

# A Potential Role for GLUT4 in Predicting Sepsis in Critically Ill Children

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## Abstract

**Background:** This study investigated serum Glucose transporter (GLUT) 4 levels and examined the relationship between serum GLUT4 levels and sepsis in critically ill children. **Methods:** This was a retrospective study of 77 critically ill children and 33 non-diabetic healthy children (controls) who were admitted between 07/2015 and 05/2016. Patient data, clinical information, and blood samples were collected on admission, alongside a large number of laboratory parameters that were routinely assessed. Critically ill patients were divided into sepsis and non-sepsis/systemic inflammatory response syndrome (SIRS). Serum GLUT4 was measured using western blotting and enzyme-linked immunosorbent assays. Insulin resistance indexes, clinical data, laboratory parameters, and inflammatory cytokines were assessed. **Results:** GLUT4 serum levels were higher in critically ill children than in healthy children (90.5 vs. 30.3 µg/L,  $P < 0.001$ ), and in septic shock compared with sepsis (116.8 vs. 64.3 µg/L,  $P < 0.05$ ), but not compared to non-sepsis/systemic inflammatory response syndrome (105.7 µg/L,  $P > 0.05$ ). Compared to healthy children, hyperglycemic patients ( $n = 48$ ) had elevated GLUT4 serum levels (30.3 vs. 103.7 µg/L,  $P < 0.001$ ). Serum GLUT4 levels were higher in patients who died ( $n = 16$ ,  $P < 0.05$ ) than in those who survived ( $n = 57$ ). Serum GLUT4 levels were positively correlated with the neutrophil count, creatine kinase levels, and glucose levels ( $P < 0.05$ ). GLUT4 levels for the diagnosis of sepsis had an area under the curve of 0.70 ( $P = 0.03$ ) when using a 51-µg/L cut-off value, resulting in 74.6% sensitivity and 80% specificity. **Conclusions:** GLUT4 serum levels might be significantly increased in critically ill children compared with healthy children, particularly those in septic shock. Serum GLUT4 could predict disease severity.

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## Keywords

Children, Critical Illness, Glucose Transporter Type 4, Hyperglycemia, Insulin Resistance

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## 1. Introduction

Stress hyperglycemia and insulin resistance are common in critically ill patients, [1], particularly in those with sepsis. [2] [3] [4] An increase in insulin resistance has been described in patients with sepsis, renal failure, and a variety of critical illnesses. [5] [6]

A family of glucose transporters (GLUTs) is responsible for clearing glucose from the bloodstream. Among them, the GLUT4 isoform is the major insulin-responsive transporter and is predominantly expressed in skeletal muscles [7] and adipose tissue. GLUT4 responds rapidly and efficiently to fluctuations in circulating insulin levels. [8] [9] Reduced GLUT4 mRNA and protein levels were reported in septic rat adipose tissue and were associated with insulin resistance. [10] Reduced intracellular GLUT4 levels play a key role in the impaired glycemic homeostasis observed in patients with metabolic syndrome, which is characterized by insulin resistance in tissues that should be insulin-sensitive. [7] During critical illness, [11] [12] the inhibitor ( $I\kappa B\alpha$ ) of the nuclear factor- $\kappa B$  kinase (NF- $\kappa B$ ) and the Jun-B pathways are activated, leading to the expression of inflammatory markers such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) that downregulate GLUT4 gene transcription. [13] This could be a possible mechanism of insulin resistance in sepsis. [10]

Because of the importance of GLUT4 in maintaining blood glucose homeostasis, its intracellular localization and plasma membrane insertion have been studied under basal conditions. The majority of GLUT4 is sequestered within a specialized, insulin-sensitive storage compartment in the form of vesicular structures. [14] [15] This reflects fast endocytosis from the plasma membrane [16] [17] [18] and slow exocytosis of GLUT4-containing vesicles, [16] [17] [18] [19] resulting in low levels of plasma membrane-inserted GLUT4 in the basal state. As glucose levels rise, the subsequent increase in circulating insulin activates intracellular signaling cascades that ultimately result in the translocation of the GLUT4 storage compartments to the plasma membrane.

We incidentally measured serum GLUT4 levels using enzyme-linked immunosorbent assays (ELISAs) and discovered a relationship between serum GLUT4 and blood glucose levels, and the severity of disease in critically ill children in the course of a previous study (data not shown). To the best of our knowledge, the GLUT4 protein has not been measured in the serum until now. Moreover, the mechanism resulting in GLUT4 release in the serum remains unclear.

The aim of this study was to ascertain the presence of GLUT4 in the serum, and consequently, to examine its relationship with the illness severity, blood

glucose, and insulin resistance in a cohort of critical care patients in the emergency department.

## 2. Material and Methods

### 2.1. Study Design and Population

This was a retrospective study of critically ill children (emergency patients grade I and II, [20] 1 month to 18 years of age) who were admitted between July 2015 and May 2016 at the emergency department at Guangzhou Women and Children's Medical Center, and of non-diabetic healthy children (control group) who were admitted for a routine medical check-up. Patients with a history of glucose metabolism disorders, or who received insulin and/or glucose in the previous 3 h were excluded.

### 2.2. Ethics

This study was approved by the ethics committee of Guangzhou Women and Children's Medical Center [2014082618]. The need for individual consent was waived by the committee because of the retrospective nature of the study.

### 2.3. Data Collection

Patient data, clinical information, and blood samples were collected on admission, alongside a large number of laboratory parameters that were routinely assessed during emergency treatment. Critically ill patients were divided into two categories: sepsis and non-sepsis/systemic inflammatory response syndrome (SIRS). The patients in the sepsis group were divided into three subgroups (Table 1) and met the criteria proposed by the American College of Chest Physicians and the Society of Critical Care Medicine Consensus Conference Committee for severe sepsis and septic shock [21]. The pediatric risk of mortality scoring system (PRISMII) [22] was used to evaluate the severity of the patient's condition at admission. The patients were divided into the euglycemia and hyperglycemia groups based on blood glucose levels (>6.1 mmol/L indicated hyperglycemia). [23] HOMA-IR [24] (homeostasis model assessment index of insulin resistance) was calculated. The patients were transferred to the pediatric intensive care unit or other inpatient departments after they were stable and were followed for 30 days from the day of admission to the emergency department. According to their prognosis, the patients were assigned to the survival and death groups. The patients in the three sepsis subgroups were compared by age, sex, and severity of disease using the PRISMII score at admittance.

### 2.4. Serum GLUT4 Detection and Quantitative Measurements

Serum GLUT4 was detected by western blotting using two monoclonal mouse anti-GLUT4 antibodies (RayBiotech, Inc., Norcross, GA, USA; and Santa Cruz Biotechnology, Santa Cruz, CA, USA). The serum sample was diluted 1:25 for western blotting. A monoclonal anti-GAPDH antibody (Sigma, St Louis, MO,

**Table 1.** Characteristics of the study population.

Parameter	Controls (n = 33)	All emergency patients (n = 77)	Sepsis			Non-sepsis/SIRS (n = 10)
			Sepsis (n = 18)	Severe (n = 37)	Shock (n = 12)	
Sex (male)	24 (72.7%)	46 (59.7%)	9 (50.0%)	23 (62.2%)	6 (50.0%)	8 (80.0%)
Age (years)	2.42 (1.42 - 4)	1.74 (0.08 - 11)	1.27 (0.25 - 3)	1.91 (0.17 - 11)	2.20 (0.25 - 10)	1.42 (0.08 - 4)
Leukocyte count ( $\times 10^{12}/L$ )	NA	15.0 $\pm$ 9.9	13.0 $\pm$ 10.9	14.8 $\pm$ 10.0	14.8 $\pm$ 9.9	20.5 $\pm$ 9.4
hs-CRP (mg/L)	NA	23.0 (1.72, 76.5)	34.0 (2.0, 145.9)	24.2 (2.8, 61.8)	75.1 (17.7, 176.5)	6.9 (0.6, 22.2)
Lactate (mmol/L)	NA	1.3 (1.0, 2.3)	1.4 (1.0, 2.4)	1.2 (0.9, 2.0)	1.2 (5.4, 23.8)	2.1 (1.5, 5.2)
Creatine ( $\mu\text{mol}/\text{dL}$ )	NA	23.0 (18.0, 29.0)	21.5 (17.5, 25.8)	24.5 (18.8, 30.0)	27.5 (17.0, 53.0)	20.0 (14.0, 29.0)
Cystatin C (mg/L)	NA	0.75 (0.62, 0.93)	0.74 (0.66, 0.95)	0.73 (0.61, 0.92)	0.82 (0.73, 1.35)	0.72 (0.61, 0.85)
Creatine kinase (U/L)	NA	132 (70, 242)	72 (53, 137)	106 (62, 230)	162 (77, 232)	264 (141, 1287)
Blood glucose (mmol/L)	4.96 $\pm$ 0.67	7.7 $\pm$ 3.5	6.0 $\pm$ 1.7	7.8 $\pm$ 2.7	6.6 $\pm$ 2.6	10.2 $\pm$ 7.4
Insulin (mU/L)	NA	6.3 (3.5, 14.2)	4.7 (1.6, 14.2)	9.5 (5.1, 14.6)	5.3 (1.3, 12.3)	5.0 (2.9, 32.2)
HOMA-IR	0.9 (0.7, 1.3)	2.3 (0.9, 5.2)	1.1 (0.3, 4.1)	2.6 (1.3, 7.9)	1.0 (0.3, 4.0)	2.2 (0.7, 8.9)
PRISMII score	NA	8 (5, 12)	5 (2, 8)	7 (4, 9)	10 (7, 15)	8 (8, 14)
Death	0	16 (20.8%)	0 (0.0%)	4 (10.8%)	8 (66.7%)	4 (40.0%)

SIRS: systemic inflammatory response syndrome; hs-CRP: high-sensitivity C-reactive protein; HOMA-IR: homeostasis model assessment index of insulin resistance; PRISM: Pediatric risk of mortality scoring system; NA: not available.

USA) was used as an internal control. The blots were scanned, and the relative protein levels were determined using the Odyssey Infrared Imaging System Version 3.0 (LI-COR Biosciences, Lincoln, NE).

A quantitative sandwich ELISA (Nava TeinBio, Inc. Cambridge, MA, USA) for GLUT4 was performed according to manufacturers' instructions. Interleukin (IL)-6 and TNF- $\alpha$  ELISA kits (Nava TeinBio, Inc. Cambridge, MA, USA) were also used according to manufacturers' instructions.

## 2.5. Statistical Analysis

Continuous variables with a normal distribution (according to the Kolmogorov-Smirnov test) were expressed as means  $\pm$  standard deviation (SD) and analyzed using the Student t-test (two groups) or one-way analysis of variance (ANOVA) with the Tukey's post hoc test (more than two groups). Those with a non-normal distribution were presented as medians and interquartile ranges (IQR) and were analyzed using the Mann-Whitney U-test (two groups) and Kruskal-Wallis analysis of variance with the post hoc Mann-Whitney U-test (more than two groups). The categorical data were presented as numbers and percentages and analyzed using the chi-square test or Fisher's exact test, as appropriate. All values, including outliers, were included in the statistical analyses. Correlations between variables were analyzed using the Spearman correlation test for variables that were found to be associated with GLUT4 with  $P < 0.20$  in

the U-test. The diagnostic value of GLUT4 for sepsis was evaluated using a receiver operating characteristic (ROC) analysis. All statistical analyses were performed with SPSS 17.0 (IBM, Armonk, NY, USA). Two-sided (except for the chi-square test) P-values < 0.05 were considered statistically significant.

### 3. Results

#### 3.1. Patient Characteristics

This study included 77 critically ill children (46 boys and 31 girls; the median age of 1.74 years; range, 1 month to 11 years; six children over 5 years old) and 33 control patients (24 boys and 9 girls; the median age of 2.85 years; range, 2 years to 7 years; five children over 5 years old (**Table 1**).

Among the 77 patients, 67 patients conformed to the criteria of sepsis (**Table 1**). In the majority of sepsis patients, the original infections were severe pneumonia (n = 37), intracranial infection (n = 19), dysentery (n = 7), intestinal perforation (n = 2), and abscess (n = 2). The patients with SIRS who conformed to the SIRS criteria in critical conditions differed in their etiology, including severe trauma (n = 4), intracranial hemorrhage (n = 4), and strong acid burns (n = 2).

As expected, high levels of hs-CRP, white blood cell count, and lactate were found in critically ill patients (**Table 1**), and the critically ill patients had higher glucose levels and HOMA-IR than healthy children ( $P < 0.05$ ). Among the critically ill patients, the PRISMII score, leukocyte counts, and blood glucose did not differ between the severe sepsis and SIRS groups. The patients with SIRS had lower levels of hs-CRP and insulin than patients with sepsis. Among all the patients who were followed, 16 patients died (**Table 1**); 13 (80%) died in the pediatric intensive care unit, and three (20%) died in the emergency room. Significant differences in the rates of death and survival were observed between the SIRS and sepsis subgroups.

#### 3.2. GLUT4 Serum Levels in Critically Ill Children

Western blotting showed protein bands at 55 kDa in the serum samples of eight children with a critical illness, including two patients who died. The optical densities were higher in the two patients who died (4.2 and 3.9) than in the two patients who survived (2.0 and 1.4) and in one control (1.8).

Serum GLUT4 levels by ELISA were higher in critically ill children than in healthy children (median 90.5 vs. 30.3  $\mu\text{g/L}$ , respectively;  $P < 0.001$ ) (**Table 2** and **Figure 1(a)**). Serum GLUT4 levels were significantly ( $P < 0.01$ ) higher in patients with septic shock than with sepsis (median 116.8 vs. 64.3  $\mu\text{g/L}$ , respectively;  $P < 0.01$ ) (**Table 2** and **Figure 1(b)**). TNF- $\alpha$  and IL-6 serum levels demonstrate changes similar to the serum GLUT4 levels in the control group and the subgroups (**Table 2**).

#### 3.3. GLUT4 Serum Levels in Patients with Hyperglycemia

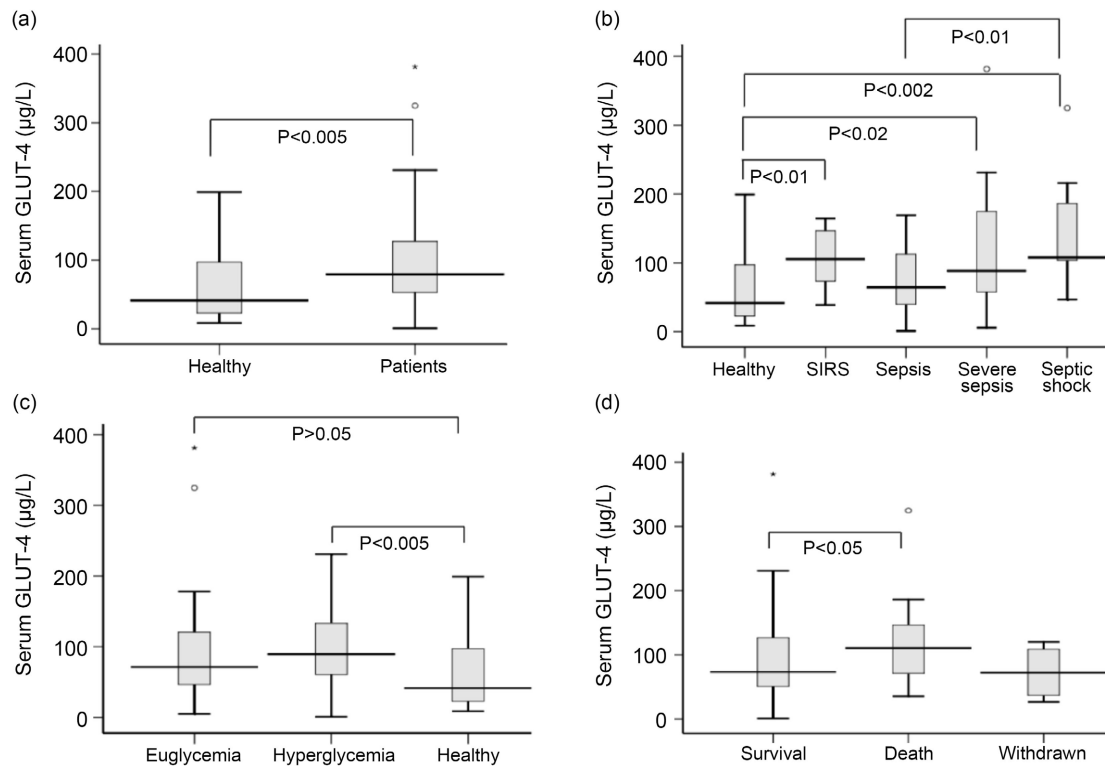
Serum GLUT4 levels [90.46 (60.97, 138.35)  $\mu\text{g/L}$ ] in critically ill patients with

hyperglycemia (n = 48, 62.3%) who had significantly increased HOMA-IR [2.3 (0.9, 5.2)] were higher than in healthy children (n = 33; 0.8 median HOMA-IR, 30.3 µg/L median GLUT4 serum levels; P < 0.001), as demonstrated in **Table 3**. **Figure 1(c)** shows that patients with hyperglycemia (n = 48, 62.3%) had higher serum GLUT4 levels than healthy control subjects (median 103.7 vs. 30.3 µg/L, respectively; P = 0.003).

**Table 2.** Comparison between healthy children and patients from the emergency room.

Parameters	Controls (n = 33)	All emergency patients (n = 77)	Sepsis			Non-sepsis/SIRS (n = 10)
			Sepsis (n = 18)	Severe (n = 37)	Shock (n = 12)	
GLUT4 (µg/L)	30.3 (23.2, 61.9) <sup>F</sup>	90.5 (50.7, 128.2) <sup>*</sup>	64.3 (34.4, 111.5)	79.2 (43.8, 135.0)	116.8 (82.8, 174.1) <sup>f</sup>	105.7 (70.0, 148.6) <sup>‡</sup>
TNF-α (ng/L)	9.2 (4.9, 17.1) <sup>F</sup>	200.4 (15.4, 315.5) <sup>*</sup>	18.2 (12.2, 117.0)	94.7 (10.6, 303.6)	529.3 (282.2, 568.6) <sup>f</sup>	278.0 (246.5, 459.0) <sup>‡</sup>
IL-6 (ng/L)	109.1 (91.3, 118.8) <sup>F</sup>	353.3 (152.2, 609.4) <sup>*</sup>	157.1 (115.9, 248.5)	254.6 (128.5, 619.5)	609.4 (464.1, 1060.6) <sup>f</sup>	447.1 (394.2, 642.8) <sup>‡</sup>

<sup>\*</sup>P < 0.01 Patients vs. Controls; <sup>‡</sup>P < 0.01 Controls vs. each subgroup; <sup>f</sup>P < 0.05 septic shock vs. sepsis subgroups; <sup>‡</sup>P < 0.05 SIRS vs. sepsis subgroups.



**Figure 1.** Serum GLUT4 levels in critically ill children. (a) Serum GLUT4 levels were significantly (P = 0.003, U-test) elevated in critically ill patients (n = 77) compared with healthy control subjects (n = 33). (b) Serum GLUT4 levels were significantly higher in patients with septic shock (n = 12) than with sepsis (n = 18) (P < 0.01, U-test). (c) Serum GLUT4 levels were significantly (P = 0.003, U-test) elevated in patients with hyperglycemia (n = 48) compared with healthy control subjects (n = 33), and similar with patients with euglycemia (n = 29). (d) Serum GLUT4 levels were significantly (P < 0.05, U-test) increased in patients who died (n = 16) compared to those who survived (n = 57). Box plots are displayed, where the bold black line indicates the median, the box represents 50% of the values, the horizontal lines show the minimum and maximum values of the calculated non-outlier values, and the open circles indicate outlier values.

**Table 3.** Comparison between healthy children and patients with hyperglycemia.

Parameters	Classification of the patients according to the blood glucose levels			P
	Healthy (n = 33)	Euglycemia (n = 29)	Hyperglycemia (n = 48)	
Glucose (mmol/L)	4.96 ± 0.67	4.95 ± 0.82	9.33 ± 3.39*	<0.001
HOMA-IR	0.9 (0.7, 1.3)	0.8 (0.3, 1.3)	4.3 (2.1, 8.4)*	<0.001
GLUT4 (µg/L)	30.3 (23.2, 61.9)	58.7 (39.4, 58.7) <sup>†</sup>	103.7 (63.9, 138.9) <sup>‡</sup>	0.009

\*P < 0.001 hyperglycemia patients vs. healthy, euglycemia patients; <sup>†</sup>P > 0.05 euglycemia patients vs. healthy subjects; <sup>‡</sup>P < 0.005 hyperglycemia vs. healthy subjects. HOMA-IR: homeostasis model assessment index of insulin resistance.

### 3.4. GLUT4 Serum Levels in Patients Who Died

As shown in **Figure 1(d)**, critically ill patients who died had significantly higher serum GLUT4 levels than those who survived (P < 0.05).

### 3.5. Correlation of GLUT4 Serum Levels with Blood Variables

**Table 4** shows that insulin levels and HOMA-IR were not significantly different among the three different ranges of GLUT4 levels. Glucose levels (P = 0.005), neutrophil count (P < 0.001), and CK levels (P = 0.017) increased with GLUT4 levels. Erythrocyte count and PRISMII score also had P < 0.20 and were included in the subsequent Spearman analyses. Parameters of inflammation, *i.e.*, TNF- $\alpha$ , IL-6, and hs-CRP, were not associated with GLUT4 levels (P > 0.05) (**Table 4** and **Figure 2**).

In the Spearman analyses (**Table 5** and **Figure 3(a)**), the GLUT4 levels were positively correlated with the glucose levels (r = 0.272, P = 0.004), neutrophil count (r = 0.471, P < 0.001), and CK levels (r = 0.247, P = 0.031) in all participants. GLUT4 levels remained correlated with neutrophil count and CK levels when considering the critical patients or the patients with sepsis (all P < 0.05).

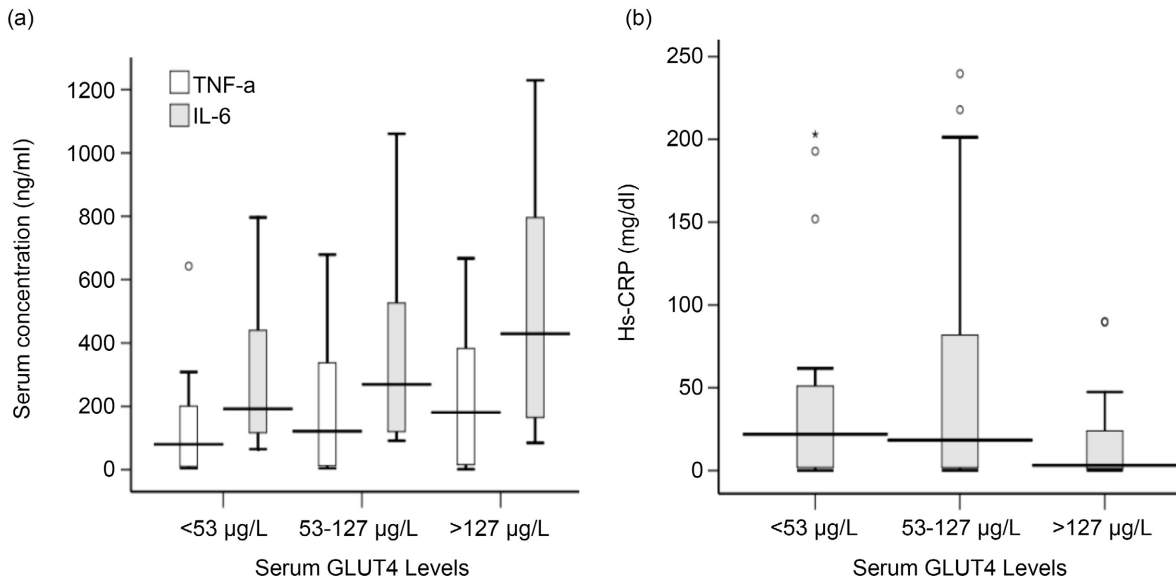
### 3.6. GLUT4 Might Have a Diagnostic Value for Sepsis in Critically Ill Children

The ROC curve (**Figure 3(b)**) indicated that GLUT4 levels for the diagnosis of sepsis had an area under the curve of 0.70 (95% confidence interval: 0.56 - 0.79, P = 0.03) when using a 51-µg/L cut-off value, resulting in 74.6% sensitivity and 80% specificity.

## 4. Discussion

Hyperglycemia and underlying insulin resistance are associated with an increase in cytokines and counter-regulatory hormones, which in turn lead to insulin resistance. [7] GLUT4 is an insulin-sensitive transporter that uptakes blood glucose into muscles and adipose tissue, but its relationship with the critical conditions is unknown. This study aimed to investigate serum GLUT4 levels in criti-

cally ill children and to examine the potential relationship between serum GLUT4 levels and illness severity. The results strongly suggest the GLUT4 serum levels might be significantly increased in critically ill children compared with healthy children, particularly those in septic shock. Serum GLUT4 could predict disease severity in critically ill children.



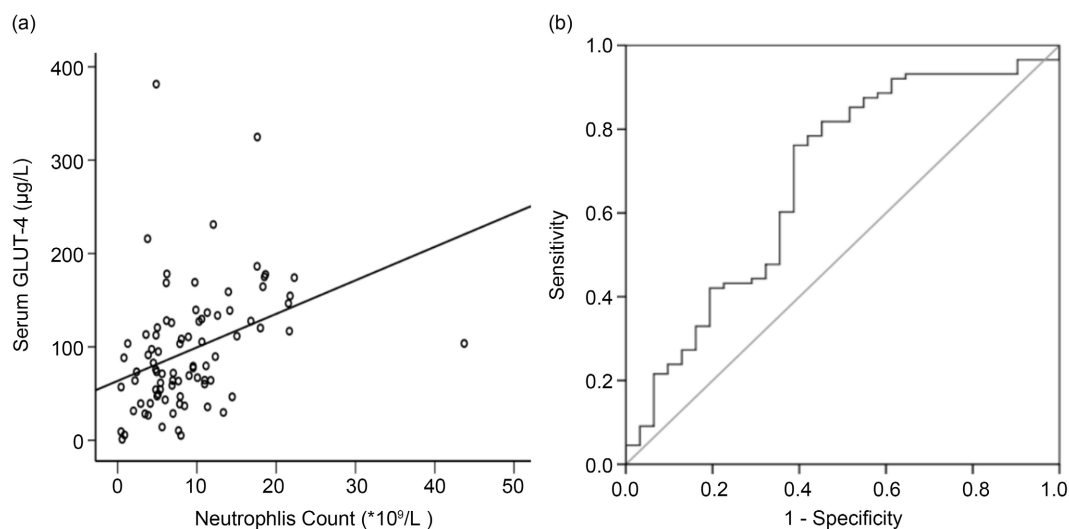
**Figure 2.** Serum levels of TNF- $\alpha$ , IL-6, and hs-CRP in groups with different GLUT4 levels. (a) The serum levels of TNF- $\alpha$  and IL-6 were consistent with the serum GLUT4 levels ( $P > 0.05$ ) in the emergency room. (b) The serum levels of hsCRP were consistent with the serum GLUT4 levels ( $P > 0.05$ ). Box plots are displayed, where the bold black line indicates the median, the box represents 50% of the values, the horizontal lines show the minimum and maximum values of the calculated non-outlier values, and the open circles indicate outlier values.

**Table 4.** Parameters associated with GLUT4 levels.

Parameter	Serum levels of GLUT4 ( $\mu\text{g/L}$ )			P
	Low (<53) (n = 39)	Middle (53 - 127) (n = 44)	High (>127) (n = 27)	
Glucose (mmol/L)	5.82 $\pm$ 1.86 <sup>‡</sup>	6.98 $\pm$ 2.68 <sup>*</sup>	8.44 $\pm$ 4.75 <sup>1</sup>	0.005
Insulin (mU/L)	6.63 (2.22, 17.36)	10.17 (3.52, 14.40)	5.08 (3.35, 8.84)	0.261
HOMA-IR	1.55 (0.57, 6.40)	2.89 (0.71, 7.84)	2.20 (1.12, 3.51)	0.347
Neutrophil count ( $\times 10^9/\text{L}$ )	5.40 $\pm$ 3.78 <sup>‡</sup>	8.45 $\pm$ 7.39	12.99 $\pm$ 6.10	<0.001
Erythrocyte count ( $\times 10^{12}/\text{L}$ )	4.13 $\pm$ 0.92	3.89 $\pm$ 1.01	4.31 $\pm$ 0.66	0.189
Hs-CRP (mg/dl)	42.46 (0.54, 84.30)	40.09 (6.38, 122.29)	12.28 (1.23, 39.61)	0.271
TNF- $\alpha$ (ng/mL)	155.87 (14.54, 262.66)	183.68 (12.24, 457.90)	282.19 (15.86, 430.30)	0.600
IL-6 (ng/mL)	388.92 (155.83, 619.03)	262.63 (119.83, 533.38)	462.34 (165.14, 796.91)	0.823
Creatine kinase (U/L)	76.50 (50.00, 162.50) <sup>‡</sup>	92.00 (49.50, 225.50)	191.00 (98.50, 306.50) <sup>‡</sup>	0.017
PRISMII score	7.0 (2.0, 8.0)	8.5 (6.0, 13.0)	8.0 (5.0, 13.5)	0.084

\* $P < 0.05$  low vs. middle; <sup>‡</sup> $P < 0.01$  low vs. high; <sup>1</sup> $P < 0.05$  middle vs. high; <sup>‡</sup> $P < 0.01$  middle vs. high. HOMA-IR: homeostasis model assessment index of insulin resistance; hs-CRP: high sensitivity C-responsive protein; PRISM: Pediatric risk of mortality scoring system.





**Figure 3.** Scatter plot of serum GLUT4 levels with neutrophils count and ROC curve. (a) The scatter plot showed the correlation coefficient  $r = 0.471$  ( $P < 0.01$ ). (b) ROC curves for the diagnosis of sepsis, showing an area under the curve of 0.7 ( $P = 0.03$ ) using 51 µg/L as the cut-off value, resulting in 74.6% sensitivity and 80% specificity.

**Table 5.** Correlation with serum GLUT4 levels.

Spearman's rho	Total population (n = 110)		All patients (n = 77)		All sepsis patients (n = 67)	
	r	P	r	P	r	P
Neutrophil count	0.471	<0.001	0.490	<0.001	0.449	<0.001
Erythrocyte count	0.116	0.269	0.221	0.053	0.221	0.072
Blood Glucose	0.272	0.004	0.221	0.053	0.129	0.296
PRISMII score	0.171	0.137	0.171	0.137	0.100	0.422
Serum CK levels	0.247	0.031	0.263	0.026	0.295	0.019

PRISM: Pediatric risk of mortality scoring system; CK: creatine kinase.

The molecular weight of the GLUT4 protein is about 55 kDa, [25] and we detected a 55-kDa serum protein by western blot using two different GLUT4 monoclonal antibodies from two companies, suggesting that GLUT4 can be released into the serum. Generally, GLUT4 remains within the cells, but as glucose levels rise, the subsequent increase in circulating insulin activates intracellular signaling cascades that result in the translocation of the GLUT4 storage compartments to the plasma membrane. [14]-[19] When circulating insulin levels decline, GLUT4 transporters are removed from the plasma membrane by endocytosis and are recycled back to their intracellular storage compartments. [16] [17] [18] [19] Unfortunately, the complexity of these regulatory processes provides numerous potential targets that might be defective and eventually result in peripheral tissue insulin resistance and possibly, diabetes. Whether serum GLUT4 is like ferritin, which is a cytosolic protein in most tissues but functions as an iron carrier in the serum where small amounts are secreted, remains unclear. Indeed,

plasma ferritin is not only an indirect marker of the total amount of iron stored in the body [26] but also an inflammatory acute-phase protein [27] because of increased levels in response to stresses such as anoxia. [28] To the best of our knowledge, the presence of the GLUT4 protein in the serum has not been described in the literature, and the mechanisms leading to GLUT4 secretion in the serum are unknown.

The present study showed that GLUT4 serum levels were significantly elevated in critically ill patients compared with healthy children, especially those in septic shock, and with SIRS and/or hyperglycemia with high HOMA-IR. Those results suggest a possible association between elevated serum GLUT4 levels and blood glucose and insulin resistance. Furthermore, we observed a discrepancy in glucose levels at different serum GLUT4 levels and found that the low (median < 53 µg/L) level group had lower glucose levels than the moderate or high-level group, suggesting that there might be an association between glucose and GLUT4 levels at a certain range. Analysis of the correlation between GLUT4 serum levels and glucose levels in different groups of the study population showed similar findings, possibly indicating that hyperglycemia in critically ill children with insulin resistance is associated with elevated serum levels of GLUT4. Nevertheless, whether a definite association exists between them is unclear, and the results require validation through further studies.

Indeed, unlike in tissues or cells, in which GLUT4 mRNA and protein levels [29] are reduced during sepsis, [10] [13] the serum GLUT4 protein levels were increased in this study. Previous findings suggest that the GLUT4 protein has a short half-life in the range of 8 - 10 h. [30] The underlying mechanism of maintaining the levels of intracellular GLUT4, membrane GLUT4, and serum GLUT4 remains unclear. A hypothesis could be that when serum GLUT4 increases, intracellular plasma, and plasma membrane GLUT4 levels are reduced, resulting in decreased glucose transport and hyperglycemia with insulin resistance. This hypothesis needs to be investigated in the future.

The present study demonstrated that the GLUT4 serum levels in the subgroups were consistent with TNF- $\alpha$  and IL-6 levels, but no significant correlations were observed. As serum GLUT4 levels are elevated in critical illness, serum GLUT4 could be a component of the systemic inflammatory response. Accordingly, GLUT4 serum levels might possibly be related to inflammatory responsive mediators (TNF- $\alpha$  and IL-6) since severe stress in critically ill children with or without infection leads to an alteration in cellular membrane permeability, possibly resulting in small amounts of GLUT4 being released from the cytoplasm [14] [15] or plasma membrane [16] [17] [18] into the serum, just like ferritin, [26] aspartate transaminase, and alanine transaminase. [31] Nevertheless, two healthy children had elevated serum GLUT4 levels. The reason for this is unknown. GLUT4 is involved in normal glucose metabolism, and those children might have an undiagnosed or asymptomatic condition associated with glucose metabolism.

Higher GLUT4 serum levels were found in sepsis and patients who died from sepsis. In this study, the ROC curve for the value of GLUT4 for the diagnosis of sepsis showed 74.6% sensitivity and 80% specificity, with an area under the curve of 0.70. This warrants exploration and validation in larger populations.

In this study, serum GLUT4 concentrations had a positive linear correlation with the neutrophil counts and with the CK levels in all critical patients. As the GLUT4 protein is expressed most abundantly in the adipose tissue and cardiac and skeletal muscle, many researchers are investigating the effect of GLUT4 expression on the plasma membrane of peripheral blood cells *in vivo* and *in vitro*. [32] [33] [34] As such, peripheral blood lymphocytes might become an interesting model system to study the effects of insulin on cellular glucose transport using a flow cytometer. The quantification of GLUT4 expression on the surface of peripheral blood lymphocytes might be a potentially useful method for the early detection of individuals at a high risk of diabetes. [35] [36] GLUT4 levels in peripheral blood mononuclear cells have been investigated using indirect immunofluorescence in sled dogs, [37] and inflammatory monocyte populations in humans, [38] [39] [40] which could be used as a method to estimate the influence of insulin on GLUT protein translocation and the dynamics of glucose uptake. GLUT4 was also detected in granulocytes and could be used to assess the immune response in diabetes [41] or the response of the plasma membrane to insulin during infection. [33] [42] In addition, GLUT4 levels were studied during perinatal and postnatal erythropoiesis and are up-regulated under anemic conditions. [43]

We also observed a positive correlation between serum GLUT4 and CK concentrations. Clinically, CK is assayed in blood tests as a marker of damage to CK-rich tissue, [44] as in myocardial infarction and rhabdomyolysis. In this study, CK levels were high in patients with septic shock patients or SIRS and could be a biomarker of the severity of the disease. [45] CK levels are observed in metabolic dysfunction associated with influenza H1N1 infection. [46] Since high CK levels were consistent with the high levels of serum GLUT4, we suggest that both probably have a similar mechanism of release into the serum, and can act as clinical indicators or biomarkers predicting the severity of the disease. This will have to be examined in future studies.

This study has limitations. The sample size was small, and no formal validation of the value of GLUT4 for critical illness could be performed. In addition, the panel of biomarkers was limited, probably masking important associations and correlations.

## 5. Conclusions

GLUT4 protein can be released into the serum in critically ill children, and have high levels, particularly in patients with septic shock. Serum GLUT4 levels were associated with glucose levels in all participants, but not with insulin or HOMA-IR. Serum GLUT4 is possibly an acute-phase protein in critically ill pa-

tients that can predict the severity of the disease or a biomarker that simplifies the detection of abnormal glucose metabolism.

### Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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