Randomized, Controlled Trial of Oral Creatine Supplementation (Not Effective) for Apnea of Prematurity

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ABSTRACT. *Background.* Hypoxic ventilatory depression in mice and muscle fatigue in adult humans are improved by creatine supplementation (CS). Because these issues may be operative in apnea of prematurity (AOP), we hypothesized that CS reduces episodes of hypoxemia and bradycardia in infants with AOP.

Methods. Infants were eligible for this double-blind, controlled trial if gestational age was <32 weeks and AOP was severe enough to require treatment with caffeine. If they had ≥1 desaturation (pulse oximeter saturation [SpO_2] ≤ 80%) or bradycardia (heart rate ≤ two thirds of baseline) per hour in an initial 6-hour recording, they were randomized to a 2-week course of oral CS (200 mg/kg per day) or placebo (P). Infants then underwent 2 additional 6-hour recordings of breathing movements, nasal airflow, heart rate, pulse oximeter saturation (SpO_2) and pulse waveforms after 7 and 14 days of treatment. Urinary creatine excretion was measured also. Recordings were analyzed for the frequency of bradycardia and desaturation, the primary outcome parameter, as well as for apnea (≥10 seconds), baseline heart and respiratory rate, and SpO_2.

Results. Of 38 infants enrolled, 34 completed the study (17 in each group). Median (range) gestational age at birth was 27 (25–30) vs 27 (25–30) weeks, and at study 29 (26–36) vs 29 (27–33) weeks. Oral CS was well tolerated; no side effects were noted. Urinary creatine excretion was low in the P group (median: 27 mmol/mol of creatinine; range: 18–102) and increased in the CS group (6949 mmol/mol of creatinine; range: 1427–11807). CS, however, had no effect on the combined rate of bradycardia and desaturation (P: 2.7 per hour [range: 0.2–10.3]; CS: 4.1 per hour [range: 0.6–12.1]), nor was there any decrease in apnea rate (P: 1.7 per hour [range: 0–4.5]; CS: 2.2 per hour [range: 0.2–5.1]).

Conclusion. Despite a significant increase in creatine excretion, suggesting good enteral absorption, CS did not, in the dose and for the duration given in this study, improve symptoms of AOP in these infants. *Pediatrics* 2004;113:e303–e307. URL: http://www.pediatrics.org/cgi/content/full/113/4/e303; *prematurity, apnea, creatine supplementation.*

ABBREVIATIONS. AOP, apnea of prematurity; CPAP, continuous positive airway pressure; PCr, phosphocreatine; CS, creatine supplementation; GA, gestational age; P, Placebo; SpO_2, pulse oximeter saturation.

With decreasing use of mechanical ventilation in extremely low birth weight infants, apnea of prematurity (AOP) has become one of the most common problems in neonatal intensive care. AOP is defined as the recurrence of a variable combination of apnea, bradycardia, and desaturation, with the latter 2 components posing a potential risk to neurodevelopment.[1] Current therapy including methylxanthines, nasal continuous positive airway pressure (CPAP), and doxapram often fails to eradicate symptoms.[2]

The pathophysiology of AOP is incompletely understood. One factor potentially involved is the so-called hypoxic ventilatory depression, the fetal response to hypoxia that persists until ~36 weeks' gestation.[3] The exact pathophysiology mediating this fetal response is unknown, but 1 substance likely involved is creatine, which is a substrate in the regeneration of adenosine diphosphate to adenosine triphosphate via the creatine kinase reaction (phosphocreatine [PCr] + adenosine 5'-diphosphate + H^+ ↔ creatine + adenosine 5'-triphosphate) during nonoxidative phosphorylation.[4,5] Neonatal mice whose dams were fed creatine (2 g/kg per day for 20 days) before delivery showed significant increases in amplitude and duration of hypoglossal bursts and in synaptic drive currents of single respiratory neurons during anoxia[6] and a reduced decrease in minute ventilation during exposure to F_{IO_2} of 0.1.[6] Also, brainstem slices obtained from mice pups of mother animals fed creatine contained twice as much PCr than control slices.[7] The human neonate is regarded creatine-deficient, because there is an increase in the ratio of PCr to nucleoside triphosphates and a threefold increase in creatine kinase activity between 24 weeks postconception and 2 to 3 months postterm.[8]

Another factor potentially involved in AOP is muscle fatigue, as evident from diaphragmatic electromyography recordings during apnea.[9] Creatine is important for the transport of high-energy phosphates in the muscle from the site of production (mitochondria) to the site of use (myofibers).[4] Depletion of muscle creatine stores is one of the classical concomitant factors of muscle fatigue.[4,5] Creatine supplementation (CS) is reported to increase muscle creatine content by >20% and can accelerate the rate

of muscle PCr resynthesis during recovery from exercise.[10,11]

Based on these considerations, we hypothesized that oral CS may improve symptoms of AOP in human infants.

PATIENTS AND METHODS

A prospective, double-blind, controlled study was conducted in spontaneously breathing preterm infants admitted to a level III neonatal intensive care unit with a gestational age (GA) <32 weeks, a postconceptional age <36 weeks, and symptoms of AOP severe enough to require treatment with caffeine ($\geq$1 bradycardia and/or desaturation per hour or >1 apnea requiring bagging over 6 consecutive hours). Exclusion criteria were severe congenital malformations, chromosomal abnormalities, presence of potential causes of apnea other than prematurity (eg, sepsis or intracranial hemorrhage higher than grade II), and refusal of parental consent. Infants were randomized by the hospital pharmacist to a 2-week course of oral creatine or placebo (P) according to a computer-generated randomization list if they had $\geq$1 desaturation or bradycardia per hour in an initial 6-hour recording (see below). Crossover was not allowed. The study protocol was approved by the ethics committee of Hannover Medical School.

The creatine-supplemented group received creatine monohydrate (200 mg/kg per day), and the P group received an equal amount of glucose. Creatine or P, provided in small capsules produced by our pharmacy, were dissolved in the infants' routine diet (fortified breast milk or preterm formula) in 8 to 12 equal doses per day according to the infants' feeding regime.

Each infant underwent three 6-hour recordings of breathing movements (thoracic impedance), nasal airflow (thermistor), instantaneous heart rate, pulse oximeter saturation (SpO_2), and pulse waveforms (Tyco Health Care, Pleasanton, CA) immediately before and after 7 and 14 days of treatment. Signals were printed onto graph paper (Edentrace plus, Tyco Health Care) at 2 mm/s. Infants were studied in their incubators at thermoneutrality and in a 15° head-tilt prone position. Infants were weighed every other day.

Recordings were analyzed by 2 of the authors (T.G. and B.B.) blinded to both treatment allocation and timing of recording. The duration of artifact-free SpO_2 signal, identified from analysis of the pulse waveform signal, was measured. Periods in which this signal was disturbed by motion were excluded from further analysis. Periods of regular and nonregular breathing patterns were identified, and their duration was measured. Regular breathing is a reproducible pattern during which the breathing movement signal is steady in rate and amplitude. It is closely related but not identical to quiet sleep.[12] Baseline values for SpO_2 and heart rate were calculated as the mean of the respective values, measured over 5 successive breaths, at the center of each episode of regular breathing pattern, at least 10 seconds away from sighs or apneic pauses. Respiratory rate was calculated as the mean of values counted over 1 minute at the center of each episode of regular breathing pattern.

In both breathing patterns, the number of desaturations, defined as a fall in SpO_2 to $\leq$80%, was counted, and the duration of each episode and the lowest value reached were recorded. The number of bradycardias, defined as a fall in instantaneous heart rate by more than one third of an infant's baseline heart rate and lasting for at least 4 seconds, and the lowest heart rate reached during each episode were recorded also. Apneas were defined as pauses in nasal airflow and/or breathing movements, measured from the end of the last inspiration before the pause to the onset of the first inspiration after the pause, both identified from the thermistor signal, for $\geq$10 seconds. Periodic breathing was defined as the occurrence of $\geq$3 apneic pauses of $\geq$4 seconds, each separated by <20 breaths and expressed as a percentage of total artifact-free recording time.[12] Apneas $\geq$10 seconds occurring during periodic breathing were handled as periodic breathing.

To account for the influence of additional apnea treatment during the study, a score was designed, with 2 points given for treatment with caffeine, 3 points for nasal CPAP, and 1 point for each 0.5 mg/kg per hour increment in intravenous doxapram. Thus, the maximum score given to an infant treated with caffeine, nasal CPAP, and 2.5 mg/kg per hour of doxapram was 10.

Before and after 7 and 14 days of treatment, a urinary sample was collected from each infant and stored at -20°C. Urinary creatine excretion was measured by gas chromatographic mass spectroscopy (INCOS XL, Finnegan, Bremen, Germany).

The primary study hypothesis was that CS would result in a significant reduction in the frequency of bradycardia and hypoxemia. We estimated that a sample size of 34 infants, 17 in each arm, would give a power of >90% to detect (or exclude) a change in the magnitude of 1 standard deviation in the combined rate of bradycardia and desaturation ([bradycardia + hypoxemia]/hour) at the 5% significance level. Secondary outcome parameters were the frequency of apnea, bradycardia and desaturation, baseline oxygenation, heart and respiratory rate, as well as the apnea therapy score (see above). Statistical analysis was performed by using the Student's t and the Wilcoxon matched-pairs tests. A P value < .05 was considered significant.

RESULTS

Of 150 infants who were eligible during the enrollment period (January 1, 2000 through August 31, 2002), 38 were randomized and 34 completed the study; 17 in the CS and 17 in the P group (Fig 1). Reasons for excluding infants after enrollment were reintubation (1 infant, 3 days after enrollment because of respiratory fatigue), necrotizing enterocolitis (2 infants), and death related to an iatrogenic stomach perforation. Patient characteristics are shown in Table 1.

There was no effect of CS on the primary outcome parameter, nor was there a significant difference in any of the secondary outcome parameters analyzed (Tables 2 and 3). Urinary creatine excretion was low in the P group and increased significantly in the CS group (Table 4).

There was no difference in the proportion of artifact-free signal and regular breathing pattern be-
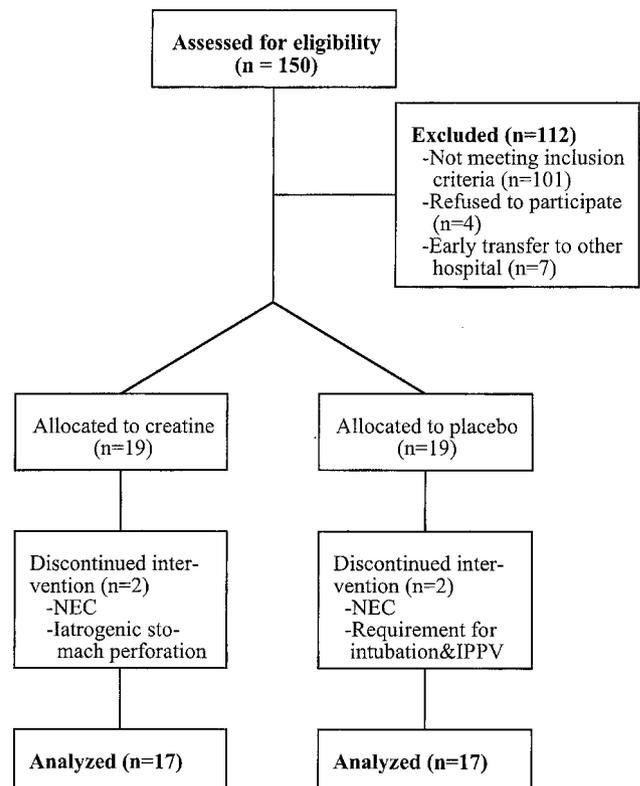

Fig 1. Flow diagram of the randomized creatine trial. NEC indicates necrotizing enterocolitis; IPPV, intermittent positive pressure ventilation.

TABLE 1. Patient Characteristics

	CS Group	P Group	P Value
Patients, n	17	17	
GA, weeks	27 (25–30)	27 (25–30)	NS
Birth weight, g	1040 (580–1400)	1015 (440–1525)	NS
GA at study onset, weeks	29 (26–36)	29 (27–33)	NS
Weight at study onset, g	895 (630–1690)	1015 (450–1740)	NS
Infants ventilated after birth, n	6	7	NS
Infants with oxygen supplementation at study onset	12	14	NS

Data are given as median (and range) where appropriate. NS indicates not significant.

TABLE 2. Data on Episodes of Apnea, Bradycardia, and Desaturation After 14 Days of Treatment

Recording	CS Group		P Group		P Value
	Median	Range	Median	Range	
Bradycardia plus desaturations per hour	4.1	0.6–12.1	2.7	0.2–10.3	NS
Isolated apneas per hour	2.2	0.2–5.1	1.7	0.0–4.5	NS
% periodic breathing	0.4	0.0–13.2	0.7	0.0–7.2	NS
Bradycardias per hour	0.9	0.0–3.0	1.0	0.2–3.5	NS
Mean heart rate nadir per min	70	59–96	70	58–86	NS
Desaturations per hour	2.7	0.2–12.1	1.4	0.0–8.9	NS
Mean Spo_2 nadir, %	70	64–79	72	65–77	NS

NS indicates not significant.

TABLE 3. Baseline Values and Treatment Scores After 14 Days of Treatment With CS or P

Recording	CS Group		P Group		P Value
	Median	Range	Median	Range	
Spo_2, %	97.3	94.3–100	97.0	93.2–100	NS
Heart rate, beats per min	161	135–173	161	142–180	NS
Respiratory rate, per min	71	43–92	71	39–96	NS
Treatment score	2	0–8	2	0–7	NS

NS indicates not significant.

tween groups, either before or after 14 days of treatment (median and range: CS before treatment, 84.5% [55.6–91.4] and 4.2% [0.3–25.7], and after 14 days of treatment, 71.6% [60.6–94.4] and 3.6% [1.1–51.1]; P before treatment, 80.9% [61.7–98.3] and 5.0% [0.8–23.9], and after 14 days of treatment, 79.6% [56.6–91.2] and 7.9% [0.9–31.9]). Data obtained after 7 days of treatment were not different from those obtained before or after 14 days of treatment (data not shown).

Oral drug or P administration was well tolerated; no side effects were noted. Weight gain throughout the study period was similar between groups (median and range: CS, 123 g/kg per week [59–220]; P, 115 g/kg per week [29–255]).

DISCUSSION

Contrary to our hypothesis, oral CS, in the dose and for the duration given in this study, did not improve symptoms of AOP in these infants. Our study was not designed to investigate mechanisms,

TABLE 4. Urinary Creatine Excretion (mmol/mol of Creatinine)

Time Point	CS Group		P Group		P Value
	Median	Range	Median	Range	
Prior to study	30	14–1899	27	18–102	NS
Day 7	5803	198–10438	21	12–125	<.001
Day 14	6949	1427–11807	30	12–145	<.001

NS indicates not significant.

but several hypotheses can be suggested to explain this failure of oral creatine to improve AOP, namely insufficient enteral absorption, dose, duration or onset of treatment, insufficient blood-brain barrier penetration, or incorrect assumptions regarding the role of creatine in the pathophysiology of AOP.

Serum levels were not measured, but urinary excretion increased dramatically in infants receiving creatine, whereas it remained low in the P group. In both groups, creatine excretion before the study was considerably lower than in term neonates (normal range: 40–360 mmol/mol of creatinine [unpublished data]), further supporting our contention that the preterm infant is creatine deficient. In adults, intestinal absorption of creatine is close to 100%.[13] In our patients, oral creatine was clearly absorbed, as demonstrated by the dramatic increase in urinary creatine excretion in the supplemented group. The failure of CS in preventing symptoms of AOP therefore does not seem to have been due to a lack of intestinal absorption.

In contrast, it is conceivable that the duration of supplementation was too short. Treatment of inborn errors of creatine metabolism (arginine:glycine amidinotransferase and S-adenosyl-L-methionine:N-guanidinoacetate methyltransferase deficiency) showed striking improvement after CS,[14,15] but steady-state concentrations for brain creatine content were reached only after several months of treatment.[16]

For ethical reasons, we could only start supple-

menting our patients postnatally and after the onset of AOP (median age: 9 days), where CS in the above-mentioned animal studies[6] started during fetal life. Thus, it is conceivable that the onset of supplementation was too late to prevent AOP in our patients and that an earlier onset of supplementation (eg, prenatally) would have been more effective.

The dose chosen (200 mg/kg per day) roughly corresponds to 20 g/day in an adult, which increased adult muscle creatine content significantly.[10,11] We do not know, however, how well oral creatine enters muscle and brain of preterm infants. The mechanisms by which exogenous creatine enters the muscle are unknown,[4] but creatine uptake, ranging from 0% to 40%,[11] seems inversely related to initial muscle creatine content.[10] The extent to which the blood-brain barrier blocks the entry of orally administered creatine into the brain is also unknown.[16] Dechent et al[17] showed that oral creatine consumption (20 g/day for 4 weeks) increased creatine concentration in intact adult volunteer brain by 3.5% to 13%. Estimating creatine brain content would require magnetic resonance spectroscopy,[18] which was not part of our study protocol, because it would have required endotracheal intubation. Thus, we do not know how well creatine reached muscle and brain in our patients.

In arginine:glycine amidinotransferase and S-adenosyl-L-methionine:N-guanidinoacetate methyltransferase deficiency, neurologic symptoms occur only several months after birth,[14,15] which suggests that these patients may have been fully provided with maternal creatine in utero and that it took some time after birth to become depleted of the maternal creatine pool. Thus, it is conceivable that a lack of PCr in the brain is less relevant to the occurrence of hypoxic ventilatory depression in preterm infants than thought originally. This hypothesis, however, is in contrast to the animal data cited above.[6,7]

There is circumstantial evidence that muscle fatigue plays a role in the pathophysiology of AOP,[19] but the increased work of breathing in preterm infants seems to be more an issue of moderate, long-lasting performance than short-term, high-intensity exercise. Availability of phosphorylated creatine and adenosine triphosphate as an energy substrate is considered to be the limiting factor for maintaining muscle force during high-intensity exercise. During prolonged moderate or submaximal exercise, however, aerobic processes dominate, and muscle glycogen content is decisive.[5] Thus, with regard to muscle fatigue, CS may not be the best choice for preventing AOP.

Was it ethically justified to supplement these infants with creatine? AOP is a disorder with potentially severe sequelae,[20] and currently established treatments received little systematic study before introducing them into clinical practice.[21] There is a large body of experience with CS in adults, particularly athletes, with very little evidence of side effects.[4,11] CS also has been well tolerated in children with inborn errors of creatine metabolism.[16] Finally, there is no animal model for AOP. Thus, we did consider this study necessary to answer a clinically important question, but we admit that our results are somewhat disappointing. We have also started a 2-year follow-up investigation of all infants enrolled in this study to be performed by a child neurologist unaware of study assignment.

Our study may have been underpowered to detect smaller group differences in the frequency of bradycardia and desaturation, but we consider this unlikely given that there was a trend toward more such episodes in the CS group. Also, the treatment score used in this study has not been validated but was based on a stepwise increment in treatment intensity for AOP, as suggested in national guidelines.[22] It was not meant to imply that, for example, CPAP is 50% more effect than caffeine but was used merely to facilitate provision of data for treatment intensity; in an analysis of individual treatments, there was also no difference between groups (data not shown).

CONCLUSIONS

We did not observe a beneficial effect of CS in these infants with AOP despite theoretical benefits derived from animal models and increased muscle performance in humans. Thus, there is currently no evidence to suggest CS for infants with AOP.

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