Factors associated with hyperglycemia and low insulin levels in children undergoing cardiac surgery with cardiopulmonary bypass who received a single high dose of methylprednisolone

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OBJECTIVES: Administering steroids before cardiopulmonary bypass in pediatric heart surgery modulates systemic inflammatory response syndrome and improves postoperative recovery. However, the use of steroids aggravates hyperglycemia, which is associated with a poor prognosis. Adult patients with systemic inflammatory response syndrome usually evolve with hyperglycemia and high insulin levels, whereas >90% of pediatric patients exhibit hyperglycemia and low insulin levels. This study aims to determine: A) the metabolic and inflammatory factors that are associated with hyperglycemia and low insulin levels in children who underwent cardiac surgery with cardiopulmonary bypass and who received a single high dose of methylprednisolone and B) the best predictors of insulin variation using a mathematical model.

METHODS: This preliminary study recruited 20 children who underwent heart surgery with cardiopulmonary bypass and received methylprednisolone (30 mg/kg) immediately after anesthesia. Among the 20 patients initially recruited, one was excluded because of the absence of hyperglycemia and lower insulin levels after surgery. However, these abnormalities were confirmed in the remaining 19 children. The C-peptide, CRP, IL-6, and adrenomedullin levels were measured before surgery, immediately after cardiopulmonary bypass, and on the first, second, and third days after cardiac surgery.

RESULTS: IL-6, CRP, and adrenomedullin increments were observed, whereas the C-peptide levels remained within reference intervals.

CONCLUSION: The multiple regression model demonstrated that in addition to age and glycemia (two well-known factors that are directly involved in glucose metabolism), adrenomedullin and IL-6 levels were independent factors associated with lower insulin concentrations. These four parameters were responsible for 64.7% of the observed insulin variances. In addition, the fact that C-peptide levels did not fall together with insulin could have grounded the medical decision not to administer insulin to patients.

KEYWORDS: Cardiopulmonary Bypass; Adrenomedullin; Insulin; Hyperglycemia; Systemic Inflammatory Response Syndrome.

Arkader R, Malbouisson LM, Del Negro GM, Yamamoto L, Okay TS. Factors associated with hyperglycemia and low insulin levels in children undergoing cardiac surgery with cardiopulmonary bypass who received a single high dose of methylprednisolone. Clinics. 2013;68(1):85-92.

Received for publication on September 3, 2012; **First review completed on** October 3, 2012; **Accepted for publication on** November 23, 2012

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INTRODUCTION

Metabolism and immunity are closely associated; therefore, a systemic inflammatory response syndrome (SIRS) produces and activates multiple proteins, including CRP, and inflammatory cascade-releasing cytokines, such as IL-6. Studies have demonstrated that IL-6 is increased earlier and returns to baseline levels more rapidly than CRP in SIRS. However, CRP levels are routinely measured in hospital laboratories because of their multiple clinical applications,

No potential conflict of interest was reported.

DOI: 10.6061/clinics/2013(01)OA14

e.g., evaluating cardiac risk and detecting inflammation and infection, whereas IL-6 is currently used as a research tool (1).

Cardiac surgery with cardiopulmonary bypass (CPB) triggers systemic inflammatory response syndrome (SIRS) and evolves with hyperglycemia and insulin resistance in adults, whereas >90% of children exhibit hyperglycemia accompanied by low insulin levels (2,3).

Insulin is synthesized as preproinsulin and turned into proinsulin. Proinsulin is then converted to insulin and C-peptide and stored in granules that await release on demand. C-peptide is secreted into the bloodstream in equimolar amounts with insulin in response to blood glucose levels (4).

A single high dose of methylprednisolone (MP) is routinely used in the majority of pediatric heart surgery centers to blunt SIRS; however, MP aggravates hyperglycemia (5,6), which is associated with a poor prognosis (7,8). Verhoeven et al. (2011) studied 49 children undergoing cardiac surgery and analyzed their blood glucose, insulin, lactate, cortisol, ACTH, IL-6, and IL-10 levels before surgery, immediately after surgery, and 12 h and 24 h after surgery. Glucocorticoids were administered to 65% of the children, and this administration was the main factor associated with hyperglycemia, which was observed in 52% of the children after surgery. However, hyperglycemia disappeared spontaneously without insulin therapy after 24 h in 94% of children, which supported the results of other studies (9). Because the postoperative morbidity was low in the study group, the authors concluded that the positive effects of glucocorticoid administration outweighed the adverse effects of iatrogenic hyperglycemia.

There is a consensus on the usefulness of intensive insulin therapy for glucose control to improve morbidity and mortality rates in adult cardiac surgical patients (10). In contrast, there have only been a few randomized controlled studies in critically ill children (8,11). Agus et al. enrolled 980 children up to 36 months of age who were undergoing cardiac surgery with CPB. The patients were randomly assigned to either undergo tight glycemic control with targeted blood glucose levels that ranged from 80-110 mg/dL or receive standard care in the intensive care unit. Continuous glucose monitoring was used to detect hypoglycemia. Overall, 444 of the 490 children (91%) who were assigned to the tight glycemic control group received insulin compared with 9 of the 490 children (2%) who were assigned to receive standard care. Normoglycemia was achieved earlier with tight glycemic control than with standard care (6 h *vs.* 16 h, $p<0.001$) and was maintained in a greater proportion of those patients (50% *vs.* 33%, $p<0.001$); however, tight glycemic control was not associated with a significantly decreased rate of associated infections (8.6 *vs.* 9.9 per 1,000 patient days, $p = 0.67$). Secondary outcomes, such as morbidity, mortality, and multiple organ system failure, did not differ significantly between the groups; therefore, it was concluded that high-risk subgroups did not benefit from tight glycemic control. In addition, 3% of the patients who were assigned to undergo tight glycemic control had severe hypoglycemia (blood glucose <40 mg per deciliter) (12). Considering the results of these studies, there is no consensus on insulin use in children despite the frequent association between hyperglycemia and cardiac surgery with CPB (7,8).

Many studies have been conducted on hyperglycemia caused by SIRS in children with sepsis (5,6,13,14); however, little is known about the factors that are involved in regulating insulin in the context of SIRS triggered by cardiac surgery with CPB and MP, which leads to hyperglycemia and low insulin levels (5,6,15,16). The development of age-appropriate immune modulatory interventions for preventing and treating SIRS abnormalities requires an investigation of the mechanisms that are responsible for the unique pediatric inflammatory response to injury. Therefore, we expanded our investigation of the clinical parameters that can be used to monitor children after cardiac surgery with CPB and MP. In addition to glycemia and CRP levels, which are typically monitored in these children, we evaluated insulin, IL-6, and adrenomedullin (ADM) levels. ADM is a novel powerful vasodilating peptide that acts as an immune modulator with bactericidal activity and as an inflammation marker during SIRS with or without infection (17). In addition, monitoring ADM levels is important due to its capacity to inhibit insulin release through a direct action on pancreatic β-cells (18,19). The ability of ADM to act specifically on insulin release supports its use as a marker in this type of pediatric patient.

Adult and pediatric patients differ according to insulin levels after a hyperglycemic stimulus that is triggered by SIRS and aggravated by MP. Additionally, there is no consensus on the routine administration of exogenous insulin to these pediatric patients. Therefore, we investigated children who were subjected to cardiac surgery with CPB and MP and who presented with hyperglycemia and low insulin levels. We monitored blood glucose, insulin, C-peptide, CRP, IL-6, and ADM levels to improve our understanding of the metabolic and inflammatory factors that are involved in glucose regulation in these children and determine the best predictors of insulin variance based on a mathematical model.

MATERIALS AND METHODS

After written informed consent was obtained from their legal guardians, 20 children from the Heart Institute in the School of Medicine at the University of São Paulo, Brazil were enrolled in the study. The inclusion criteria included the presence of ventricular or atrial defects that required surgical correction with CPB, aged over one year, the presence of hyperglycemia and a decrease in insulin levels after surgery. The exclusion criteria included the presence of an infection one month before surgery, pre-existing endocrine diseases, abnormal glucose and/or insulin values before the study, the need for insulin administration during the hospital stay or for corticosteroids up to 30 days before surgery, renal failure, pulmonary hypertension or congestion, the need for diuretics or oxygen therapy, overt cardiac failure, an inability to provide blood samples for all of the laboratory tests during the five study time points, and the refusal of parents or legal guardians to participate.

Anesthetic procedures, surgical procedures, methylprednisolone administration and postoperative care

On the day of surgery, the children received midazolam ($0.5\ mg.kg^{-1}$) as premedication 30 minutes before surgery. Anesthesia was induced and maintained with 2.0% sevoflurane inhalation and a target-controlled infusion system of remifentanil ($0.20\ \mu g.kg^{-1}.min^{-1}$). Then, an esophageal thermometer, an indwelling bladder catheter, a 22G-24G arterial catheter (right radial artery) and a central venous line (right

internal jugular vein) were inserted. Arterial and central venous blood pressure, core temperature, heart rate, oxygen saturation and remifentanil dosing were continuously recorded. Intraoperative mechanical ventilation was set using pressure control ventilation with airway pressure (15-10 cmH_2O) to achieve a tidal volume of 8-10 $mL.kg^{-1}$, a PEEP of 5 cmH_2O, an inspiratory oxygen fraction of 0.6 and an inspiratory time of 33%. The respiratory rate was adjusted to maintain end-tidal CO_2 at 35 cmH_2O.

All of the patients received 30 $mg.kg^{-1}$ of MP and 30 $mg.kg^{-1}$ of cefuroxime immediately after anesthesia was induced.

After sternotomy and systemic intravenous anticoagulation with 400 U/kg of heparin, large bore aortic, superior and inferior vena cava cannulas were inserted. Non-pulsatile CPB was performed using a hollow fiber membrane oxygenator (Terumo CapioxC RX05 Cardiovascular Systems, Ann Arbor, MI, USA) with uncoated polyvinyl chloride bypass tubing and a non-occlusive roller pump. Hypothermia (28-32℃) was induced in all of the patients. The CPB circuit was primed with normal saline, mannitol, sodium bicarbonate, calcium chloride, heparin and red blood cells to achieve a hematocrit of 20%. The circuit volumes were 450 mL for the patients <10 kg, 800 mL for the patients 10-15 kg and 1.0-1.2 L for the patients >15 kg. The pump flow rates were 200 mL/kg for the children <5 kg, 150 mL/kg for the children between 5 and 9 kg, 125 mL/kg for the children between 10 and 17 kg, and 100 $mL.Kg^{-1}$ for the children >17 kg. After initiating CPB, hypothermia (28-32℃) was induced in all of the patients. An initial dose of 30 $mL.kg^{-1}$ was used to induce cardioplegia, followed by 10 $mL.kg^{-1}$ every 10-20 minutes. At the end of CPB, the vascular cannulas were removed and anticoagulation was reverted with protamine sulfate. Vasoactive drugs were infused at the discretion of the attending anesthesiologist. The same surgical team performed all of the procedures.

After surgery, the patients were transferred to the Pediatric Intensive Care Unit (PICU). The patients were weaned from the vasoactive drugs within the first 12 h after surgery. Fluids were restricted to 30 mL/kg/d during the first 24 h and increased to 60 mL/kg/d while the glucose infusion rate was maintained at 2 $mg.kg^{-1}.min.^{-1}$.

Blood sampling and laboratory tests

An initial blood sample (basal time, BT) was collected from a peripheral vein to determine the pre-surgical glycemia, insulin, C-peptide, IL-6, CRP, and ADM values. Intraoperative and postoperative blood samples were taken using a catheter after CPB (ACPB) and on the first (POD1), second (POD2), and third postoperative days (POD3) to analyze the parameters. The dead space and the extension of the indwelling venous catheter were cleared before blood collection.

Plasma glucose (glycemia) was analyzed using the hexokinase enzymatic method. Normal glucose levels ranged from 60-125 $mg.dL^{-1}$ (5).

Serum insulin levels were determined using a luminescence enzyme immunoassay (Auto DELFIA automatic immunoassay system, Perkin Elmer Life and Analytical Sciences, Shelton, CT, USA). The reference interval was 2-16 $\mu U.L^{-1}$, and the detection limit was 3 $\mu U.L^{-1}$.

Serum C-peptide levels were determined using a luminescent immunoassay (Immulite; Diagnostic Products, Los Angeles, California, USA), and the normal range varied from 1.1-4.4 $ng.mL^{-1}$.

Serum CRP concentrations were determined using immunonephelometry (nephelometer-2, Dade-Behring, La Défense, France); the reference values were ≤5.0 $mg.L^{-1}$.

Serum IL-6 levels were measured using a highly sensitive EIA kit (Human Interleukin-6 Quantikine HS ELISA, R&D Systems, Minneapolis, USA). The detection limit was 0.7 $pg.mL^{-1}$.

Serum ADM concentrations were measured using the EIA Kit from Phoenix Pharmaceuticals (Inc., Belmont, CA). The detection range was 0.01-100 $ng.mL^{-1}$, and the reference interval was <0.5 $ng.mL^{-1}$.

Statistical analysis

The normal distribution of data was studied using the Skewness, Kurtosis and Shapiro-Wilk tests (20). For measurements over time, the Friedman analysis of variance test was used to establish the overall group differences. Multiple comparisons were performed using the Wilcoxon test.

The variation in the insulin concentrations was tested for normality. Because the distribution was normal, a multiple regression analysis with the Bonferroni correction was used to assess the independent variables associated with low insulin levels.

To evaluate the influence of glucose, C-peptide, CRP, IL-6, and ADM on insulin levels after cardiac surgery with CPB and MP, the concentration variations were calculated as the difference between the first postoperative day (POD1) levels and the baseline time (BT) levels (i.e., POD1-BT).

All of the variables were initially tested, and a backward stepwise elimination of the variable with the largest p-value was performed at each step to enable the model to be refitted. In each subsequent step, the least significant variable was removed from the model until all of the remaining variables had individual p-values<0.05.

The IL-6 and CRP concentrations both increased; therefore, we selected one inflammation marker (IL-6) to avoid artificial increments of R^2.

The variation in the glucose levels (glycemia) and the patients' ages (in months) were arbitrarily included in the model irrespective of their p-values because of their central roles in pancreatic function and insulin regulation.

The fitness in each step was assessed using the adjusted coefficient of determination (adjusted R^2).

The collinearity and multicollinearity among the independent variables were tested using the variance inflation factor (VIF). The beta coefficients (ß) of the variables were used to express the effect of the different variable sizes in the final model. The coefficients of semi-partial determination (spR^2) were assessed to evaluate the relative importance of each variable with respect to the other variables. The limit of agreement between the observed and predicted insulin variations, which were obtained using the multiple regression equation, was performed according to the method proposed by Bland and Altman (21).

The data were expressed as the mean±standard deviation or the median [25^{th} and 75^{th} percentiles] according to the data distribution. In the boxplots, the bottom and top of the box were the 25^{th} and 75^{th} percentiles, respectively. The line within the box was the median, and the lower and upper whiskers were the 1.5 IQR of the lower quartile and the 1.5 IQR of the upper quartile, respectively (22). The

statistical analyses were performed using the STATA 11.1 statistical package (StataCorp., College Station, Texas, USA).

■ ETHICS

This study was approved by the Institutional Ethics Committee (protocol CAPPesq number 675/05).

■ RESULTS

The study group consisted of 19 children, ten girls and nine boys, after excluding one child because of the absence of hyperglycemia and lower insulin levels after surgery. The study group was a convenience sample that was limited by the number of children who met the inclusion criteria of the study, whose parents or legal guardians agreed to participate and who underwent five successful blood samplings. We recruited children with congenital heart defects of moderate severity who were clinically stable to ensure that the five blood samplings would not damage their health. The homogeneity of the participant heart disease was essential to ensure the internal and external validity of the study and allow for the extrapolation of the data to populations of children with congenital heart defects of moderate severity who were subjected to cardiac surgery with CPB and MP in pediatric intensive care units.

The mean age of the patients was 39 ± 16 months, and the mean weight was 14 ± 3.8 kg. Atrial and ventricular defects were corrected in 10 and 9 children, respectively. The mean CPB duration was 55 ± 14 min., and the mean aortic cross-clamp length was 25 ± 6 min. None of the children needed inotropic support or experienced surgical complications. The patients were all discharged from the pediatric intensive care unit (PICU) within 72 hours.

Many parents and legal guardians did not consent to participate, primarily because of the volume of blood samples that were needed for the five study time points and/or the fear of increased risks to their children. In addition, this study was limited by its budget because most of the exams are not routinely performed in hospital laboratories, and several exams needed to be tested in triplicate to comply with the manufacturers' instructions, particularly the enzyme immunoassays that were used to evaluate IL-6 and ADM.

Figure 1 shows the insulin, glycemia, C-peptide, ADM, IL-6 and CRP levels throughout the study period. Compared with the BT values ($p<0.05$), the insulin levels at the post-CPB time point and at the POD1 time point had decreased by 72% and 74%, respectively. On POD2 and POD3, the insulin levels increased by 47% with respect to the BT values ($p = \text{NS}$). Glycemia increased significantly from the BT time point to the POD1 time point (87 $mg.dL^{-1}$ [68-88] to 172 $mg.dL^{-1}$ [155-207], $p<0.05$) but returned to the BT levels on POD3. The C-peptide levels (Figure 1) did not significantly change. The ADM levels at the BT time point were slightly higher than normal, and 323% increments were observed on POD1 ($p<0.05$); however, the levels returned to BT levels on POD3. There was a significant increase in the IL-6 and CRP levels, which peaked on POD1 and returned to BT levels on POD3.

During the fitting process, several variables were removed from the initial model (step-by-step) in the following order: 1) the variation in the C-peptide values, 2) the aortic cross-clamp duration, and 3) the CPB duration. Table 1 shows the final multiple regression model that was used to predict insulin levels, including patient age expressed in months and the variations in the glycemia, ADM and IL-6 values. Taken together, these parameters explained 64.7% of the insulin reduction on POD1, $F(4, 14) = 9.22$, $p<0.001$. The following four tested variables reached statistical significance and were successfully correlated with insulin variation according to their semipartial R^2: age (0.55, $p<0.001$), glycemia variation (0.17, $p = 0.01$), ADM variation (0.18, $p = 0.009$), and IL-6 variation (0.2, $p = 0.007$).

The diagram displayed on the left side of Figure 2 shows the observed insulin values and the predicted values that were calculated using the multiple regression equation. The following final equation was used to predict insulin variation ($\mu U.L^{-1}$):

$$-16.3 + 0.14 \times \text{age} + 0.03 \times \text{glycemia}\left(\text{mg.dL}^{-1}\right) + 0.29 \times \text{ADM}\left(\text{ng.mL}^{-1}\right) - 0.17 \times \text{IL} - 6\left(\text{pg.mL}^{-1}\right),$$

where -16.3 was a negative constant, patient age was expressed in months and the glycemia, ADM and IL-6 variations were the difference between the POD1 and BT values. The diagram displayed on the right side of Figure 2 shows that the 95% limit of agreement for the observed and predicted insulin values according to the Bland and Altman analysis was within the interval -2.76 to+2.76 $\mu U.L^{-1}$.

■ DISCUSSION

A better understanding of the complex interactions between metabolism and inflammation in critically ill children, such as children with congenital heart defects of moderate severity, will lead to appropriate general and metabolic support for these patients.

In this study, all 19 children had hyperglycemia and low insulin levels caused by SIRS, which was induced by cardiac surgery with CPB and attenuated by MP. The insulin resistance state, which is characterized by decreased peripheral use of glucose in skeletal muscles and the liver (13), is more prone to occur in the context of prolonged hyperglycemia that is caused by multiple mechanisms, including the counter regulation of gluconeogenesis and glycogenolysis and the downregulation of glucose transporters (23).

To our knowledge, this is the first study that has demonstrated variations in insulin concentrations and their association with inflammatory markers and other factors in pediatric cardiac surgery with CPB and MP, which leads to SIRS, hyperglycemia and low insulin levels. This study has several limitations, such as the lack of MP evaluation on the study time points, although it is well-known that MP effects are exerted during the first 12 h after surgery (24). In addition, most of the findings in this study occurred at the ACPB time point and persisted until the POD2 and POD3 time points, according to the parameter. Another limitation was the convenience sample, which was composed of only 19 patients who were evaluated over time (five study time points). However, age, glycemia, and the IL-6 and ADM levels of the patients reached strong statistical significance; therefore, the results indicate that these four parameters in the proposed mathematical model were consistently correlated with insulin variation in this study.

In adult patients with SIRS, hyperglycemia is frequently associated with high insulin and C-peptide levels because of a

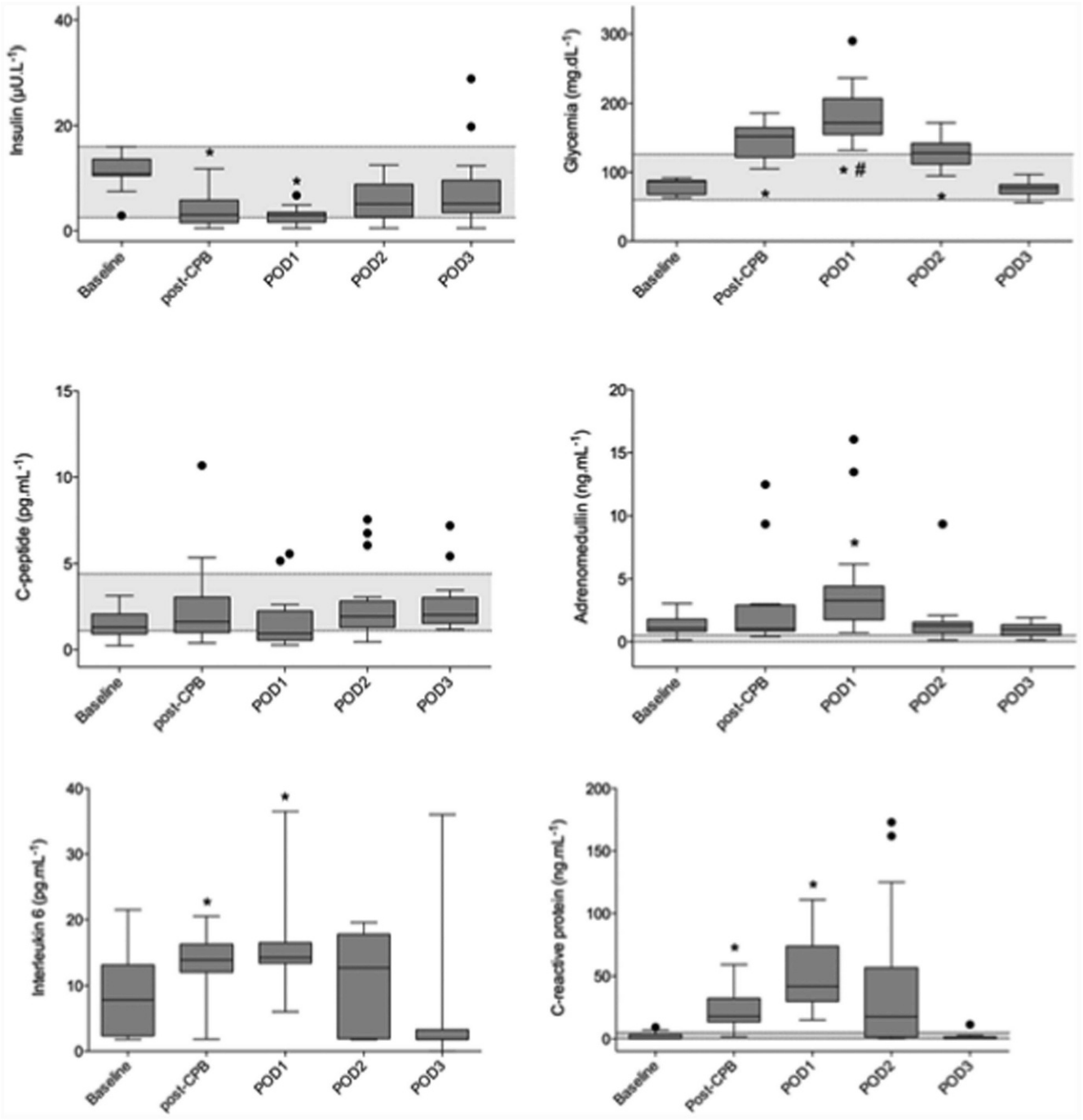

Figure 1 - Insulin, glycemia, C-peptide, ADM, IL-6, and CRP levels throughout the study period. The shaded area in each diagram represents the normal range of the test. *indicates a statistically significant difference between the value at the study time point and the baseline value (Wilcoxon).

peripheral resistance to insulin (2). In contrast, Vignewaran et al. described reduced insulin levels in children after cardiac surgery with CPB, which supported our findings; however, the authors did not monitor the other laboratory parameters (25). Bialkowski et al. reported hyperglycemia accompanied by insulin reduction during and after cardiac surgery with CPB in children; however, the authors did not use MP. In contrast to our findings, the C-peptide levels were reduced in the study along with insulin levels (3), most likely because of more pronounced and/or prolonged hyperglycemia.

As expected in pediatric patients after cardiac surgery with CPB and MP, glycemia levels increased and insulin levels decreased in all 19 patients and reached maximum and minimum levels at the POD1 time point, respectively. The glycemia and insulin levels returned to baseline levels at the POD3 and POD2 time points, respectively. These findings were in agreement with those of other studies (26). During this study, all of the patients had glycemia that exceeded 125 mg.dL^{-1}, which was the threshold adopted in this study; however, these values were higher than 150 mg/

CLINICS 2013;68(1):85-92

Table 1 - Statistical analysis results show the coefficient (Coef), the standard error (Std err.), the *p* value, the 95% confidence interval, the variance inflation factor (VIF), the 1/VIF, the ß coefficient, and the coefficients of the semipartial determination (spR2) of the four independent variables (age, glycemia, ADM and IL-6) that were significantly correlated with insulin variation.

	Coef.	Std. Err.	*p*-value	95% CI			VIF	1/VIF	ß coef.	SP R^2
Age (months)	0.14	0.03	<0.001	0.08	-	0.19	1.23	0.81	0.82	0.55
Glycemia variation ($mg.dL^{-1}$)	0.03	0.01	0.01	0.01	-	0.05	1.18	0.84	0.43	0.17
ADM variation ($ng.mL^{-1}$)	0.29	0.1	0.009	0.09	-	0.5	1.09	0.92	0.43	0.18
IL-6 variation ($pg.mL^{-1}$)	-0.17	0.05	0.007	-0.28	-	-0.05	1.03	0.97	-0.48	0.2
Intercept	-16.34	1.59	<0.001	-19.74	-	-12.94				

dL^{-1}. None of the patients received insulin, most likely because the hyperglycemia levels were not persistently high. Decisions related to patient care were made by the attending physicians at the Heart Institute without interference from the researchers.

In this study, it is likely that the main factor associated with hyperglycemia was a single high dose of MP, which was administered in all of the children; this result supported previous data (5).

The decreased insulin response after cardiac surgery with CPB in these children may be caused by the greater vulnerability to beta cell dysfunction that is observed in critically ill children compared with adults. Therefore, adults who are subjected to cardiac surgery with CPB evolve with hyperglycemia and resistance to insulin, whereas insulin concentrations are reduced in the majority of children. Preissig and Rigby (8) hypothesized that beta cells are extremely sensitive to rapid physiological changes; therefore, these cells may become dysfunctional if these changes occur quickly and exceed a certain threshold. Changes may be induced by multiple factors, such as hypothermia during CPB, vasopressors to maintain blood pressure, elevations of proinflammatory cytokines, including IL-6, and the use of glucocorticoids, such as MP (1,9,12,25). Excluding the use of vasopressors, all of these factors were present in this study. The acute and temporary nature of the physiological disturbance that is induced by SIRS may explain the low insulin levels and the unaltered C-peptide levels. In this context, the C-peptide is a more stable marker of beta cell function than insulin because of its longer half-life, lower first-pass hepatic extraction and lack of adherence to the CPB circuit (4,27). Because of this stability and the temporal nature of the hyperglycemia that was triggered by SIRS and aggravated by MP in this study, the C-peptide levels were not as reduced as the insulin levels; therefore, this marker could not be used to predict insulin behavior. Nevertheless, the monitoring of C-peptide levels was useful and could have led to the medical decision not to administer insulin to patients.

Regarding the CRP and IL-6 levels, there were similar variations in these two parameters, including a significant increment after surgery and a return to baseline levels at the POD3 time point. According to the multivariate analysis, the insulin reduction was directly proportional to patient age and glycemia, whereas the ADM and IL-6 increments followed the magnitude of inflammation (28). To elaborate the mathematical model, we arbitrarily included the IL-6 levels instead of the CRP levels. However, these markers demonstrated similar behavior. Because only CRP levels are routinely evaluated after cardiac surgery in pediatric intensive care units, this parameter could replace the IL-6 levels in the model.

Several studies in adults have found that ADM concentrations increased during CPB (17,29). Komai et al. were the first to describe increased ADM levels in 14 children after cardiac surgery with CPB (29). This trend was later confirmed by Szekely et al., Takeuchi et al. and Sekine et al. (18). These authors found that ADM can inhibit insulin/C-peptide exocytosis via the activation of G proteins that are located on the insulin/C-peptide cellular receptor. We observed a slight elevation in the ADM concentrations at the BT time point; however, the children in our study were not healthy because of

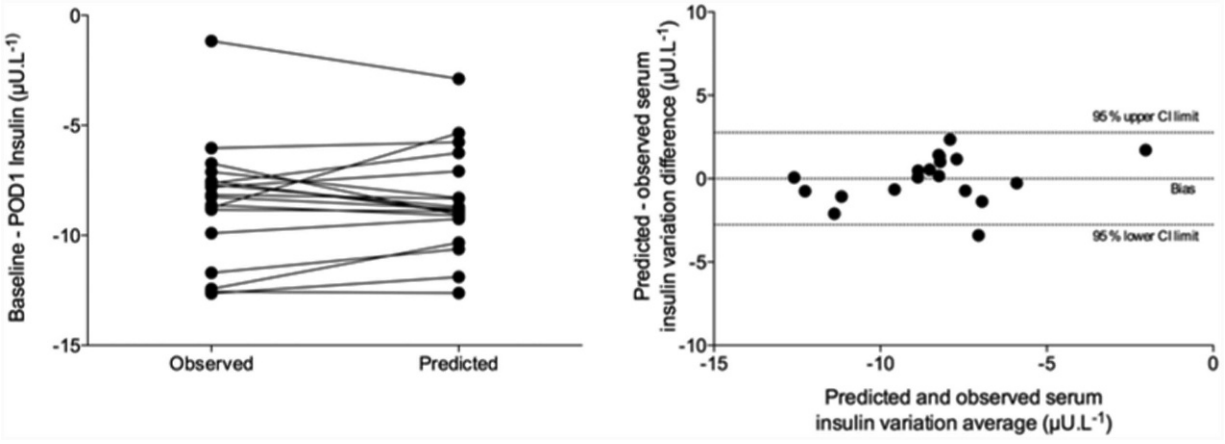

Figure 2 - The diagram on the left shows the observed insulin values and the predicted values that were calculated using the multiple regression equation. The diagram on the right shows that the 95% limit of agreement for the observed and predicted insulin values was within an interval of -2.76 to+2.76 µU.L-1 (Bland and Altman).

previous heart conditions, and this finding was expected. In addition, a strong ADM increment occurred after CPB, which was accompanied by a decrement in insulin levels but not in C-peptide concentrations. The latter is secreted in equimolar amounts with insulin; therefore, ADM may have temporarily affected insulin release but did not inhibit C-peptide exocytosis or the inhibition was not strong enough to reduce the C-peptide concentrations in this study. We expected to observe C-peptide levels below the reference interval.

The results of this study were based on a convenience sample of 19 children; however, the strict application of the study inclusion criteria and the homogeneity of the patient heart conditions enabled us to evaluate the role of all of the laboratory parameters during SIRS. Therefore, the semipartial R^2 analysis indicated that patient age, glycemia, and the ADM and IL-6 levels were independent factors that were associated with insulin variation. These findings were confirmed in the multiple regression analysis with the Bonferroni correction, which demonstrated that these four parameters were responsible for 64.7% of the observed insulin variance. Although significant, this percentage indicates that childhood SIRS that is induced by cardiac surgery with CPB and MP and leads to hyperglycemia and low insulin levels is a complex process that involves more factors than those investigated in this study.

In our patients, the insulin levels decreased, but there were no significant variations in the C-peptide levels during the study. Therefore, the insulin levels were normal in these children, which indicated that hyperglycemia did not persist for a long time period because glycemia returned to BT levels at the POD3 time point, and the insulin concentrations returned to BT levels one day earlier (POD2). Considering the potential hazards of iatrogenic hypoglycemia that is caused by exogenous insulin and the deleterious effects of hyperglycemia in these patients, insulin administration should be considered when a concomitant reduction in the insulin and C-peptide levels occurs in children with sustained hyperglycemia after cardiac surgery with CPB and MP. Therefore, the equation proposed in this study, modified or not by the replacement of IL-6 by CRP, and of ADM for another inflammation or metabolic marker could be useful to predict most of the insulin variation in pediatric intensive care units. In addition, the monitoring of insulin and C-peptide levels in parallel with glycemia is useful in the medical decision not to administer insulin to patients. Therefore, insulin and C-peptide exams are advised for these patients.

ACKNOWLEDGMENTS

This study was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), grant number 2006/59214-6.

AUTHOR CONTRIBUTIONS

Arkader R co-wrote the article and was responsible for collecting all of the biological material during the five study days, conducting the follow-up of patients and collecting the medical records to query the results of the lab tests, which were performed at the INCOR. Malbouisson LM co-wrote the article and assisted in the description of this particular portion of the study and was our statistics expert. Del Negro GM was responsible for supervising the laboratory tests that were not included among the routine laboratory tests in the study, specifically the adrenomedullin and interleukin-6 tests. She oversaw the completion of routine tests, such as blood glucose and hormone, at the clinical laboratory of the Instituto da Criança. Yamamoto L conducted the non-routine laboratory tests (adrenomedullin and interleukin). Okay TS conceived the study, oversaw the writing of the article, and was responsible for obtaining the funding from FAPESP and the doctoral thesis orientation.

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