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Oral Sucrose for Heel Lance Increases ATP Utilization and Oxidative Stress in Preterm Neonates

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Abstract

Objective—To examine the effects of sucrose on pain and biochemical markers of adenosine trisphosphate(ATP) degradation and oxidative stress in preterm neonates experiencing a clinically required heel lance.

Study design—Preterm neonates that met study criteria (n=131) were randomized into three groups: (1) control; (2) heel lance treated with placebo and non-nutritive sucking (NNS); and (3) heel lance treated with sucrose and NNS. Plasma markers of ATP degradation (hypoxanthine, xanthine and uric acid) and oxidative stress (allantoin) were measured before and after the heel lance. Pain was measured using the Premature Infant Pain Profile (PIPP). Data were analyzed using repeated measures ANOVA and Spearman rho.

Results—We found significant increases in plasma hypoxanthine and uric acid over time in neonates who received sucrose. We also found a significant negative correlation between plasma allantoin concentration and PIPP in a subgroup of neonates who received sucrose.

Conclusion—A single dose of oral sucrose, given before heel lance, significantly increased ATP utilization and oxidative stress in premature neonates. Because neonates are given multiple doses of sucrose per day, randomized trials are needed to examine the effect of repeated sucrose administration on ATP degradation, oxidative stress and cell injury.

Premature neonates experience many painful procedures as part of their standard care in the neonatal intensive care unit (NICU) (1, 2). To prevent or treat procedural pain, the use of oral sucrose is recommended by many national and international clinical guidelines based on results from multiple randomized clinical trials that determined sucrose to be effective in reducing signs of pain (3). However, there are no studies to date examining the effects of sucrose, a disaccharide of fructose and glucose, on neonatal cellular ATP metabolism. This is despite the well-documented relationship between fructose metabolism and reductions in ATP synthesis in adult animals (4), in children ages 11 months to 12 years (5) and in healthy

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adults (5, 6). Inhibition of ATP synthesis, as a consequence of sucrose administration, may reduce a premature neonate's already modest ATP stores (7). Additionally, there is evidence that sucrose administration does not attenuate the tachycardia that often accompanies painful procedures (8). In neonatal pigs and newborn lambs, tachycardia significantly increased glucose oxidation and myocardial oxygen requirement (9), which paralleled significant reductions in phosphocreatine/ATP and significant elevations in ADP and inorganic phosphate (10). More importantly, sucrose may not be an effective analgesic as evidenced by its lack of impact on neonatal nociceptive circuits in the brain and spinal cord (11). Together, these considerations imply that oral sucrose administration may alter ATP metabolism and may have adverse cellular effects in neonates with limited energy stores.

The aim of this study is to examine the effects of a single dose of oral sucrose on behavioral/physiological markers of pain and biochemical markers of ATP metabolism and oxidative stress in premature neonates experiencing a clinically required heel lance. Heel lance was chosen because it is a frequent painful procedure in the NICU, as shown in 26 different clinical trials (12). Pain was quantified using the Premature Infant Pain Profile (PIPP) (13). ATP metabolism was quantified by measuring plasma concentrations of purines (hypoxanthine, xanthine and uric acid), which are well-documented markers of ATP utilization and breakdown, and oxidative stress, measured as plasma concentrations of allantoin, a well-accepted in vivo free radical marker (14).

METHODS

We conducted a prospective double-blind randomized controlled study at Loma Linda University Children's Hospital neonatal intensive care unit. Study protocol and informed consent documents were approved by the Loma Linda University Children's Hospital Institutional Review Board. Subjects included in the study were premature infants ≤ 36.5 weeks gestation who: (1) weighed ≥800 grams; (2) had a central catheter in place; and (3) required a heel lance. Exclusion criteria included neonates: (1) with unstable oxygenation and hemodynamic status; (2) receiving opioids or sedatives or any anti-epileptic medications; (3) diagnosed with intraventricular hemorrhage ≥ grade 3 or (4) with facial or multiple congenital anomalies that might alter the pain response. The heel lance was performed for an accurate measurement of blood glucose from neonates receiving glucose-rich total parenteral nutrition through a central catheter. Parents of premature infants who met study criteria were approached for informed consent as soon after birth as possible. With consent, subjects were randomized into one of 3 groups: (1) control; (2) placebo with non-nutritive sucking (NNS or pacifier); or (3) sucrose (Sweet-Ease; Inspiration Healthcare Ltd, UK)) with NNS (Figure 1;available at www.jpeds.com). Randomization was performed by a research pharmacist using a permuted block randomization table generated by the study statistician.

The experimental procedure is described in Figure 2 (available at www.jpeds.com). Investigators collaborated with the clinical staff to sample approximately 0.8 ml of blood from a central catheter before ("0" min) and five minutes after the heel lance to measure purine and allantoin levels. In control neonates who did not receive a study drug or undergo a heel lance, similar samples were collected at "0" and five minutes from baseline. The time period of five minutes after heel lance for blood sample collection was based on previous investigations, which showed plasma levels of purines and organic hydroperoxides significantly increasing five minutes after conditions such as incomplete ischemia (15). These data were validated by unpublished preliminary studies in our laboratory, where we found increases in plasma purines compared with baseline five minutes after heel lance, and purine values that were less than baseline, 20–30 minutes after heel lance. Blood samples

were centrifuged within five minutes to separate the plasma which was then stored at −80°C. All samples were analyzed within one week of acquisition.

Heel Lance Procedure and Administration of Study Drug

The study drug was prepared immediately before the experimental procedure by the research pharmacist and labeled as “study drug” to ensure blinding. The dose of sucrose was based on previously published studies in premature infants (16–19). Neonates randomized to the sucrose group received a single dose of 24% sucrose in the following volumes: 2 ml for neonates >2 kg, 1.5 ml for neonates 1.5 – 2 kg and 0.5 ml for neonates that were <1.5 kg. The study drug was administered slowly via syringe to the anterior tongue along with a pacifier (NNS) two minutes before the heel lance. Multiple studies showed that sucrose was most effective when given approximately two minutes before heel lance (8, 20–25). Neonates randomized to the placebo group received an equal volume of sterile water to the anterior portion of the tongue along with a pacifier. The neonate’s face was videotaped by trained research staff to record facial action at “0” minutes, during the heel lance and up to 30 seconds post heel lance.

Pain Assessment

To assess pain, we used the Premature Infant Pain Profile (PIPP), an instrument designed to assess acute pain in preterm neonates(13). This scoring system includes seven items, each graded from 0 to 3. Two items describe baseline characteristics of the neonate (gestational age and behavioral state), two items are derived from physiologic measurements (heart rate and oxygen saturation), and three items describe facial actions (brow bulge, eye squeeze, and nasolabial furrow). Baseline pain was scored before the heel lance (0 minute) over a 30-second window. Procedural pain was scored from the time of heel lance to 30 seconds post the lance. Facial actions were recorded with a digital camera with real-time counter that allowed for intensive slow motion stop frame, videocoding and playback. Previous work on validation of the PIPP score showed an ability to differentiate painful from non-painful or baseline events (13, 26).

Measurement of purines

Purine metabolites were measured as previously published by our laboratory (27). Specifically, plasma was removed, transferred to separate Eppendorf tubes, and immediately centrifuged in Eppendorf 5702R (Pittsburgh, PA) centrifuge, for 30 minutes at 18000 × *g*. The supernatant was transferred to Microcon centrifugal filter devices (Millipore Corp.; Bedford, MA), 200 μl per device, and spun for 90 min at 14000 × *g*, 4°C. Filtrate was removed, and 150 μl was transferred to an Eppendorf tube containing 1×10^{-7} mol of 2-aminopurine (internal standard). HPLC (Waters 996 PDA, 715 Ultra Wisp Sample Processor; Millipore Corp.) analysis was done in the same day, or the tubes were frozen at −80°C until analysis. Previous HPLC analysis of plasma demonstrated that purines remained stable with freezing.

Three 45 μl injections were used for each sample onto a Supelcosil LC-18-S 15 cm × 4.6 mm, 5 μm column (SGE; Austin, TX), with the following isocratic conditions: 50 mM ammonium formate buffer, pH 5.5, flow rate 1.0 ml/min. Hypoxanthine, xanthine and uric acid were quantitated by obtaining peak areas at appropriate retention and wavelengths (28). Once the peak area of 2-aminopurine at approximately 10.8 minutes and 305 nm was determined, the area ratios of hypoxanthine, xanthine, and uric acid to 2-aminopurine were determined and converted to micromolar concentrations using standard curves. Samples were analyzed in triplicates and values with a coefficient of variation of less than 10% were included in the final analyses. The limits of detection for the purines are as follows: 1.58 μM hypoxanthine, 1.32 μM xanthine and 5.0 μM uric acid.

Measurement of allantoin

Allantoin was measured in plasma using an adaptation of the method developed by Gruber et al (29, 30). Plasma (50 μl) was transferred to an Eppendorf tube containing 5×10^{-10} mol internal standard (50 μl 10 μM [^{15}N]-labeled allantoin). Spiked samples were simultaneously deproteinized and extracted by the addition of 100 μl acetonitrile. These samples were then vortexed and centrifuged at 20000g, 4°C for 5 minutes and the supernatant was dried under N_2. After drying, 50 μl of MTBSTFA in pyridine (1:1 vol/vol) was added and the derivatization reaction was facilitated by incubation at 50°C for 2 h. Analysis was performed on Agilent 6890N Network GC System connected to an Agilent 5973 Inert Mass Selective Detector. Separation was performed using an Agilent 122–5532G capillary column (25.7 m length, 0.25 mm internal diameter). Helium was used as the carrier gas at a flow rate of 1.5 mL/min. Derivatized product (1 μl) was injected in split mode (split ratio 20:1, split flow 29.4 mL/min, total flow 33.8 mL/min). The initial column temperature was set at 100°C, and held at that temperature for 2 min, before being increased to 180°C at a rate of 10°C/min. The column was held at this temperature for 4 min and then increased to 260°C at a rate of 20°C/min. This temperature was maintained until the end of the run. Allantoin was quantified using selected ion monitoring mode with the 398.00 m/z ion being monitored for allantoin and the 400.00 m/z for DL-Allantoin-5-^{12}C; 1-^{15}N. The ion abundance ratios (398.00/400.00) were converted to micromolar concentrations by use of a standard curve.

Statistical Analyses

A repeated measures ANOVA with one between factor (type of intervention) and one within factor (time) was used to compute the minimum sample size needed for this study. The sample size was based on the following assumptions: (1) the significance level was set to 0.05; and (2) required power was 80%. After adjusting for a 10% drop out rate, we enrolled 42–45 participants per group for a total of 131 subjects.

To analyze the data, assumptions of normality and equal variance were assessed. Demographic data for categorical variables were analyzed using Chi-square test. Repeated measures ANOVA for one between subject factor (group) and one within subject factor (time) were assessed to evaluate the effect of the heel lance on plasma purines and allantoin concentrations over time. Interaction terms in the General Linear Model were used for this purpose. The interaction terms assess the differences between the groups over time. Correlations between purines, allantoin and biobehavioral markers (PIPP) were examined using Spearman rho. All statistical analyses were performed using SPSS Statistics for Windows Version 20. Differences were considered significant at $P < 0.05$.

Results

Of the 151 subjects consented between the months of July 2009 to February 2012, 131 subjects were randomized into one of three groups: control (n=42), heel lance and placebo (n=45) or heel lance and 24% sucrose (n=44) (Figure 1). All subjects randomized to the heel lance groups were given a pacifier (non-nutritive sucking, NNS) immediately before, during and after study drug administration. There were no significant differences between the groups (Table I).

Effects of oral sucrose on behavioral and physiological markers of pain

There were no significant differences in baseline pain score between the three groups (Table II). Sucrose significantly attenuated the increase in pain score in response to heel lance, compared with placebo (Table II). The heart rate response to heel lance was highest in the sucrose group ($P < 0.001$). Heart rate increased by 11% in the sucrose group, compared with 6% in the placebo group and 0.5% in the control group. We observed no significant changes

in mean oxygen saturation in response to heel lance in any of the three groups. These data suggest that the lower pain scores in the sucrose group were due to significant reductions in the behavioral components of the Premature Infant Pain Profile scoring tool, and not from physiological markers of pain such as heart rate or oxygen saturation.

Effects of oral sucrose on markers of ATP metabolism (purines) and oxidative stress (allantoin)

There were no significant differences in baseline and five minute purine and allantoin levels in any of the groups. However, although plasma purine and allantoin concentration decreased over time in subjects randomized to the control and placebo groups, we observed a significant increase over time in plasma hypoxanthine and uric acid in neonates who received sucrose before the heel lance (Figure 3, A and B). This effect persisted even when analysis was limited to subjects less than 33 weeks gestation at the time of sampling. Xanthine concentration remained stable over time in each of the three groups. Plasma allantoin concentration increased over time in those who received sucrose; however, this effect was not statistically significant (data not shown).

Effects of oral sucrose on plasma allantoin in neonates with a minimal pain response to heel lance

We found that 63% of neonates who received sucrose demonstrated a minimal response to heel lance, defined as an increase in PIPP score of < 33%. When we examined the effect of sucrose in this subgroup, we found that plasma allantoin concentration increased significantly over time (Figure 3, C). When we examined the correlation between the percent change in PIPP pain score over time and the percent change in allantoin concentration over time, we found a significant negative correlation (Spearman's rho, −0.378, P = 0.014), suggesting that although sucrose significantly decreased the pain scores, it also increased markers of oxidative stress in this subgroup of premature neonates.

Discussion

Although oral sucrose given before a single heel lance significantly decreased behavioral markers of pain, consistent with the findings of numerous clinical investigators(8, 18, 25, 31), it also increased markers of ATP utilization, as evidenced by significant increases over time in plasma hypoxanthine and uric acid concentrations. The relationship between sucrose, ATP utilization/depletion and increased purine production is well documented in adult animal and human literature (6, 36, 37) (Figure 4). Sucrose is a disaccharide of glucose and fructose. It is hydrolyzed by sucrase, an enzyme secreted by epithelial cells of the villi in the small intestine Figure 4, A). Both glucose and fructose are rapidly absorbed from the GI tract through GLUT5 (fructose) and SGLT/GLUT2 (glucose) transporters in the apical membrane and transferred to the portal circulation via GLUT2 transporters in the basolateral membrane of enterocytes (Figure 4, B). The expression of these glucose transporters are upregulated by exposure of intestinal lumen to fructose solutions (38) or by prior exposure to corticosteroids (39). Once in circulation, glucose uptake is insulin-dependent and fructose uptake is independent of insulin (4). Inside the cell, fructose is rapidly phosphorylated into fructose-1-phosphate by the enzyme fructokinase (Figure 4, C). The activity of fructokinase is four-fold higher than glucokinase (the enzyme that phosphorylates glucose). Moreover, fructokinase activity is relatively unregulated, being limited only by fructose concentration (40). Fructose-1-phosphate is split by aldolase (aldolase B) into glyceraldehyde and dihydroxyacetone phosphate, a member of the glycolysis sequence of intermediates. The third enzyme in the fructose pathway is triokinase, which catalyzes the phosphorylation of glyceraldehyde to glyceraldehyde-3-phosphate, another intermediate in the glycolytic pathway.

These fructose-related biochemical reactions are significant because each phosphorylation step requires ATP (37). As ATP is consumed, it is degraded to ADP, leading to an increase in ADP concentration (4). Simultaneously, inorganic phosphate levels decrease because they are sequestered in fructose-1-phosphate or the mitochondria to generate ATP necessary to maintain fructose phosphorylation (Figure 4, D) (4). As inorganic phosphate concentration decreases, oxidative phosphorylation is inhibited, reducing ATP synthesis and rapidly depleting ATP (4, 6, 36, 37). ATP and ADP catabolism results in increased concentration of purines such as uric acid (Figure 4, E) (5, 6, 41). These observations have been documented in adult animals as well as in children ages 11 months to 12 years (5) and in healthy adults (5, 6). We show similar effects in preterm neonates, in which a single dose of sucrose significantly increased plasma hypoxanthine and uric acid concentrations.

We also found that neonates in the sucrose treatment group had the largest increase in heart rate compared with those in the control or placebo groups, providing additional evidence that sucrose does not attenuate the tachycardia that accompanies painful procedures, but may increase it. This increase in heart rate may be due to the stimulatory effect of sucrose on the sympathetic nervous system, as shown in rats (32, 33) and healthy young adults (34). Interestingly, in humans, the sympathetic response to sucrose ingestion was enhanced under conditions of acute moderate hypoxia (35). Together, these data suggest that the apparent analgesic effect of oral sucrose may come at a price, namely tachycardia, which in turn contributes to increased ATP utilization.

An additional finding of this study is the effect of sucrose administration on plasma allantoin concentration in neonates with reduced pain responses (PIPP score increased by < 33%). We found that in this subgroup, plasma allantoin concentration significantly increased over time, compared with neonates with a larger pain response (defined as having an increase in PIPP score of ≥34% compared with baseline) (Figure 3, C). The demographics of this subgroup of neonates were not significantly different from those with a larger pain response. Although newborns with reduced pain responses had significantly lower baseline heart rates (151 ± 11.6 /min vs. 159 ±14.4 /min, P = 0.028), they tended to have a greater percent change in heart rate with the heel lance procedure compared with those with larger pain responses (12 ± 7 % vs., 9 ± 7 %, P = 0.290). These data suggest that changes in heart rate due to sucrose administration may be more predictive of oxidative stress than changes in facial or behavioral activity. Additional studies are required to examine the relationship between sucrose administration, pain reactivity and oxidative stress.

A limitation of this study is that although statistical power of more than 80% was achieved for hypoxanthine and uric acid, it was not achieved for allantoin. A larger sample size may be required to adequately examine the effect of sucrose treatment on allantoin. It is possible that a significant increase in allantoin may not be always evident five minutes after the heel lance procedure.

In this prospective, randomized double blind study, we made the observation that a single dose of oral sucrose, given before a heel lance, significantly increased markers of ATP utilization and oxidative stress in premature neonates over time. This suggests that the apparent analgesic effect of oral sucrose may come at a price, namely increased ATP utilization. Because neonates can be exposed to numerous painful procedures per day requiring multiple doses of sucrose, randomized trials should be performed to examine the effects of repeated sucrose administration not only on markers of ATP breakdown and oxidative stress but also on cellular injury. If it is determined that the metabolic risks of using sucrose in neonates is indeed greater than the known benefits of reducing behavioral indices of pain, additional studies need to be performed to identify alternative effective substances or methods to prevent or treat pain in neonates.

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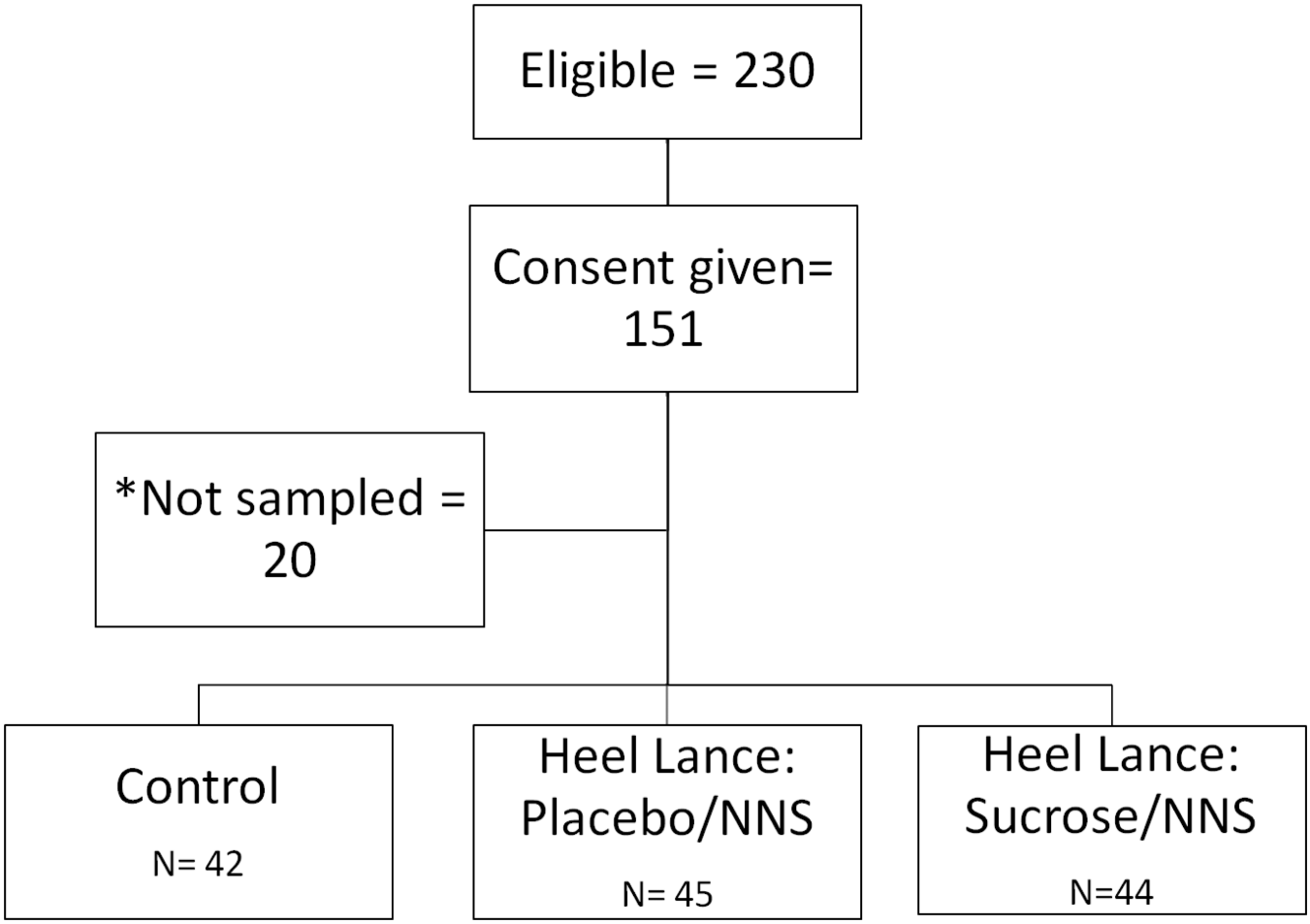

Figure 1.
(online): Enrollment flow chart

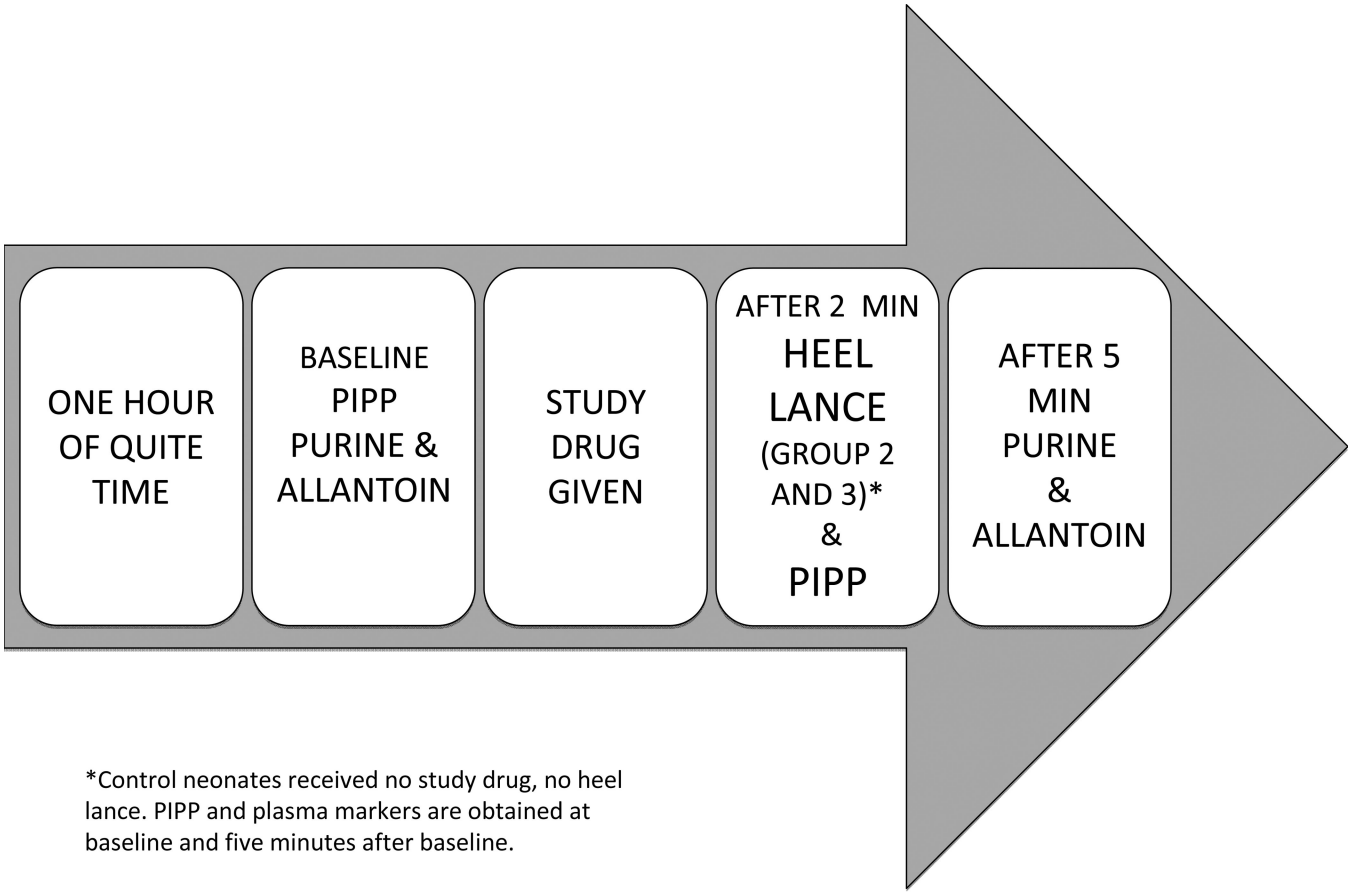

Figure 2.

(online): Study procedure. * Reasons why subjects were not sampled include: Change in acuity or failure to meet study criteria after consent was obtained (n=9); central line will not draw or lipids were being infused preventing sampling (n=5); line was discontinued before sampling could be scheduled (n=6).

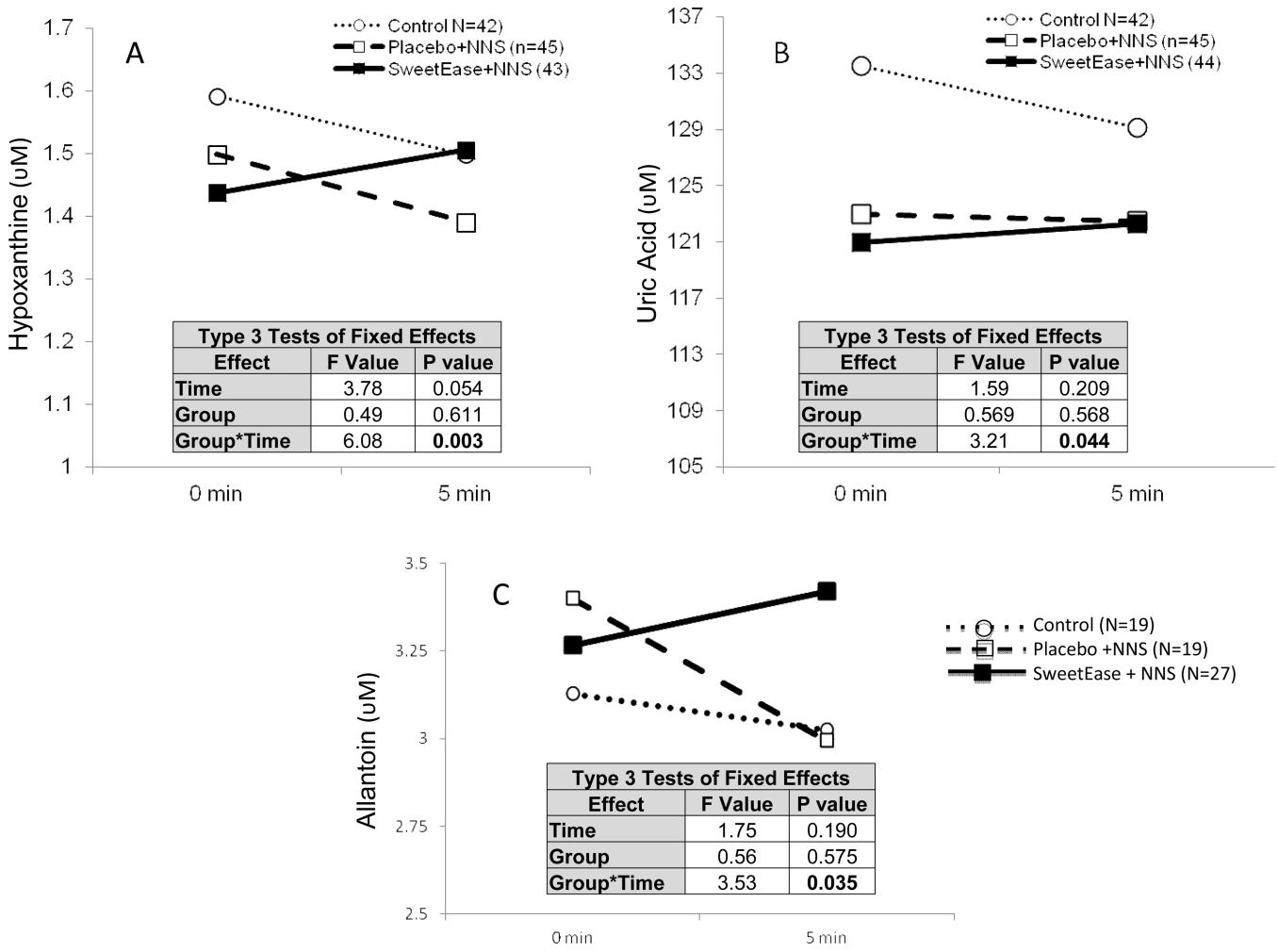

Figure 3.

A and B, Plasma hypoxanthine and uric acid concentration increased over time in preterm neonates who received oral sucrose before a clinically required heel lance. C, In neonates with minimal pain response (<33% increase in PIPP with heel lance), plasma allantoin concentration increased over time.

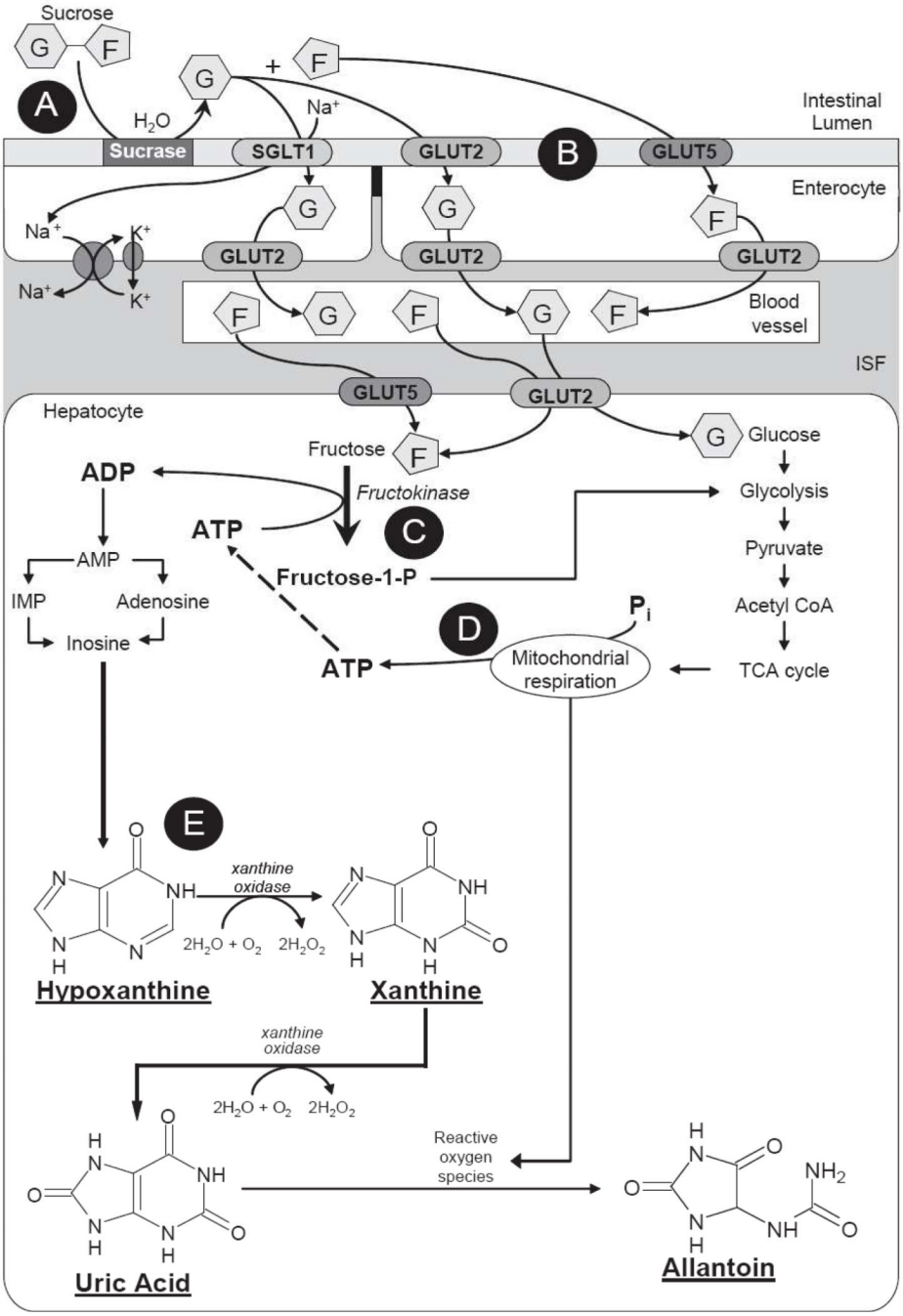

Figure 4.
Sucrose metabolism

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Table 1

Subject Demographics

	Control (n=42)	Heel Lance, Placebo-NNS (n=45)	Heel Lance, *SweetEase*-NNS (n=44)	F value	P value
EGA, weeks	30.5 ± 2.6	30.3 ± 3.2	30.1 ± 3.1	0.218 *	0.804
Birthweight (g)	1456.4 ± 502	1498.4 ± 706	1374.1 ± 552	0.499 *	0.608
Apgar, 1 min	5 ± 3	5 ± 3	6 ± 2	0.961 *	0.385
Apgar, 5 min	7 ± 2	7 ± 2	7 ± 2	1.008 *	0.368
Sex	Male 25 (60%) Female 17 (40%)	Male 22 (49%) Female 23 (51%)	Male 22 (50%) Female 22 (50%)	1.175 **	0.556
Race				4.277 **	0.831
Caucasian	15 (36%)	14 (31%)	19 (43%)		
Hispanic	15 (36%)	21 (47%)	15 (34%)		
African-American	6 (14%)	7 (16%)	6 (14%)		
Asian	2 (4%)	2 (4%)	2 (4.5%)		
Other	4 (10%)	1 (2%)	2 (4.5%)		
Condition at time of sampling					
F_iO_2 (%)	0.25 ± 0.07	0.26 ± 0.09	0.23 ± 0.03	1.783 *	0.172
EGA, weeks	32.5 ± 2.3	32.6 ± 2.6	33.1 ± 2.1	0.759 *	0.470
SNAPPE-II	7.8 ± 10.8	7.9 ± 11.9	8.3 ± 11.6	0.020 *	0.980
Mode of O_2 delivery				6.861 **	0.334
Spontaneous RA +	19	19	24		
Nasal Cannula	6	11	11		
NCPAP ++	6	6	1		

	Control (n=42)	Heel Lance, Placebo-NNS (n=45)	Heel Lance, *SweetEase*-NNS (n=44)	F value	P value
NIPPV[+++]	11	0	8		
Hemoglobin	13.1 ± 2.4	12.7 ± 2.3	12.5 ± 2.2	0.846[*]	0.432
Hematocrit	38.7 ± 6.5	37.6 ± 6.4	36.9 ± 5.7	0.844[*]	0.433

[*] Repeated Measure ANOVA

[**] Chi-Square Test

[+] RA – room air

[++] NCPAP – Nasal continuous positive airway pressure

[+++] NIPPV – Non invasive intermittent positive pressure ventilation

Table 2

Pain Score, Heart Rate and Oxygen Saturation

	Control (n=42)	Heel Lance, Placebo-NNS (n=45)	Heel Lance, *SweetEase*-NNS (n=44)	F value	P value
Pain Score					
Mean (Min-Max)					
Baseline	3.9 (1–8)	3.8 (1–8)	3.8 (1–7)	0.233 *	0.792
Procedural	5.9 (2–15)	6.3 (2–12)	4.6 (2–10)	6.216 *	0.003 +
Heart Rate					
Baseline	154.4 (12.8)	155.9 (14.4)	154.1(13.3)	0.206 *	0.814
Procedural	154.9 (13.9)	164.9 (14.6)	170.5 (14.7)	14.480 *	<0.001 ++
Oxygen Saturation					
Baseline	96.5 (0.5)	96.2 (0.5)	96.2 (0.5)	0.123 *	0.885
Procedural	96.2 (0.6)	95.8 (0.6)	96.4 (0.6)	0.235 *	0.791

* Repeated Measure ANOVA

+ Sweet Ease group significantly lower than control or placebo groups

++ Control group significantly lower than both heel lance groups