# Altered Carnitine Homeostasis in Children With Increased Pulmonary Blood Flow Due to Ventricular Septal Defects

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**Objectives:** Congenital heart disease with increased pulmonary blood flow results in progressive pulmonary vascular endothelial dysfunction and associated increased perioperative morbidity. Using our ovine model of congenital heart disease with increased pulmonary blood flow, we have previously demonstrated progressive endothelial dysfunction associated with disruption in carnitine homeostasis, mitochondrial dysfunction, decreased nitric oxide signaling, and enhanced reactive oxygen species generation. However, potential alterations in these parameters in patients with congenital heart disease have not been investigated. The objective of this study was to test the hypothesis that children with increased pulmonary blood flow will have evidence of altered carnitine homeostasis, mitochondrial dysfunction, decreased nitric oxide levels, and increased reactive oxygen species generation.

**Design:** A prospective single-center cohort study.

**Setting:** A tertiary care cardiac ICU/PICU.

**Patients:** Arterial blood samples from 18 patients with congenital heart disease associated with increased pulmonary blood flow (ventricular septal defect), 20 with congenital heart disease without increased pulmonary blood flow (tetralogy of Fallot), and 10 without heart disease (controls) were obtained.

**Interventions:** Plasma levels of total carnitine, free carnitine, acylcarnitine, and lactate-to-pyruvate ratios, an indicator of mitochondrial function, were determined and compared. In addition, levels of superoxide and hydrogen peroxide were determined and compared in patients with ventricular septal defect and controls. Statistical analysis was performed using an unpaired *t* test and analysis of variance.

**Measurements and Main Results:** Baseline acylcarnitine levels ($25.7 \pm 13$ vs $12.7 \pm 8.3$; $p < 0.05$), the acylcarnitine-to-free carnitine ratio ($0.8 \pm 0.1$ vs $0.3 \pm 0.05$; $p < 0.05$), and the lactate-to-pyruvate ratio were higher in ventricular septal defect ($27.5 \pm 3.8$ vs $11.1 \pm 4.1$, $p < 0.05$) than tetralogy of Fallot; there were no differences between tetralogy of Fallot and control. Superoxide and $H_2O_2$ levels were also higher in ventricular septal defect compared with controls, and NOx levels were lower in ventricular septal defect patients compared with tetralogy of Fallot and controls ($p < 0.05$).

**Conclusions:** These data suggest that increased pulmonary blood flow from ventricular septal defect results in altered carnitine and mitochondrial homeostasis, decreased nitric oxide signaling, and increased reactive oxygen species production. These data are consistent with our animal data demonstrating that altered carnitine homeostasis results in mitochondrial dysfunction, increased reactive oxygen species production, and decreased bioavailable nitric oxide. Since disruption of carnitine metabolism may contribute to endothelial dysfunction, carnitine supplementation may attenuate endothelial dysfunction associated with increased pulmonary blood flow and warrants further investigation. (*Pediatr Crit Care Med* 2017; XX:00–00)

**Key Words:** carnitine; congenital heart disease; nitric oxide; pulmonary blood flow; pulmonary hypertension

Pulmonary vascular dysfunction is perhaps the most important complication for children with common congenital heart defects (CHDs) that result in increased pulmonary blood flow (PBF) and pressure, such as large ventricular septal defects (VSDs) and AV septal defects (1, 2). Beginning immediately after birth, the pulmonary vasculature in these infants is subjected to pathologic mechanical forces, which result in early functional abnormalities of

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the vascular endothelium (3, 4). Normally, repairing these defects in infancy or early childhood prevents the development of irreversible pulmonary vascular disease. However, perioperative pulmonary vascular dysfunction, particularly following cardiopulmonary bypass, continues to contribute to the morbidity and mortality of these patients (5, 6). Integrated in vitro and in vivo data from our laboratory and others demonstrate that exposure to these abnormal mechanical forces results in early and progressive abnormalities in pulmonary vascular endothelial structure and function including progressive decreases in bioavailable nitric oxide (NO) and increases in oxidative stress (3, 7–12). Using our clinically relevant lamb model of CHD, we have generated compelling data demonstrating that increased PBF impairs carnitine homeostasis, a vital role in cellular energy production. Our in vivo and in vitro animal data further suggest that this altered carnitine homeostasis leads to mitochondrial dysfunction, increased reactive oxygen species (ROS) production, and decreased bioavailable NO (7–11, 13). However, potential alterations in these parameters in patients with CHD have not been investigated.

Carnitine is present in the body as free carnitine (FC) or as acylcarnitines (esterified form). Adequate carnitine levels and optimal activities of carnitine-dependent enzymes are needed to allow the carnitine system to work. The main function of L-carnitine is the transport of long-chain fatty acids from the cytosol to the mitochondrial matrix for β-oxidation and adenosine triphosphate production. L-carnitine, however, also plays a key regulatory role in intermediary metabolism by modulating the cellular acyl-CoA/CoA ratio. CoA is an obligate cofactor for many enzymes involved in intermediary metabolism. It remains compartmentalized in limited pools within the cell, mainly in the mitochondria, and is normally kept in equilibrium with carnitine. The reversible transfer of acyl groups from CoA to carnitine ensures the vital maintenance of free CoA pools within the mitochondria and prevents the accumulation of poorly metabolized short-chain acyl-CoA compounds which are exported out of the mitochondria as carnitine esters. Therefore, the carnitine system is crucial for normal mitochondrial function, as the accumulation of acyl groups and the unavailability of free CoA result in a metabolic roadblock within the mitochondria with subsequent impaired oxidative metabolism, increased mitochondrial ROS generation, and decreased energy production (14–16).

Thus, we hypothesized that children with increased PBF will have evidence of altered carnitine homeostasis, mitochondrial dysfunction, decreased NO signaling, and increased ROS generation. To this end, we determined and compared plasma levels of carnitine, lactate-to-pyruvate ratios (an indirect determinant of mitochondrial function), bioavailable NO (NOx) levels (a marker of NO generation), and ROS in children with CHD associated with increased PBF (VSD), children with CHD without increased PBF (tetralogy of Fallot [TOF]), and children without heart disease (controls).

## MATERIALS AND METHODS

We conducted a prospective cohort study in the PICUs at the University of California, San Francisco Benioff Children's Hospital, San Francisco. Parents of all eligible patients were approached for informed consent and study enrollment. Eligible subjects included all infants (< 1 yr old) with a large VSD or TOF undergoing elective surgical repair. Echocardiographic criteria used to define that the VSD was large were 1) a nonrestrictive flow pattern across the VSD; 2) a VSD size larger than the aortic annulus; and 3) evidence of left atrial and left ventricular enlargement. Ten patients admitted in the PICU without heart disease served as controls. The University of California, San Francisco Hospital review board approved the study.

Blood samples were obtained from an arterial catheter less than 24 hours preoperatively. The samples were placed immediately on ice in chilled EDTA-treated tubes. Plasma was frozen at –80°C within 15 minutes of collection until analysis.

### Plasma Acylcarnitine and FC Levels

Plasma acylcarnitine and FC levels were determined using high-performance liquid chromatography (13). NOx levels were determined by chemiluminescence (11, 13).

### Lactate and Pyruvate Levels

Lactate and pyruvate levels were determined by spectrophotometric measurements as previously described (13).

### Plasma Superoxide Levels

Plasma superoxide levels were estimated by electron paramagnetic resonance assay using the spin-trap compound 1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine·HCl in the presence and absence of polyethylene glycol-superoxide dismutase, as we have previously described (22).

### Plasma Hydrogen Peroxide Levels

Plasma hydrogen peroxide levels were determined using a modified $H_2$DCFDA oxidation method (17, 18). Five microliters of patient's plasma was incubated with 25 μM $H_2$DCFDA (Calbiochem) for 15 minutes in the dark. The samples were measured with excitation at 485 nm and emission at 530 nm in Fluoroskan Ascent FL (Thermo Electron, Waltham, MA). The values were normalized with protein concentration in their respected plasma samples.

### Statistical Analyses

Statistical analyses were performed using an unpaired *t* test and one-way analysis of variance for differences between more than two groups. Analyses were performed using SAS 9.4 (SAS Institute, Cary, NC). A *p* value of less than 0.05 was considered significant.

## RESULTS

The median age of the patients with VSD (4, 3–7 [mo, interquartile range (IQR)]) and the patients with TOF (3.5, 3–7 [mo, IQR]) were similar. Control patients were older (16.0, 1–7.2 [mo, IQR]) ($p < 0.05$). Eleven of the 48 patients (45.8%)

were male. Diagnoses within the control group admitted to the PICU included pertussis, seizures, other neurologic disorders, pyloric stenosis, and jejunal atresia.

Baseline acylcarnitines levels (25.7 ± 13 vs 12.7 ± 8.3 μM; $p < 0.05$) and the acylcarnitines-to-FC ratio (0.8 ± 0.1 vs 0.3 ± 0.05; $p < 0.05$) were higher in patients with VSD than TOF and controls, suggestive of abnormal carnitine homeostasis in children with VSD (**Figs. 1, *A–B***). The lactate-to-pyruvate ratio was higher in patients with VSD (27.5 ± 3.8 vs 11.1 ± 4.1; $p < 0.05$) than in patients with TOF and controls, suggestive of mitochondrial dysfunction in patients with VSD (**Fig. 1*C***). There were no differences in the lactate-to-pyruvate ratio between patients with TOF and control. NOx levels were decreased in children with VSD compared with children with TOF and controls, suggestive of decreased bioavailable NO in children with VSD (**Figs. 2*A***), and superoxide and $H_2O_2$ levels were increased in patients with VSD compared with controls, suggestive of increased ROS production in VSD patients (**Figure 2, *B–C***) ($p < 0.05$).

## DISCUSSION

Endothelial dysfunction is well documented in pulmonary vascular diseases. For example, Giaid and Saleh (19) and Glaid et al (20) demonstrated decreased NO and increased endothelin-1 gene expression in lungs from patients with advanced pulmonary vascular disease. In addition, human data suggest a role for increased ROS production and oxidative stress in advanced disease (17–20). Since most forms of pulmonary vascular disease present clinically in advanced forms, little is known about the initiating events that lead to its development. Importantly, in CHD, the initiating events, abnormal mechanical forces, and natural history are better understood. This provides a framework in order to elucidate early perturbations in vascular function. In fact, Rabinovitch et al (2) demonstrated structural abnormalities of the vascular endothelium in young children undergoing surgical repair of CHD with pulmonary hypertension, and Celemajer et al (3)identified functional abnormalities of the endothelium in children with CHD and increased PBF prior to the establishment of pulmonary hypertension.

Using our clinically relevant large animal model of CHD with increased PBF, we have previously demonstrated a mechanism for flow/pressure-induced endothelial dysfunction mediated via the down-regulation of peroxisome proliferator-activated receptor γ that results in altered carnitine homeostasis, mitochondrial dysfunction, and a ROS-mediated decrease in bioavailable NO (21, 22). The current study is the first investigation seeking to examine these potential aberrations in humans with CHD. Infants with VSD represented those with increased PBF, infants with TOF represented those with normal or decreased PBF, and infants and children admitted to

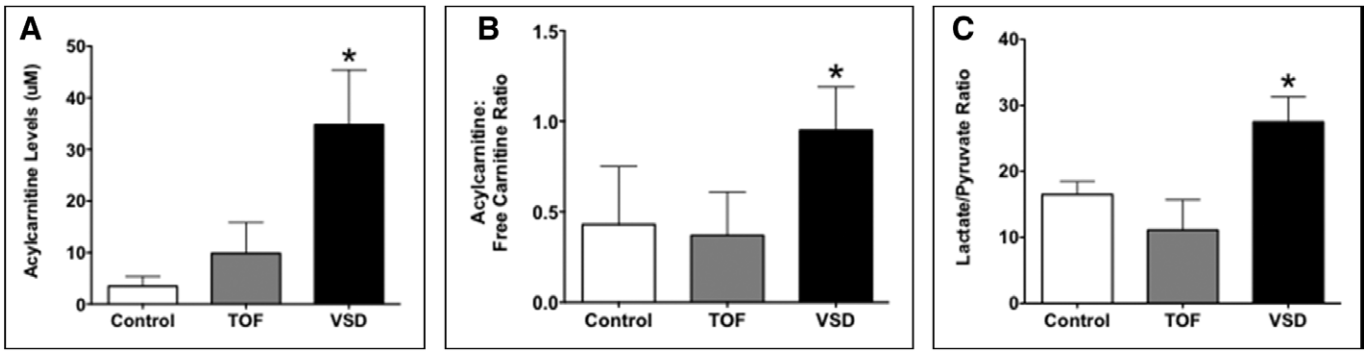


**Figure 1.** Plasma acetylcarnitine levels (**A**), the acetylcarnitine-to-free carnitine ratio (**B**), and the lactate-to-pyruvate ratio (**C**) were higher in ventricular septal defect (VSD) than tetralogy of Fallot (TOF) and controls. There were no differences between TOF and control. *$p < 0.05$ versus control and TOF (analysis of variance). Values are mean ± SD. *n* equals to 18 for VSD; 20 for TOF, and 10 for control.

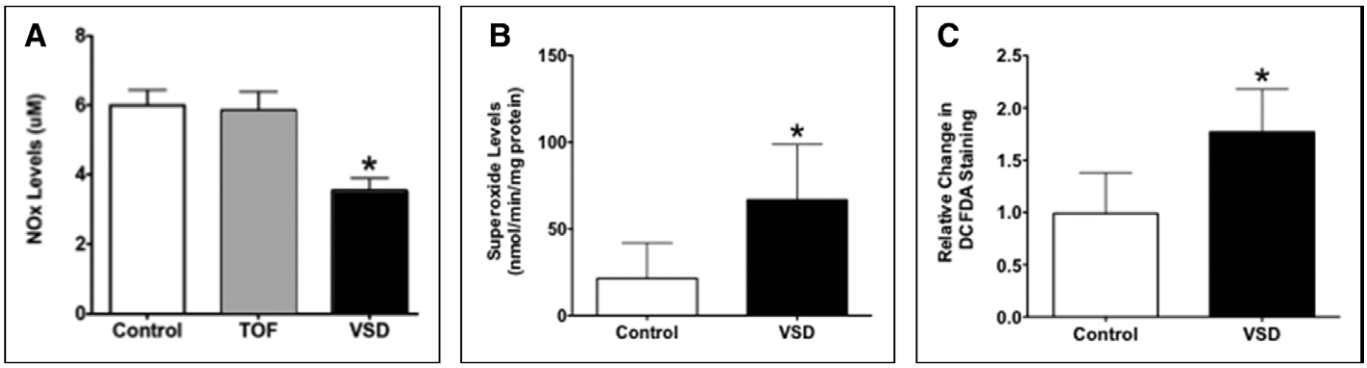


**Figure 2.** Plasma NOx levels were decreased in ventricular septal defect (VSD) patients compared with tetralogy of Fallot (TOF) and controls (**A**), and superoxide (**B**) and $H_2O_2$ (**C**) levels were increased in VSD compared with controls. *$p < 0.05$ versus control (analysis of variance). Values are mean ± SD. *n* equals to 18 for VSD; 20 for TOF, and 10 for control.

the ICU without CHD served as controls. Supporting the animal data, we found that infants with increased PBF had altered carnitine homeostasis, altered lactate-to-pyruvate ratios, increased plasma superoxide and hydrogen peroxide levels, and decreased NO metabolites (NOx) compared with children with TOF or control children. As only children with VSD, and not children with TOF, exhibited these aberrations, our data are highly suggestive that altered biomechanical forces associated with increased PBF are responsible for the disruption of carnitine homeostasis.

A few limitations are noteworthy. Most importantly, all samples were obtained from blood, negating our ability to localize any differences to the pulmonary vasculature and potentially diluting any potential differences. Despite this limitation, all blood samples were consistent with the animal data. Next, our control group was older than both CHD groups. However, there were no differences between the controls, and younger patients with TOF suggesting that age was not a confounding factor. Last, none of these patients underwent direct measurements of PBF via cardiac catheterization; the status of PBF was assumed by the lesion and echocardiographic findings.

In summary, this study demonstrates early alterations in carnitine homeostasis that are associated with altered mitochondrial function, increased ROS, and decreased bioavailable NO. This is consistent with our previous animal investigations that used a clinically relevant ovine model of increased PBF (14, 21, 22). In fact, we also demonstrated in this model that carnitine supplementation normalized the noted aberrations and endothelial dysfunction. Since disruption of carnitine metabolism may contribute to endothelial dysfunction, carnitine supplementation may attenuate endothelial dysfunction associated with increased PBF and warrants further investigation.

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