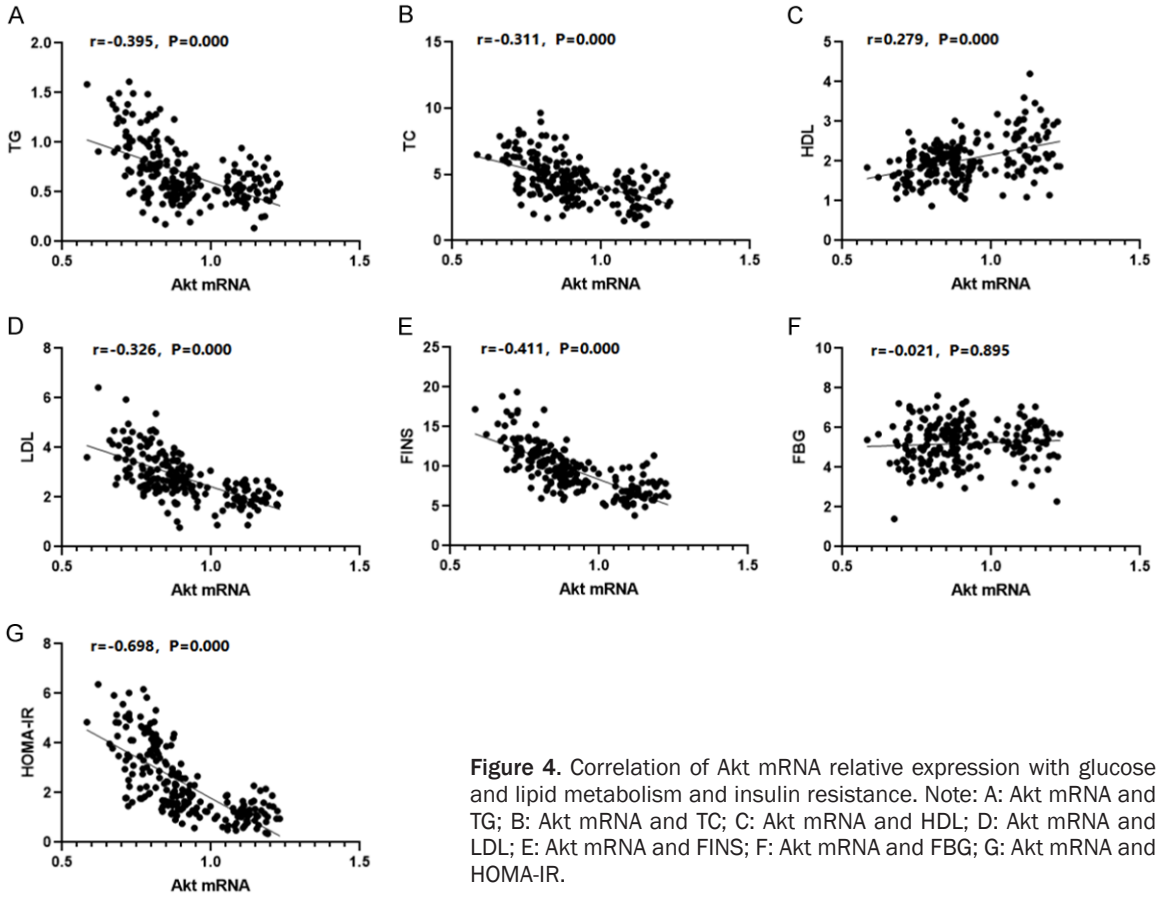

Figure 4. Correlation of Akt mRNA relative expression with glucose and lipid metabolism and insulin resistance. Note: A: Akt mRNA and TG; B: Akt mRNA and TC; C: Akt mRNA and HDL; D: Akt mRNA and LDL; E: Akt mRNA and FINS; F: Akt mRNA and FBG; G: Akt mRNA and HOMA-IR.

that PI3K/Akt signaling pathway can regulate the occurrence and progression of childhood obesity by involving in glucolipid metabolism and IR process.

In summary, the inhibition of PI3K/Akt signaling pathway in children with simple obesity is correlated with the abnormal glucolipid metabolism and IR, and thus participated in the occurrence and progression of obesity.

Disclosure of conflict of interest

None.

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