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BIOEQUIVALENCE OF THE PRECURSORS ALFA-LINOLENIC ACIDS (LNA) AND EICOSAPENTAENOIC ACID (EPA) IN THE SYNTHESIS OF DOCOSAHEXAENOIC ACID (DHA) USING A MULTI-COMPARTMENTAL MODEL WITH STABLE ISOTOPE METHODOLOGY

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Introduction: Docosahexaenoic acid (22:6n3; DHA), an essential fatty acid (EFA), is a major component of the nervous system and is required for optimal neuronal function. Human infants can synthesize DHA from dietary alpha-linolenic acid (18:3n3;LNA) with eicosapentaenoate (20:5n3; EPA) and docosapentaenoate (22:5n3; DPA) as intermediates. It is of interest then to determine whether LNA or its partially metabolized form, EPA, would be a better substrate to support DHA biosynthesis. Previously we have used isotopic ratios to report a higher DHA formation from LNA(x5) compared to EPA.

Objective: To determine kinetic parameters that reflect the flow of tracer in different compartments using a multi-compartmental physiologic model, as an indirect indicator of the n-3 metabolic pathway fatty acids occurring in the liver.

Subjects and Methods: Term neonates (n=11) were administrated a single oral dose of 20 mg of d5-LNA and 2 mg of 13C-EPA per kg of BW. Blood was then sampled at 0, 4, 8, 24, 48, 96, 168 hr after administration, and, tracers were simultaneously detected in plasma using CG/EM. Physiologic compartmental model, developed in the WInSAAM software, was used to determine the coefficients of the in vivo rate constants of the labeled n-3 fatty acids in plasma. A greater rate constant coefficient for the conversion of d5 DPA to d5 -22:6n3 (0.05 hr-1) than for 13C-DPA to 13C-DHA (0.014 hr-1) was determined from the model calculations on seven infants.

Results: This resulted in an hourly synthetic rate of 47 nmol (or 16 mcg) for the LNA-derived DHA compared to 17 nmol (or 5.9 mcg) for the EPA-derived DHA (P=0.041). However, half-lives were the same for both labeled-22:6n3 species.

Conclusion: Compartmental modeling is a useful tool for calculating biosynthetic rate parameters that are needed for determining n-3 fatty acid substrate utilization for DHA supply.

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EFFECT OF TIMING OF CORD CLAMPING ON POSTNATAL HEMATOCRIT VALUES AND CLINICAL OUTCOME IN TERM INFANTS. A RANDOMIZED, CONTROLLED TRIAL.

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Background: Delayed cord clamping at birth increases iron stores in newborns, which correlates with their iron stores at 6, 9 and 12 months of age. This effect may reduce iron deficiency in infancy. However, there are very few randomized studies about the effects of delaying cord clamping on hematological parameters in the newborn and neonatal outcome Objectives: To determine the effect of umbilical cord clamping timing on venous hematocrit (Hct) and clinical outcomes in term neonates within the first month of life.

Subjects: Term neonates (>37 weeks of gestation) born to mothers with normal pregnancies and uncomplicated vaginal or caesarean births.

Methods: After written informed consent infants were randomized to have the cord clamped either within the first 15 seconds (group 1), or at one minute (group 2), or at three minutes (group 3). An independent observer measured the cord clamping time using a chronometer. When certain unexpected situations occurred (no spontaneous breathing at birth, meconium stained amniotic fluid and nuchal cord) the cord was clamped immediately. The Hct was obtained from the antecubital veins. The analyses were performed in an intention to treat basis. Main outcome measure: venous Hct measured at six hours after birth. Secondary outcome measures: venous hct and bilirubin levels between 24 and 48 h after birth and neonatal outcome.

Results: 273 newborns were recruited (group 1= 90, group 2= 91 and group 3 = 92). The median time of cord clamping was 14" (Q25:7-Q75:15) in group 1; 60" (Q25:60-Q75:61) in group 2 and 180" (Q25:180-Q75:181) in group 3. Venous Hct (mean) at 6 hours were: 53.5 (SD= 6.9) in group 1; 57.0 (SD= 5.8) in group 2 and 59.4 (SD = 6.1) in group 3. The differences between means were not statistically significant. The rate of infants with Hct >65% was similar between group 1 and group 2 (4.4% and 5.9%, respectively) but was significantly higher in group 3 (14.1%) vs. group 1 (RR=3.1, CI 95%: 1.14-9.02, p= 0.04). All polycythemic infants were asymptomatic and none needed partial exchange transfusion. There were not significant differences between the groups in any of the secondary outcome measures.

Conclusions: The cord clamped at least one minute after birth increases Hct levels within a physiological range. Moreover, no harmful effects were observed, thus the practice seems to be safe. Delayed cord clamping should be recommended in term newborns in order to reduce the iron deficiency during infancy.

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FACTORS ASSOCIATED WITH NOSOCOMIAL INFECTION IN PRETERM INFANTS < 1250 GRAMS

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Background: Nosocomial infection is a very frequent event among small preterm infants. Its occurrence increases neonatal morbidity and mortality. A better understanding of the associated variables could contribute to reduce the infection rate.

Objective: To determine factors independently associated with nosocomial sepsis in preterm infants with birth weight < 1250 grams.

Design/methods: Cohort study. We assessed all preterm infants < 1250 grams born in our institution between January 1st, 1993 and December 31st 2003. Infants who died within the first 3 days of life and those with congenital infections or major malformations were not considered for inclusion. A multiple regression analysis was performed considering presence of proven bacterial sepsis (positive blood or CSF culture) as dependent variable, and gestational age, respiratory distress syndrome (RDS), surfactant administration, patent ductus arteriosus, length of mechanical ventilation (MV), parenteral nutrition and indwelling catheters, as independent variables.

Results: During the study period, 191 infants < 1250 g were born in our Hospital. We excluded 21 infants who died within the first 72 hours after birth, one with a complex cardiac anomaly, and five because of missing data, thus 164 infants were included for analysis. Mean birth weight was 939 grams, and mean gestational age, 28 weeks. Overall mortality was 24.2%. The rate of small for gestational age was 29%. Sixty two patients had proven bacterial sepsis (38%). The regression analysis showed the following variables as significatively associated with sepsis: RDS (OR = 3.9 CI 95%: 1.07- 14.6), length of MV (OR = 1.02 CI 95%: 1.00 - 1.03) and days on parenteral nutrition (OR = 1.07 CI 95%: 1.03 - 1.11). Increasing gestational age (OR 0.88 CI95%: 0.73-1.05) and the administration of surfactant (OR = 0.26 CI 95%: 0.07 - 0.92) were associated with a lower incidence of sepsis.

Conclusions: In our cohort of preterm infants < 1250 grams, the risk of nosocomial sepsis was increased by the presence of RDS, days on parenteral nutrition and length of MV. Surfactant administration seems to have a protective effect.

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VLBW TWINS VERSUS SINGLETONS MORBIMORTALITY OUTCOMES

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Objective: To describe and compare clinical outcomes of VLBW twins to those singletons infants admitted in the Neocosur data base network.

Methods: With data collected on line between 10/2000 and 05/2004, biodemographics information were analyzed. We selected complete twin pairs (260 NB) and compared perinatal outcomes until discharge with singletons. Statistics: chi square ($p<0.05$)

Results: Neocosur network (16 South American centers) enrolled 2,875 VLBW infants with BW between 500-1500 g. Multiple pregnancies represented 17.6% (n= 483). Distribution was as follows: twins: 87% (n= 483), Triplets: 12% (n= 59), Quadruplets: 1% (n= 6). Paired twins have more prenatal care and better Apgar at 1 min. They receive more surfactant and need ventilatory support. NEC and ROP incidence was significative in twins. No difference was found in survival rates.

Table 1. Perinatal Characteristics: Singletons versus Twins. Neocosur network (10/00-05/04)

Maternal and newborns characteristics	Singletons n = 2.392	Paired Twins n = 260	p value <
Prenatal care	77.4%	91.4%	0.0001**
Cesarean section	62.8%	68.9%	0.05
Antenatal Steroids	67.6%	73.3%	0.07
GA (weeks)	29.1 s	28.7 s	0.05
BW (g)	1081.7 g	1050.4 g	0.09
Apgar score 1' < 3	16.1%	8.1%	0.001**
Morbimortality			
Malformations	7.2 %	6.7%	0.800
RDS	71.9%	79.3%	0.01
Surfactante use	52.8%	61.7%	0.007 **
Ventilatory support	65.1%	76.6%	0.001 **
CCH G III-IV	10.5%	10.8%	0.877
NEC	10.5%	16.4%	0.005 **
BPD	25.4%	28.9%	0.322
Oxigen supply at 36 weeks	21.0%	23.5%	0.464
ROP	29.2%	43.0%	0.001 **
Mortality	26.9%	26.6%	0.08

Conclusions: Multiple gestation have an important contribution to Neocosur network intensive care units. Twins VLBW compared with singletons show significative major morbidity even perinatal mortality is similar. Some results can be explained for better prenatal control in multiple pregnancy.

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DYNAMIC OF ANTI-TRANSGLUTAMINASE AUTOANTIBODIES IN THE FOLLOW-UP OF CELIAC CHILDREN WITH GLUTEN FREE DIET: COMPARISON OF IGG AND IGA.

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Introduction: During the last few years, autoantibodies against tissue transglutaminase (tTGase) have proven to be the most sensitive and specific humoral markers for celiac disease. Traditionally, IgA subclass immunoglobulins are determined in celiac patients, but IgG antibodies also exist, and at least at the time of diagnosis, perform equally well as IgA, with the additional advantage that IgG are also present in IgA-deficient individuals. Nevertheless, little is known on the outcome in terms of frequency and titres, of both antibodies once gluten is removed from diet.

Aim: To investigate the evolution of IgA and IgG autoantibodies against tTGase in celiac patients on gluten-free diet (GFD)

Methods: Serum samples from 69 celiac children were taken at diagnosis and after 15 days, 1, 2, 4, 6, 12, 18 and 24 months on GFD. The presence of tTGAse autoantibodies was determined with a radioassay using in vitro transcribed-translated human recombinant tTGAse, and immune complexes were precipitated with protein A- or anti-IgA-agarose for IgG and IgA, respectively. Antibody levels were calculated using an 8-point serial two-fold dilution of a positive sample, and are expressed as a standard deviation score (SDS), relative to the mean and SD of 100 non-celiac controls. According to the presence of HLA-DR3, patients were subdivided into homozygous, heterozygous or non-DR3.

Results: The evolution of the prevalence of IgA and IgG class autoantibodies against tTGase is shown on the table. No differences were observed when patients were stratified according to their HLA status.

	DX	15	30	60	120	180	360	540	720
IgA	88	86	77	70	52	48	34	7	1
IgG	85	80	75	70	56	43	22	1.7	4.3

Conclusions: Overall, there are no differences in the performance of IgG and IgA class autoantibodies in the evolution of celiac patients. Antibody positivity is above 30% during the first year after removal of gluten, but decreases to less than 5% during the second year. HLA-DR3 does not seem to influence the frequency of neither IgA nor IgG tTGase autoantibodies.

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INTESTINAL HEM IRON UPTAKE IS AN ENDOCYTIC-MEDIATED PROCESS

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Background: Hem-iron is the main source of iron intake for humans in developed countries, which is best absorbed than inorganic iron (Fe). Iron deficiency is one of the most prevalent nutritional deficiencies in developing countries. One of the strategies to improve Fe nutrition is to increase hem-Fe intake in the population.

Objective: To study in vitro the mechanisms of hem-Fe uptake and transport at the intestinal cells.

Methods: Caco-2 Cells were grown in Iscove′s media, supplemented with 10% SFB, hem-Fe (Hb-Fe55) (0,1–100 uM Fe). We determined in cellular extracts, total intracellular Fe by AAE and heme oxygenase and DMT1(Divalent Metal Transporter 1) by Western Blot. We studied endocytic process, temperature effect and hem-Fe uptake and transport. The radioactivity was counted in the cells and in basolateral media.

Results: Cells exposed to hem-Fe showed lower intracellular Fe than cells exposed to inorganic Fe. Hem-Fe uptake was inhibited by acidification and K+ depletion. The highest uptake and transport were observed in cells incubated with hem-Fe. Heme oxygenase 1 expression increased and DMT1 expression decreased when hem-Fe increased in the incubation media.

Conclusions: Hem-Fe uptake in the intestinal epithelia cells would be by a inorganic Fe independent mechanism by a saturable and endocytic process dependent. Financing: FONDECYT 1030633 Chile.