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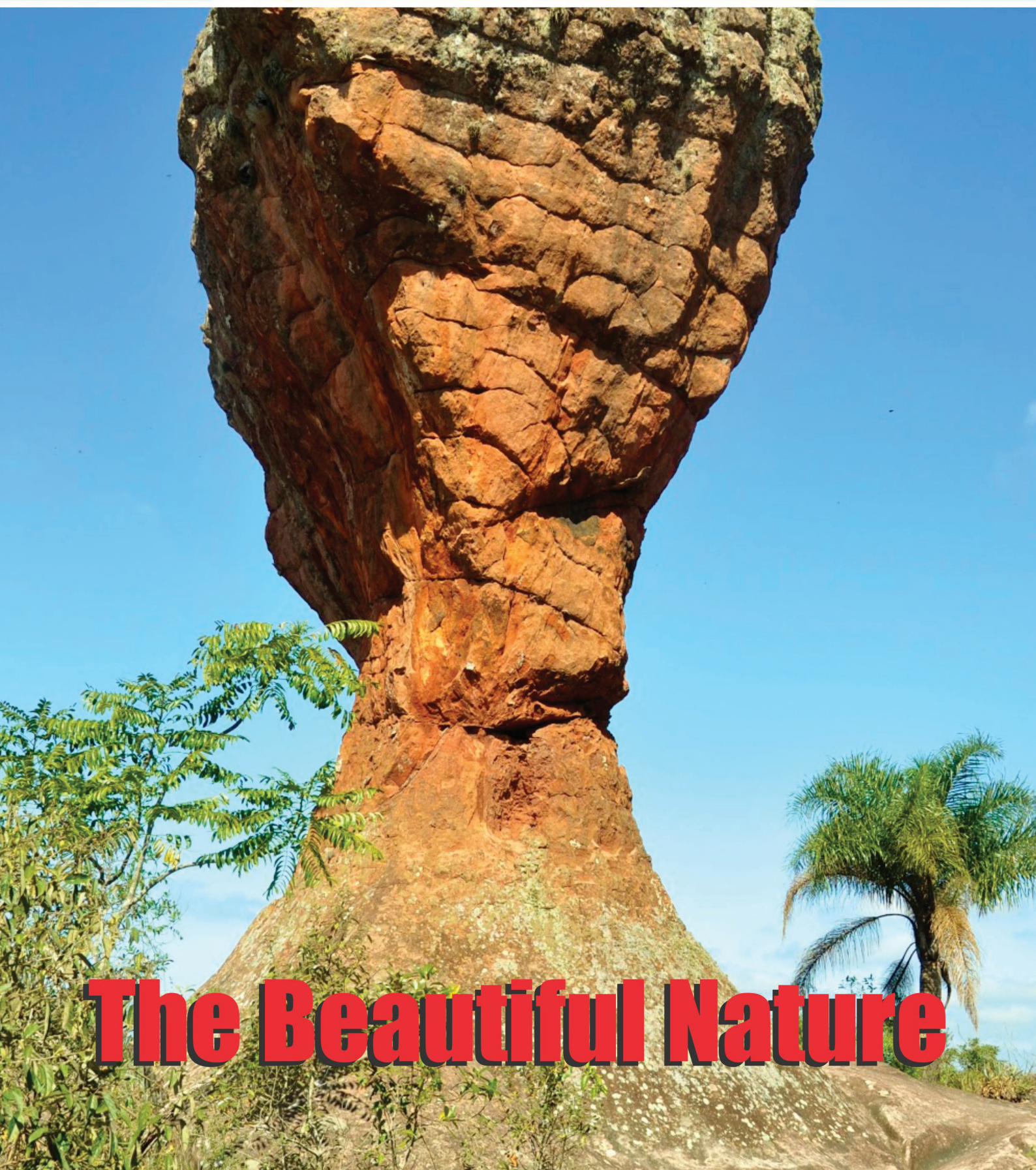

The Beautiful Nature

EDITORIAL

Against Pandemic Cardiometabolic Diseases Let's Celebrating the 1st Meeting of Ibero-American Chapter of DOHaD International Society

Diseases that perturb the metabolism, such as diabetes and hypertension do not stop to increase worldwide, including our country. While, public health police has been done the obesity and overweight and its comorbidities numbers do not decrease. What is wrong? Do governments must to intensify their polices against cardiometabolic diseases? Are the strategies in this combat right? Do target people not cooperating? Are the Economic interests perhaps involved, which neutralize public health efforts? Or there are something else that we cannot see?

Maybe, many factors are involved in those unsuccessful attacks against cardiometabolic diseases. While, in the last two decades it has been enormous the scientific production about diseases that disturb metabolism, causes of obesity are not fully nominated neither their mechanisms.

Fortunately, a new concept how disease or health are developing has been emerged in the last 20 years. Developing origins of health and diseases (DOHaD) concept is based that perinatal phase is sensible to change the control of metabolism imprinting these alterations to later life. Any stressful condition during pregnancy, such bad nutrition, induces low body weight to babies, which is high risk to develop cardiometabolic diseases when they turn to adult life.

While, with certain timidity, few countries start some public health polices based on DOHaD concept to reduce cardiometabolic diseases, is not enough. It is urgent that polices must to be spread all over the world and also intensify it. Although, more studies must be conducted to better understanding mechanisms that are involved in the DOHaD concept. Then, clinicians, epidemiologists and experimental biologists, among others people involved with health problems must be invited to know what means DOHaD.

Last year the readers of ***Endocrinologia & Diabetes Clínica e Experimental*** [Vol 14(1), 2013] were granted with the program and the abstract of 3rd International Symposium of Metabolic Programming and Stress that was held in Morretes/PR – Brazil. Many researchers and PhD students from Brazil and abroad participated, and one recommendation at the end of meeting was organizing a Brazilian Society to DOHaD Studies.

A group of Brazilian researchers participated in the 8th Meeting of DOHaD International Society in Singapore, November 2013. It was a big discussion with colleagues from all Ibero-America to spread the DOHD in our countries. The decision was to create an Ibero-American Chapter of the DOHaD International Society like that the Japanese, Chinese, French, Australian and Newzeland recently created.

In Ponta Grossa/PR – Brazil, from 13th to 16th next November we will be proud to receive the 4th version of the International Symposium on Metabolic Programming and Stress (4ISMPS). The idea to discuss DOHaD concept in Brazil during the 4ISMPS get out of ours frontiers and we will received around 100 researchers from all Ibero-America, also to discuss the DOHaD concept in the 1st Meeting of Ibero-American Chapter of DOHaD society at the same time of 4ISMPS.

It is a privilege to have in Paraná those events and do also to ***Endocrinology & Diabetes Clínical e Experimental*** that will collaborated spreading the program meeting as well as the ideas that will be discussed during the meetings.

We holpe that ***Endocrinology & Diabetes Clínical e Experimental's*** readers will integrated the efforts to bring DOHaD concept close to Brazilian reality and we can see further practical results that can be translated to reducing prevalence of obesity, hypertension and diabetes, among others cardiometabolic diseases, in our country.

Professor Dr. Paulo Mathias Cezar de Freitas Mathias and Dr. Júlio Cezar de Oliveira
Chairs of the 4th International Symposium on Metabolic Programming and Stress and the 1st Meeting of Ibero-American Chapter of DOHaD Society.

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4TH INTERNATIONAL SYMPOSIUM ON METABOLIC PROGRAMMING AND STRESS AND THE 1ST MEETING OF IBERO-AMERICAN CHAPTER OF DOHAD SOCIETY

LECTURES

Designer G Protein-Coupled Receptors as Useful Tools to Identify Novel Targets for the Treatment of Obesity and Type 2 Diabetes

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Background: G protein-coupled receptors (GPCRs) regulate essentially all physiological functions, including body weight, and energy and glucose homeostasis. GPCRs are linked to different functional classes of heterotrimer G proteins, including Gs and Gi- and Gq-type G proteins. Because endogenous GPCRs are generally expressed in multiple tissues, it has been very challenging to study the physiological consequences of activating specific GPCR signaling pathways in distinct cell types in vivo.
Methods: To address this issue, we have used muscarinic receptor-based designer receptors as novel experimental tools. These new designer GPCRs, which are commonly referred to as DREADDs (designer receptors exclusively activated by designer drug), are unable to bind acetylcholine, the endogenous muscarinic receptor agonist, but can be activated by clozapine-N-oxide (CNO), an otherwise pharmacologically inert compound, with high potency and efficacy. During the past few years, we and others have developed DREADDs endowed with distinct G protein coupling properties.
Results: We expressed DREADDs with different G protein coupling selectivities in distinct cell types of the mouse that are critical for the regulation of food intake and maintaining glucose homeostasis. These cells include specific neuronal subpopulations of the hypothalamus (AgRP neurons), pancreatic beta cells, and hepatocytes. We found that selective activation of Gs signaling in AgRP neurons leads to a long-lasting increase in food intake and that stimulation of multiple GPCR-G protein pathways in hepatocytes promotes hepatic glucose output, leading to impaired glucose tolerance.
Conclusions: These studies provide a framework for the development of novel classes of drugs that can modulate specific GPCR signaling pathways for the treatment of obesity and type 2 diabetes.
Key-words: G protein-coupled receptors, transgenic mice, food intake, glucose homeostasis, type 2 diabetes

Protein malnutrition programming of mitochondria function and insulin secretion in malnourished obese mice

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Background: Early life protein malnutrition predisposes obesity development impairing insulin secretion. On the other hand, taurine (TAU) reestablishes insulin secretion in malnourished and obese rodents. Considering that mitochondrial metabolism plays a key role on insulin secretion stimulus/coupling, and TAU effect on previously malnourished obese mice is poorly understood, we investigated the programming of early malnutrition and TAU effects outcomes on mitochondrial control of insulin secretion.
Methods: Male C57BL-6 mice were treated with normo or restricted protein diet (C and R). After that, animals received high fat diet (HFD) (36% fat) (CH and RH). CH and RH also received TAU (5% in the drinking water) throughout the treatment (CHT and RHT).
Results: R showed lower body mass but increased glucose and insulin tolerance. On the other hand, HFD increased body weight and fat content, lowering glucose and insulin tolerance. Insulin secretion, mitochondrial enzymes as well as pancreatic islets transcription factors PGC-1α and TFAM content were higher in CH and RH. TAU reestablished most parameters in CHT, but not in RHT.
Conclusions: TAU reestablishes mitochondrial metabolism and insulin secretion via PGC-1α/TFAM pathway. Protein malnutrition programming blunted this effect.
Key-words: Pyruvate-citrate shuttle, taurine, pancreatic beta-cell, malnutrition, obesity
Financial support: FAPESP and CNPq.

Leptin as imprinting factor to programming obesity and insulin resistance

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Background: Several imprinting factors can increase the probability of obesity during development. There are windows of higher developmental plasticity that are critical for the action of these imprinting factors. Our group has studied several imprinting factors such as, protein malnutrition, litter size reduction (an experimental model of early overfeeding), oil supplementation during lactation and maternal high fat diets, early weaning and maternal nicotine exposure during lactation. Interestingly, in each one of these alterations, leptin serum alterations both in the mothers and pups and in the content in the milk

were observed. It seems that leptin is a hormone that play a central role in the adaptation to future environment conveying the nutritional information aquired during gestation and lactation. Those leptin early changes may alter the neural plasticity, changing the neural pathways of hypothalamic orexigenic and anorexigenic peptides, such NPY, AGRP, MSH and CART. Those precocious alterations seems to regulate peripheral changes predisposing the animal to an obesogenic phenotype. Insulin resistance is developed gradually, first changing insulin action on skeletal muscle and then upon its action on adipose tissue. Those alterations depends on changes in the adrenal system, both in epinephrine production, as well as corticosterone. Finally, important lipid profile changes increases the risk of tissue lipoperoxidation, due to a reduced antioxidant system, both enzymatic and non-enzymatic, leading to an extra-adipose tissue lipid deposition, especially in the liver. The common characteristic of all these models is leptin resistance that can help to explain most of these alterations.

Key-words: Metabolic programming, hypothalamus orexigenic pathways, neural plasticity

Cortisol stress response after nutritional recovery in previously undernourished children

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Undernutrition is a form of stress with long-term consequences. However, the effect of complete nutritional recovery on the stress response in undernourished children has not been investigated. In addition, conflicting results have been described in undernourished children with either an elevated or normal cortisol stress response. The current study aimed to investigate the cortisol response in undernourished children after treatment and compare this response with that of well-nourished and undernourished children. A cross-sectional study was undertaken in children (6-16 years) separated into 4 groups: control (n=41), stunted (n=31), underweight (n=27), and recovered (n=31). Salivary cortisol was collected over the course of 10 hours: upon awakening, before and after an unpleasant stimulus, and in the afternoon before and after a pleasant stimulus. In a two-way analysis of covariance adjusted for age, cortisol upon awakening was highest in the stunted and lowest in the underweight groups (control=5.05 nmol/L(3.71-6.89, 95% Confidence Interval (CI)), stunted=6.62 (3.97-11.02), underweight=2.51 (1.75-3.63) and recovered=3.46 (2.46-4.90); P=0.005). Girls had higher cortisol concentrations upon awakening in relation to boys (P=0.021). Undernourished groups showed an elevated cortisol response to both the unpleasant stimulus and at the last measurement (16:00) compared to the control and recovered groups (Area under the curve in response to an unpleasant stimulus: control=2.07(1.69-2.45), stunted=2.48(1.91-3.06), underweight=2.52(2.07-2.97), recovered=1.68(1.26-2.11); P=0 .042.16:00:control=2.03(1.75-2.39), stunted=2.51(1.97-3.19), underweight=2.61(2.16-3.16),recovered=1.70(1.42-2.03); P=0.009). Lower free T4 was found in the recovered and stunted groups (control=1.28pmol/L(1.18-1.39), stunted=0.98(0.87-1.10), underweight=1.10(1.01-1.21) and recovered=0.90(0.83-0.99); P<0.001). A multivariate two way mixed between-within subjects analysis of covariance showed a lower cortisol concentration in the recovered group throughout the circadian rhythm and during the unpleasant stimulus compared with the other groups (P=0.017 and P=0.023, respectively). A hypocortisolism response was found in the recovered children. The return of these children to a normal stature and weight suggests that this response represents an adaptation. Potential adaptive mechanisms are discussed.

Key-words: Cortisol, stress, undernutrition

Flaxseed supplementation during lactation programs adiposity and hormonal function in adult offspring

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Background: Flaxseed is the seed from the flax plant (Linum usitatissimum L), called linseed, which is a member of the Linaceae family, which presented 32–45% of its mass as oil, of which 51–55% is alpha-linolenic acid (ALA) and 15–18% is linoleic acid. Flaxseed has potential health benefits, such as improvement in lipid profile, glycemia and cardiovascular function. In this study we evaluated the effects of flaxseed supplementation during lactation on endocrine and metabolic factors in the adult offspring.

Methods: Lactating rats were divided into: (1) Controls (C), diet containing 20% casein; (2) Flaxseed (F), diet with additional 25% of flaxseed. The treatment started at birth, day 0 of lactation, and ended at weaning. After weaning, all pups received a standard diet until 180 days old. Only male offspring were studied and were sacrificed at 21 or 180 days old. Body weight (BW) and food intake were monitored. The blood and tissues were collected at 21 and 180 days old.

Results: During lactation, F pups had higher BW and at weaning, they presented lower total and subcutaneous fat mass and higher subcutaneous adipocyte área, lower total cholesterol and triacylglycerol, hypoinsulinemia and hyperleptinemia. At 180 days, F pups had lower glycemia, hypoinsulinemia and hypoadiponectinemia and higher subcutaneous and visceral adipocyte areas, with no change in body fat mass. F pups showed lower T3 levels at weaning, probably caused by lower liver D1 activity and higher TSH levels characterizing a profile of hypothyroidism. At 180 days old, F pups had lower T4 and thyroid D1 and D2 activities and higher BAT D2 activity.

Conclusions: The maternal flaxseed supplementation modifies adiposity, glucose homeostasis and thyroid function at weaning and persisted at adulthood. The present data constitutes a warning against extensive use of flaxseed during lactation.

Key-words: Flaxseed, lactation, programming, glucose homeostasis and rats

Effects of hydroalcoholic extracts from Syzygium cumini leaf on metabolic parameters of polycystic ovaries syndrome rats.

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Background: Polycystic ovaries syndrome (PCOS) affects 5-10% of women and is considered the most frequent endocrinopathy within female population. Along with the reproductive abnormalities, PCOS women have metabolic comorbidities as hyperinsulinemia, dyslipidemia, obesity, insulin resistance and Diabetes Mellitus type 2. In spite of the association between PCOS and metabolic disorders, we hereby investigate the effects of a hydroalcoholic extract (HE) from *Syzygium cumini* leaves on metabolic parameters of rats injected with monosodium L-glutamate (MSG), a new PCOS model characterized by our group.

Methods: Female Wistar rats received MSG (4.0g/kg, S.C., PCOS group) or saline (0.1mL/10.0g, S.C., CTR group) between day 2 and 10 after birth on alternate days. At day 90, PCOS animals received oral saline (PCOS group), Metformin 200mg/kg (PCOS-Met group) or HE 500mg/kg (PCOS-Syz) for 30 days. Glucose tolerance test (GTT) was performed two days before the end of the treatment. At day 120, all animals were anesthetized for collection of: retroperitoneal and visceral fat pads and blood for metabolic biochemistry. Results are expressed as mean±SEM and compared with ANOVA/Newman Keuls for $p<0.05$.

Results: PCOS-Met and PCOS-Syz showed a significant reduction on retroperitoneal fat pads (2.84±0.18 and 2.28±0.28g/100g, respectively) when compared with PCOS (3.85±0.39g/100g). However, there was no difference on visceral fat pads. PCOS-Met and PCOS-Syz also had diminished glycemia (91.73±6.58 and 97.13±3.93mg/dL, respectively) when compared with PCOS (113.8±1.32mg/dL) with no difference to CTR (102.3±2.04mg/dL). On regards of GTT, PCOS had increased area under curve (10277±1100) whereas PCOS-Met and PCOS-Syz presented a significant reduction (6029±606.9 and 6045±711.1), resembling CTR (7289±863.7). Interestingly, PCOS-Met animals had increased triglyceridemia (148.1±17.46mg/dL) compared to PCOS (97.68±17.99mg/dL), whose mechanisms are under investigation.

Conclusion: These results suggest that the HE from *Syzygium cumini* leaves is a promising agent for treatment of metabolic disorders in PCOS, being as effective as Metformin.

Key-words: Polycystic Ovary Syndrome, Obesity, *Syzygium cumini*

How glucocorticoids can be useful for elaboration of experimental models of metabolic programing?

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Background: Glucocorticoids (GCs) are largely known by their antiinflammatory and immunosuppressive actions in the clinic practice. However, when administered in excess, GCs induce several adverse metabolic effects that include glucose intolerance and insulin resistance and, in some cases, diabetes. The precise mechanisms whereby GCs cause such dysregulation on glucose homeostasis (GH) are not fully elucidated, but their negative impact on glucose metabolism turn GCs an interesting insult to challenge the GH.

Methods: The present rat model consisted of male and female adult (or aged) Wistar rats that received daily injection of dexamethasone (1mg/kg, b.w., i.p.) for 5 consecutive days (DEX), whereas control rats received saline. Some DEX groups were concomitantly treated with the leucine metabolite HMB or arjunolic acid (AA) or sitagliptin or metformin to verify if any of these components could be effective in attenuate the GC impact on GH. A group of female rats received dexamethasone before the gestation to investigation of their offsprings.

Results: DEX rats exhibited increased glycemic values, hyperinsulinemia, hyperglucagonemia and hypertriacylglyceridemia ($p<0.05$) in association with glucose intolerance and reduced insulin sensitivity. Insulin and glucagon secretion are both increased in response to glucose stimulus in islets from the DEX rats ($p<0.05$). The beta- and alpha-cell masses are also increased in the DEX rats ($p<0.05$). The GC may impact differently on GH depending on rat gender or age. The most of dexamethasone effects on GH are not attenuated by HMB, AA and any of glucose lowering drugs. Offsprings from those mothers that received GCs before the gestation period become smaller ($p<0.05$).

Conclusions: Altogether, it can be concluded that dexamethasone effectively impairs the GH for the most experimental conditions evaluated, which turn this GC an interesting insult to challenge the GH on experimental models of metabolic programing. These data also reinforce the need for strategies to decrease the GC side effects on GH.

Key-words: Glucose homeostasis, dexamethasone, leucine, metformin

The impaired endothelium-dependent relaxation in placental arteries of intrauterine growth restricted fetuses is regulated by oxidative stress and correlates with epigenetic changes in eNOS in umbilical artery endothelium.

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Background: Intrauterine growth restricted (IUGR) fetuses show placental vascular dysfunction, impaired eNOS-mediated relaxation and increased oxidative stress. By the other hand in IUGR-derived umbilical artery endothelium (HUAEC), eNOS expression is influenced by DNA methylation status. We studied the role of oxidative stress in the vascular reactivity of

IUGR-chorionic arteries (CA) as well as the effect of histone deacetylase (HDAC) and nitric oxide (NO) on the expression of key NOS pathway genes; arginase-2 (ARG2) and eNOS.
Methods: Vascular rings from control and IUGR-CA were mounted on a wire-myograph. Responses to CGRP (10-10–10-6M) in the presence/absence of SOD inhibitor, DDC (10-5M), GPx inhibitor (MS, 10-3M), ONOO-donor (SIN-1, 1.6×10-6M) and antioxidant (NAC, 10-5M) were determined. HUAEC were exposed to HDAC inhibitor (TSA, 0.1-10μM) with/without NOC-18 (NO donor, 100μM) and L-NAME (NOS inhibitor, 100μM). Chromatin accessibility at ARG2 and NOS3 promoters was analyzed and mRNA levels determined by qPCR.
Results: IUGR-CA presented lower CGRP relaxation (29.72±7.21% Kmax) compared with control-CA (47.06±3.96% Kmax). Pre-incubation of IUGR-CA with NAC normalized the CGRP-response (78.05±7.06% Kmax). Pre-incubation with MS and DDC blocked the CGRP-response in IUGR-CA (5.7±8.7% Kmax; 3.8±12.1% Kmax, respectively). SIN-1 induced a concentration-dependent constriction in IUGR-CA (–58.57±13.11% Kmax). In control and IUGR-HUAEC, TSA up-regulated arginase-2 and down-regulated eNOS expression, the later was not NO-dependent. Only in control-HUAEC the induction of ARG2 by TSA was potentiated by NOC-18 (~12 fold) and blocked by L-NAME. NOC-18 induced ARG2 in control- and IUGR-HUAEC (~3 fold), whilst L-NAME reduced its expression only in control cells. Changes in ARG2 expression were paralleled by changes in promoter chromatin accessibility.
Conclusion: Oxidative stress impairs eNOS-dependent relaxation in IUGR-CA and the restitution of glutathione improves the IUGR vascular dysfunction. HDAC activity has a differential effect on eNOS (induction) and arginase-2 (reduction) expression in HUAEC. Apparently NO enhances chromatin accessibility at the arginase-2 promoter, which can be potentiated by HDAC inhibition.
Key-words: Intrauterine growth restriction, oxidative stress, nitric oxide, epigenetics, HUAEC
Financial support: FONDECYT 1120928/1130801; EM and IC hold CONICYT PhD fellowships (Chile).

Strategies to assess causality in observational studies

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***Background*:** It is difficult, if not impossible, to establish causality when the main exposure is not randomly assigned by the researcher, a problem that occurs in observational studies. The aim of the present research is to describe some of the strategies used in the design and analysis of observational studies which help to improve inference in the context of life course epidemiology studies.
Methods: We applied examples of longitudinal cohort studies that investigate intrauterine effects of maternal smoking during pregnancy (main exposure) on two child outcomes: offspring psychological problems and growth.
Results: Different strategies for assessing causality were considered: controlling for a wide set of measured confounding variables; comparing the associations of maternal smoking during pregnancy and offspring outcomes with those of her partner´s smoking during pregnancy. As a way of dealing with unknown and unmeasured confounders (maternal-paternal approach); exploring whether there is a dose-response for intensity of maternal and partner smoking and offspring outcomes associations; searching for specific effects of maternal smoking on offspring outcomes; and cross-cohort comparisons where confounding structures differ systematically across populations.
Conclusions: Each of the strategies had advantages and limitations. The most frequent problems were the absence of key confounding variables and the lack of statistical power to test for differences in the maternal and paternal associations. Research studies, limited by data availability, might integrate as many strategies for assessing causality as possible.
Key-words: Intrauterine effects, causality, smoking, cohort studies

Developmental origins of health and disease: Effects on metabolism and ovarian function

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Background: There is now considerable epidemiological and experimental evidence indicating that early life environmental signals, including nutrition, affect subsequent development and long term metabolic function. These signals induce highly integrated responses in endocrine-related homeostasis, resulting in persistent changes to the developmental trajectory producing an altered adult phenotype. This phenomenon has been termed developmental programming, whereby early life events trigger processes that prepare the individual for particular circumstances that are anticipated in the postnatal environment. However, where the intrauterine and postnatal environments differ markedly, such modifications to the developmental trajectory may prove maladaptive in later life. Evidence that reproductive maturation and function is similarly influenced by early life events is now emerging from both animal studies and human populations. This should not be surprising, since the primordial follicle pool is established early in life and is thus vulnerable to early life events. Clinical and experimental studies have demonstrated that early life adversity is associated with a decline in ovarian follicular reserve, changes in ovulation rates and altered age at onset of menarche – effects that are often accompanied by negative impacts on metabolism. But the underlying mechanisms regulating the relationship are unclear. We have shown that early life deficits accelerate reproductive maturation, and at least in rodent models, results in a loss of ovarian follicles associated with key indicators similar to those observed in accelerated ovarian aging. Our research studies aim to investigate how early life nutritional cues facilitate adaptations in the developing ovary in a manner that alters long-term postnatal function.
Key-words: Developmental programming, ovarian follicles, reproductive maturation

Prenatal growth and the metabolic syndrome in Chilean school-age children

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Background/Aims: We firstly studied the prevalence of obesity, metabolic syndrome (MS) and insulin resistance (IR) in Chilean children (Mardones F et al. Nutr Hosp 2013; 28(6):1587–1593). Secondly we assessed the association of prenatal growth with nutritional status, MS, and IR (Mardones F et al. Biomed Research International 2014. doi:10.1155/2014/472017).

Methods: We conducted a cross-sectional study in 20 public schools of Santiago, Chile. Anthropometry, blood pressure (BP) and pubertal status were assessed. A blood sample was obtained for determination of lipids, blood glucose and insulin. In addition, a retrospective cohort study was designed linking present data of children with perinatal records. 3325 subjects were enrolled. Linear associations were assessed using the Cochrane-Armitage test. Odds Ratios and non-linear associations were computed.

Results: 3325 children had a mean age of 11.4 ± 1 years old (range 10-15 years). The prevalence of obesity, MS and IR was 16.1%, 7.3% and 25.9%, respectively. The prevalence of IR and MS was higher in obese children. MS and IR were strongly associated with an OR of 8.0 (95%, CI = 5.9–10.7). Regarding prenatal growth, 3290 children were analyzed and the strongest positive association was between birth weight (BW) and obesity [OR, 2.97 (95%, CI = 2.01–4.40) at BW ≥ 4,000g compared to BW 2,500–2,999]. The strongest inverse association was between birth length (BL) and stunting [OR, 8.70 (95%, CI = 3.66–20.67) at BL < 48cm compared to BL 52–53cm. A U-shaped association between BL and BP ≥ 90th percentile was observed. Significant ORs were also found for MS and IR. Adjustments for present fat mass increased or maintained most prenatal growth influences.

Conclusions: Prenatal growth influences MS, IR, and nutritional status. Prenatal growth was more important than present body composition in determining these outcomes.

Key-words: children, prenatal growth, insulin resistance, metabolic syndrome, prevalence study, retrospective cohort study.

Developmental programming influences on the life-course trajectory of aging

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***Background*:** Extensive human epidemiological and controlled animal studies indicate that sub-optimal environments during plastic developmental stages result in developmental programming defined as responses to challenges during fetal and early neonatal life affecting growth and differentiation, altering phenotype and increasing chronic disease susceptibility. Few studies link the developmental origins of disease hypothesis to early aging. We hypothesize that developmental programming also alters phenotype in ways predisposing to premature and/or augmented aging. Of the several maternal challenges that lead to offspring programming we focus on maternal under- and over-nutrition and stress hormones which can produce among other outcomes, dysfunctional offspring metabolic, neuroendocrine and reproductive dysfunction. We studied aging process in offspring rats whose pregnant mothers ate control (C), protein restricted (R) or obesogenic (O) diet. After birth, mothers and offspring ate the C diet. Interaction of developmental programming with aging involves basic physiological mechanisms concerning cell signaling systems, oxidative stress, altered adrenal and reproductive steroid production and epigenetic gene modifications. Offspring showed age and maternal nutrition related changes in weight, fat accumulation, metabolism parameters, oxidative stress markers and antioxidant enzymes and fertility. Developmental programming is considered important in predisposing to chronic disease. Our data show that programming by maternal R and O nutrition plays an important role in aging trajectory. Understanding how particular developmental programming outcomes alter the life-course trajectory including aging will enable identification of markers that predict early aging. Early prediction is essential to the development of intervention strategies and therapeutic management.

Key-words: Developmental programming, oxidative stress, aging, maternal nutrition, gene-environment interactions.

The fetal programming of food preferences

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Background/aims: Intrauterine growth restriction results from a failure to achieve a higher growth potential and is associated with many maternal conditions, such as chronic diseases (e.g. infections, hypertension), exposures (tobacco smoke, drugs), and malnutrition. These early adversities induce a series of adaptive physiological responses aimed at improving survival, but imposing increased risk for developing chronic non-transmittable diseases (obesity, type 2 diabetes, cardiovascular disease) in the long term. Our group has been describing evidence that IUGR also leads to the programming of food preferences, exploring the moderators of this association, as well as its putative mechanisms.

Methods: We use data from prospective human cohorts, especially a birth cohort from Canada (Maternal Adversity, Vulnerability and Neurodevelopment, the MAVAN project, in collaboration with McGill University, University of Toronto and McMaster University), in which children were extensively studied in relation to their habitual food intake, laboratory snack test and questionnaires. Another source of information is the PROTAIA project (Universidade Federal do Rio Grande do Sul – UFRGS), which had prospective data on feeding behavior as well as brain imaging in response to food cues. Finally, a rodent model was established to explore brain mechanisms involvement, especially the programming of mesolimbic dopaminergic pathways involved in food reward.

Results: The fetal programming of food preferences is evident in different human studies, as well as in the animal model. Peculiar functioning of specific brain areas seem to be involved, but the association seems to be modifiable by some environmental variations, which opens an opportunity for intervention.
Conclusions: Fetal growth impairment is associated with altered feeding behavior and preferences through the life course. The chronic, persistent alteration in food preferences in individuals exposed to fetal growth restriction possibly contributes to the development of these adult diseases, what could be seen as another facet of the well-known "thrifty phenotype hypothesis" described by Barker and Hales in 1992, the "thrifty behavior".
Key-words: Feeding behavior, IUGR, dopamine

Gestational diabetes and miscarriages

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Background/aims: We aimed to analyze the prevalence of miscarriages among a group of women presenting gestational diabetes.
Methods: This study included 500 pregnant women. Oral glucose tolerance test was determined using a charge of 75g. Normality criteria for glycemia during fasting was: <100mg/dL, and after 2hours: <140mg/dL. Odds ratios (OR) were used to estimate the strength of the association between glycemia levels and miscarriages and also between glycemia levels and birth weight. Fisher test was also applied in those analyses. Predictive ability of glycemia levels and low birth weight was studied using ROC curves.
Results: 18 women presented gestational diabetes (3.6%). Women presenting miscarriage or initiating spontaneous abortion: 44.4%. Low birth weight reached 18.7%. Fasting glycemia levels higher than 92mg/dL had a risk six times higher of presenting a low birth weight. Predictive ability of glycemia levels and low birth weight reached 33%. Postprandial glycemia levels higher than 140mg/dL had a risk three times higher of presenting a birth weight >3.5k. Predictive ability of glycemia levels and low birth weight reached 33%. Fasting glycemia levels higher than 92mg/dL had a risk 1.5 times higher of presenting a miscarriage or initiating spontaneous abortion: OR= 1.5 IC 95% (0.16).
Conclusions: These results stress the need to test the diagnosis of gestational Diabetes Mellitus during the first trimester. Women presenting miscarriages could be at risk of gestational diabetes when their fasting glycemia levels reach figures over 92mg/dL.
Key-words: Gestational diabetes mellitus, spontaneous abortion, birth weight

Infant feeding and growth patterns in children < 4y in Mexico: Associations with adiposity and cardiometabolic risks at 4-5 y of age.

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Background/Aims: Study the association of breastfeeding and rapid weight gain (RWG) in children < 4y with adiposity and cardiometabolic markers at 4-5y.
Methods: Analysis of a Mexican birth cohort of 727 children with prospective breastfeeding (BF) and anthropometric information, of whom 524 provided a non-fasting blood sample. We used path analysis to model associations of BF and adiposity: BMI, sum of skinfolds (SSF) and arm circumference (AC) and cardiometabolic markers: serum total-cholesterol (TCH) and LDL-cholesterol (LDL-C), triglycerides (TG), insulin concentration and blood pressure (BP) at 4-5y. RWG was defined as a change of ≥1.0 Z-score of weight-for-height for the age periods 0-6, 6-12, 12-24 and 24-48mo. Associations of RWG and the outcomes were analyzed using multivariate multiple linear models adjusting for potential confounders.
Results: Non BF (NBF) or partially BF (PaBF) infants at 3mo. had higher BMI, SSF and AC, respectively, than exclusively or predominantly BF (E-PreBF) (P<0.01). NBF children had higher TCH than children E-PreBF. An inverse association between E-PreBF and insulin, mediated through AC was documented (P<0.05). Relative to children without RWG, those with RWG from 6-48mo. had higher adiposity indicators at 5y (BMI, SSF and AC) and higher systolic and diastolic BP at 5y. RWG at older age intervals (12–48mo.) was associated with greater levels of adiposity and BP compared to younger age periods. Only RWG from 12-24mo. was associated with higher insulin concentration (P<0.05) at 4y.
Conclusions: E-PBF at 3mo. was associated with lower adiposity and TCH in children at 4y. Additionally, breastfeeding > 12mo. was associated with lower adiposity. Children with RWG 12-48mo. had greater adiposity and BP than infants 0-12mo. and those with RWG from 12-24mo. had higher insulin concentrations at 4-5y of age.
Key-words: Breastfeeding, cardiometabolic disease, blood pressure
Financial support: National Institute of Child Health and Development, NIH (HD043099), March of Dimes Foundation (6FY04-69) and National Public Health Institute (INSP).

Breastfeeding duration modulates food intake, satiety responsiveness and BMI in IUGR children at 4 years of age

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Background/Aims: Intrauterine growth restriction (IUGR) is implicated in the risk for adulthood diseases such as overweight, preceded by altered feeding behavior during childhood. Our objective was to investigate the effects of breastfeeding duration on these outcomes in IUGR children.
Methods: 273 children from the cities of Montreal and Hamilton, Canada, were recruited from an established prospective birth cohort (the Maternal Adversity, Vulnerability and Neurodevelopment – MAVAN project). At 48-months of age, mothers completed the Children Eating Behavior Questionnaire (CEBQ) and a Food Frequency Questionnaire. Linear regression models were built to evaluate the effect of breastfeeding duration reported at 12-months of age on the CEBQ scores and food preferences described at 4-years of age according to birthweight status (IUGR or not).
Results: Longer duration of exclusive breastfeeding was related to decreased caloric intake and increased satiety responsiveness in IUGR children. Total breastfeeding duration was associated with decreased food responsiveness reported of the CEBQ and decreased BMI at 48-months in IUGR children.
Conclusions: As IUGR is related to the development of overweight during the life course, as well as its morbid consequences, these results suggest that longer breastfeeding duration may be particularly important for these children.
Key-words: Breastfeeding, feeding behavior, appetite, IUGR

4TH INTERNATIONAL SYMPOSIUM ON METABOLIC PROGRAMMING AND STRESS AND THE 1ST MEETING OF IBERO-AMERICAN CHAPTER OF DOHAD SOCIETY

SHORT COMMUNICATIONS

Maternal cafeteria-style diet during lactation induces obesity and alters leptin and insulin crosstalk in the hypothalamus in offspring at adulthood

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Background/Aims: Childhood obesity is associated with increase in the obese in adulthood. During pregnancy and lactation the pups are vulnerable to nutritional or hormonal changes of the mother. This phenomenon has been called as 'metabolic programming'. Our aim was to evaluate the impact of maternal consumption of cafeteria-style diet during lactation on the composition of breast milk, adiposity and glucose homeostasis in the offspring at adulthood.
Methods: Female Wistar rats were divided into two groups: control (CO, n=14) fed standard rodent chow (Nuvilab®, Brazil) and cafeteria group (CAF, n=14) fed with a cafeteria-style diet consisting of standard chow triturated (33%), Nestle® condensed milk (33%), sucrose (7%) and water (8.6%). The rats were fed their respective diets and water ad libitum during the lactation period (21 days). With 91-days-old offspring (n=12/group) were euthanized for collection of blood and tissue samples.
Results: The CAF mothers breast milk contains more cholesterol (+20%, p<0.006), triglycerides (+77%, p<0.003) and protein (+37%, p<0.02). In adulthood (91-days old), CAF offspring had overweight (+13%, p<0.0001) and hyperphagia (+18%, p<0.03), which was associated with an increase in the adiposity of this animals (1.5-fold, p<0.006) and adipocyte hypertrophy (2.2-fold, p<0.002). In the leptin and insulin hypothalamic crosstalk decreased IRβ (-53%) ObRb (-39%) and JAK2 (-49%), suggesting hypothalamic leptin and insulin resistance, which was associated with an increase in the hypothalamic NPY content (+ 41%). The CAF offspring also presented insulin resistance (HOMA +108%, p<0.04, and Kitt -28%, p<0.01).
Conclusions: Therefore, maternal consumption of cafeteria-style diet during lactation changes the composition of breast milk. This excess of the energy promoted obesity combined with changes in glucose homeostasis and persistent hyperphagia in the offspring at adulthood. It is therefore, concluded that lactation is a critical period in the formation of neuroendocrine circuitry that controls food intake and energy expenditure.
Key-words: Diet, lactation, metabolic programming, obesity.
Financial support: CAPES, CNPq, Fundação Araucária.

Breast-feeding duration in Chile. National information from the Ministry of Health.

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Background/Aims: Iround 985,754 children < 6 years age are included in the Chilean Nutritional Surveillance System: this figure means about 80% of coverage. Chile has surveyed breast duration during the last 20 years as part of that system. At the end of year 2011 a law was passed increasing maternal post-natal leave for working women from three to six months. It has been reported that around 50% of women in their fertile ages are working outside their homes. Proportions of exclusively breast-fed infants at 1st, 3rd and 6th months of age are presented from year 2011 to year 2013 in this report.
Methods: Infants covered by the National Health Services monthly visit the health clinics and the health personnel register their type of feeding at these health check-ups for growth and health, including vaccinations. Exclusive breast-feeding is defined when no other type of artificial milk, liquids or solids are ingested; occasional intake of low amounts of water or syrups are accepted for this definition. The numerator of these proportions corresponds to those exclusively breast-fed infants that visited the clinics for the sixth month check-up. The denominator of these proportions corresponds to those infants that visited the clinics for the sixth month check-ups.
Results: Exclusive breastfeeding at 6th month of age was 41% in year 2011 meanwhile that proportion reached 45% in year 2013, after the increase in the maternity leave for working women. Those percentages were significantly different as far as each group had a very high sample size reaching more than 100,000 infants each.
Conclusions: Exclusive breast-feeding duration significantly increased after roughly two years of the legal increase in the post-partum maternity leave for working women. This positive change may be due to that increase although further studies are needs to prove the possible causal association.
Key-words: Exclusive breast-feeding duration; post-partum maternity leave.

Expression of Adiponectin receptors 1 and 2 in primary culture of human umbilical artery endothelial cells (HUAEC) from large fetuses of obese women.

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Background/Aims: Maternal obesity is directly related to fetal macrosomia and large-for-gestational-age (LGA) fetuses, which associates with higher risk of cardiometabolic disease in adulthood, a concept known as "fetal programming". Fetal growth is determined by the placenta, mainly by regulating nutrient transport and its vascular function. Macrosomic fetuses show lower umbilical artery vascular resistance and increased umbilical blood flow. Maternal plasma levels of adiponectin, an energy homeostasis regulatory adipokine, are negatively related to her BMI and to fetal birth weight. Growing evidence suggests that adiponectin induces a nitric oxide-dependant vasoactive function through the Adiponectin Receptor–APPL1–PI3K–AMPK–eNOS pathway. In this study we aimed to determine if HUAEC express adiponectin receptor 1 (AdipoR1) and 2 (AdipoR2). Additionally we studied the differential expression of AdipoR1 and AdipoR2 in LGA fetuses of obese pregnant women compared to appropriate-for-gestational-age (AGA) fetuses of normal weight pregnant women.
Methods: Primary cultures of HUAEC were obtained from the umbilical cord of term single pregnancies of AGA babies from normal weight women (A/N) and LGA babies from obese
women (L/O). The mRNA and protein levels of AdipoR1 and AdipoR2 were measured by qPCR (Sybr Green) and Western blot using primary monoclonal AdipoR1 (anti-rabbit, 1:250) and polyclonal AdipoR2 (anti-goat, 1:500) antibodies, respectively.
Results: HUAEC express the mRNA and protein for AdipoR1 and AdipoR2. A/N and L/O groups express comparable mRNA levels for AdipoR2, however L/O showed a significant decrease in AdipoR1 expression compared to A/N. AdipoR2 did not show significant changes between groups.
Conclusions: This study demonstrates for the first time that both adiponectin receptors are expressed in HUAEC. The physiological role of a decreased expression of AdipoR1 in L/O HUAEC, and the participation of the AdipoR1 signaling pathway in this condition needs to be further elucidated.
Key-words: Adiponectin, AdipoR1, AdipoR2, LGA, HUAEC.
Financial support: Fondecyt N°1120928. EMM holds a CONICYT PhD fellowship (Chile).

Evaluation of drug therapy and quality of life in type 1 diabetic patients with conventional insulin treatment

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Background/Aims: The Type 1 Diabetes Mellitus is a chronic, dependent on insulin replacement disease, affecting much of the young population. According to the Department of the Health System in the city of Ponta Grossa/PR, Brazil counted up 119 DM1, 620 patients with DM2 and hypertensive patients with diabetes totaled-4940, however, the same site under the Regional Health Department data unchanged as 338 DM1 patients, DM2 1,178 and hypertensive patients with diabetes 9,680 individuals. This disparity between the data demonstrate the importance of research to meet the diabetic population using the SUS. Quantify the current prevalence of DM1 and evaluate the use of NPH and regular insulin, which do not reach the expected results, and producer of other health problems, is the goal of this work.
Methods: This observational retrospective study as a source of secondary data collection from medical records of patients seen at the Municipal Center Specialties, Basic Health Units of Ponta Grossa/PR – Brazil and Hospital Santa Casa de Misericordia of Ponta Grossa/PR – Brazil and primary data contact with the patients themselves. Inclusion criteria are DM1 patients, the users of these sites mentioned above, we made use of NPH and regular insulin. Exclusion criteria were those that did not fit this profile.
Results: We recently began collecting data and as expected, it was found in the first 626 records evaluated 210 diabetic patients without accurate description of the type 1 or 2, and only 10 were DM1 and users of NPH and regular insulin.
Conclusions: We conclude, in this initial research, that the medical records, the criteria of responsibility and the future of treatment are in an unsatisfactory state to which the user has an adequate quality of life.
Key-words: Diabetes Mellitus type 1, insulin NPH, regular insulin.

Guinea pig intrauterine growth restricted foetuses show altered systemic and umbilical vascular function.

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Background/Aims: Intrauterine Growth Restriction (IUGR) relates with altered placental vascular reactivity and long term cardiovascular risk. However whether vascular changes observed in umbilical arteries (UA) reflect the alterations present in systemic arteries (SA) in the IUGR foetus is unknown. We aimed to determine the effect of foetal growth restriction on NO-dependent relaxation in UA and SA, and determined the expression NO-related proteins in cultured endothelial cells from these vessels as a signature of vascular programming, in Guinea pig.
Methods: IUGR was induced by implanting ameroid constrictors in both uterine arteries at 35 days of gestation. Foetal

growth and umbilical blood velocity was followed by Doppler Sonography. At term, foetuses were extracted, weighed and dissected. NO-dependent vasoactive responses of foetal UA and SA were studied by wire myography. Endothelial cells from fetal aorta, femoral and UA were cultured and characterized by immunocytochemistry. The gene/protein expression of eNOS and arginase-2 ware determined by qPCR and western blot.

Results: Uterine artery occlusion increased UA resistance and reduced foetal, placental weight, and induced asymmetric IUGR. IUGR UA showed a lower NOS-dependent relaxation (7.2±1.02%KCl vs. 41.3±7.0%KCl), and increased sensitivity to an NO-donor (SNP, 7.17±0.29 vs. 6.08±0.07). IUGR aortas showed higher response (57.2±0.7%KCl vs. 22.5±0.9%KCl) and decreased sensitivity (5.86±0.05 vs. 6.89±0.13) to acetylcholine, and higher relaxation to SNP (103.5±5.3%KCl vs. 70.6±9.5%KCl) compared to controls. Cultured cells from foetal vessels presented markers of endothelial cells, and expressed eNOS and arginase-2 as reported for human endothelial cells.

Conclusions: Guinea pig IUGR foetuses present altered NO-mediated vascular reactivity in UA and aorta, characterized by a higher NO- and decreased eNOS-dependent agonist- sensitivity. Notably, changes in UA reactivity in IUGR guinea pigs are comparable to those observed in human IUGR umbilicoplacental vessels.

Key-words: IUGR, vascular programming, endothelium.
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Influence of ethanol and lycopene consumption during lactation in enzymes and peptides involved in liver detoxification of the offspring

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Background/Aims: Lycopene's derivatives, as well as oxygen free radicals, stimulate transcription of phase II detoxifying enzymes, such as glutathione-S-transferases and glutathione reductase (GR). However, oxidative stress due to ethanol consumption may cause a disorder in this system's activation. This effect can also affect the adult offspring of rats that consumed ethanol during the lactation. Therefore, this study intends to evaluate if lycopene consumption is capable of diminish the effects of ethanol exposure.

***Methods*:** Lycopene powder and/or water with 20% ethanol were administered to lactating Wistar rats. They were distributed in control group (CG), lycopene group (LG), ethanol group (EG) and ethanol+lycopene group (ELG). The offspring (n=72) was randomized and sacrificed at 12th (n=24), 30th (n=24) and 90th (n=24) days. The offspring liver's weight, total glutathione (TG), glutathione disulfide (GSSG), reduced glutathione (GSH), GSSG/GSH, and enzymatic activity of glutathione peroxidase (GPx), GST, and GR. Rats weight was also accessed.

***Results*:** At 12th days: body weight was lower in EG and ELG than in CG; liver's weight was lower in ELG than in CG; TG and GSH were lower in ELG compared to CG, LG and EG (both p<0.001); LG compared to CG had lower levels of GR (p=0,160) and GPx (p=0.035). At 30th days: no difference was founded in any variables. At 90th days: GSSG and GSSG/GSH were higher in EG, in comparison with all others.

***Conclusions*:** Although EG and ELG has had a lower body weight at 12th days, this was not seen in those groups at 30th and 90th days. However, EG also had worse liver detoxification at 90 days. This could show that ethanol exposure through lactation can damage the detoxification system at a low level, which is only seen when accumulated with lifelong stress. Lycopene exposure may have a deleterious effect during lactation, but a protective effect in the long term.

Key-words: Glutathione, lycopene, ethanol, lactation, offspring

Intrauterine programming and dyslipidemia in Chilean children

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Background/aims: Dyslipidemias are multifactorial: Genetic factors interact with postnatal environmental factors (diet, physical activity, cultural, nutritional status). Intrauterine environment may play a role in its programming. We aimed to study the relationship between gestational age (GA), body weight (BW) and height (BH) at birth and the presence of dyslipidemia at school age.

Methods: A cross-sectional study of a retrospective cohort (2,871 school-age children, 10 to 14 y.o.) was done. Anthropometry and fasting blood sample for blood lipids assessment were performed. We analyzed the relationship between birth data and total cholesterol (TC), LDL cholesterol (LDL-C), HDL cholesterol (HDL-C) and triglycerides (TG), as well the association with clinical forms of dyslipidemia.

Results: GA <37 weeks (n=164; 5.7%), ≥37weeks (n=2707; 94.3%). BW <2500g (n=134; 4.7%), 2500g to 4000g (n=2483; 86.5%), >4000g (n=254; 8.85%). BH <48cm (n=386; 13.4%), 48 to 50cm (n=1466; 51.06%) and >50cm (n=1019; 35.5%). Children with antecedent of GA <37 weeks had higher prevalence of isolated hypertriglyceridemia (12.2% vs. 9.2%; p=0.04) and atherogenic dyslipidemia (low HDL-C + high TG; 8.5% vs.6.1%; p=0.059). There was a tendency to higher combined dyslipidemia in children with GA ≥37 weeks (4.2% vs. 1.8%; p=0.066). Children with BW > 4000g had a non-significative tendency to higher prevalence of isolated hypercholesterolemia and isolated low HDL-C. Those with BW <2500g had a tendency of more isolated hypertriglyceridemia and atherogenic dyslipidemia. Also, children with BH <48 cm had more isolated hypertriglyceridemia and those with BH >50 cm had more low HDL-C. In multiple regression analysis, only sex, nutritional status and family history of dyslipidemia were significative variables for dyslipidemias.

Conclusion: This study in school-age children shows a higher prevalence of some dyslipidemias in those who were premature, with weak associations to extremes of weight and height at birth.

Key-words: Dyslipidemia, pediatrics, prenatal programming

Effects of histidine and cimetidine on the progression and differentiation in mice melanoma

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Background/Aims: Previous studies have demonstrated the relationship of histidine on the differentiation of some cell types, such as hepatocytes. One of the main characteristics of tumors, such as melanoma, is cell without differentiation. It is known that this type of cancer produces large amounts of histamine, having a direct relationship with histaminergic. The purpose of our work is study the effects of a histidine supplementation on the differentiation and tumor progression. Histidine is converted to histamine by histidine decarboxylase, and its presence in high pathophysiological process of cancer suggests a role of importance.
Methods: Melanoma B16F10 cells were cultured, subsequently performed in vitro cell viability MTT method. Cells were treated with histidine, cimetidine or the association of histidine/cimetidine, and evaluated 24, 48 and 72hours, after treatment. The in vivo assay, cells were injected subcutaneously into C57BL/6 mice. The animals were divided into 4 groups of 8 animals (4 males and 4 females aged between 8 and 10 weeks), as follows: control, histidine, cimetidine and association histidine/cimetidine. After 10 days of implantation of the cells, the animals were treated orally for 12 days with the drugs at doses of 21.4mg/kg histidine, 343.3mg/kg of cimetidine association between both 21.4mg/kg histidine/343.3mg/kg of cimetidine and assessed for tumor growth.
Results: The dose-dependent response of histidine, cimetidine and the association histidine/cimetidine melanoma cells in vitro showed that the concentrations of 0.01 to 10μM histidine limited cell viability while overdosing 20μm stimulated growth after 72h of treatment. The concentrations of cimetidine study led to inhibition of cell proliferation. The effects of histidine and cimetidine and combination on tumor growth in vivo showed that all treatments have limited he growth of tumors until the sixth day, but the end of the ten day treatment with cimetidine only, histidine/cimetidine combination showed limitation in the final volume of the tumor.
Conclusions: Histidine plays an important role in tumor progression and stimulating or inhibitory depending on the dosage/day of treatment. Cimetidine, and association cimetidine/histidine inhibited tumor growth. Future experiments will help to elucidate the mechanism related to these events.
Key-words: Histidine, cancer, melanoma

Effects of aerobic training on reduced response to angiotensin II in intrauterine growth restricted rats: role of renin angiotensin system and oxidative stress

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Background/Aims: The aim was to evaluate the effects of intrauterine growth restriction (IUGR) and aerobic training (AT) on vascular response to Angiotensin II (AngII).
Methods: Pregnant Wistar rats were submitted to ad libitum or 50% of ad libitum diet throughout gestation. At birth, pups were weighted. Adult male offspring of both groups were assigned to Sedentary Control (SC), Sedentary Restricted (SR), Trained Control (TC) and Trained Restricted (TR). After AT (10 weeks, 5 days/week, 1h/day; 50–60% of maximum exercise capacity), thoracic aorta was excised to the following studies: cumulative concentration-response curves to AngII (10-9–10-5M), superoxide production by dihidroethidium staining (DHE, 5μM). The protein expression, by western blot, of AT1 and AT2 receptors, p47phox subunit of NADPH oxidase enzyme and manganese isoform of superoxide dismutase enzyme (MnSOD). Results are shown as mean±SEM, Student's t test or two way ANOVA were used, P<0.05; (Ethics Committee/UNIFESP 0092/10).
Results: AT restored the response to AngII (SC: 0.37g±0.66; SR: 0.66g±0.33, TC: 0.40g±0.03; TR: 0.38g±0.03) and the elevated superoxide concentration (SC: 18.1±0.9; SR: 25.6±0.7; TC: 20.0±0.9; TR: 20.9±0.3) in aorta from TR rats. The increased superoxide concentration was reduced after both AT1 antagonism (Losartan:10-4M; SC: 22.9±0.6; SR: 22.0±1.5; TC: 24.4±0.8; 23.9±0.5), and blockage of NADPH oxidase (Apocynin: 10-4M; SC: 22.5±0.6; SR: 23.±1,7; T: 23,5±0,7; T: 24,3±0,7) in aorta from SR group. However, high levels of superoxide was found after the AT2 antagonism (PD: 123,319; SC: 24.5±0.5; SR: 30.2±1.1; TC: 24.4±1.1; TR: 28.9±0.6) in aorta from TR rats. Furthermore, AT normalized the protein expression of AT2 (SC: 1.0±0.04; SR: 0.8±0.05; TC: 1.0±0.06; TR: 1.0±0.07), p47phox (SC: 1.1±0.06; SR: 1.4±0.05; TC: 1.2±0.03; TR: 1.1±0.04) and MnSOD (SC: 1.2±0.07; SR: 0.8±0.06; TC: 1.0±0.03; TR: 1.1±0.10) in TR group. Protein expression of AT1 was similar among groups. ***Conclusions:*** AT was effective in restoring vascular response to AngII and superoxide concentration in IUGR rats. These effects may be due to normalization in AT2 receptors, p47phox and MnSOD observed in TR group.
Key-words: Intrauterine growth restriction, aerobic training, angiotensin II, oxidative stress.
Financial support: FAPESP, CAPES, CNPq.

Association between neonatal body iron status with maternal body iron status and the expression of transferrin-1 receptor and ferroportin in full term placenta.

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Background/Aims: Maternal iron deficiency and anemia could negatively affect neonatal iron status, and no studies are available on their relationship with transferrin and ferroportin expression in human placenta. To evaluate the association between biochemical parameters of maternal and neonatal iron status, and placental transferrin receptor-1 and ferroportin expression.
Methods: Full term pregnant women (n=24), were engaged in a cross-sectional study and three experimental groups were defined according to serum ferritin and hemoglobin values: iron deficiency and anemia; iron deficiency with no anemia; and control group. All neonates were born by cesarean delivering, between 37 to 39 weeks of pregnancy, and were immediately bled by umbilical vein puncture for serum ferritin levels quantification and to evaluate neonatal iron status. The transferrin receptor-1 (TfR 1) and ferroportin (Fpn) expression were evaluated in villous tissue and trophoblast cells. Data were analyzed by Mann-Whitney U Test or Student t test.
Results: Ninety six percent of patients did intake iron supplements frequently, lasting of supplement intake significantly correlated with maternal serum ferritin levels, and tended to be lower in women with iron deficiency and anemia, compared with control group. Iron deficiency was found in 22% of neonates with no differences between groups (P>0.05). TfR1 and Fpn expression did significantly correlated in villous tissue (P<0.01), but their expression did not significantly differed between groups (P>0.05). Neonatal ferritin significantly correlated (P<0.05) with Fpn expression. TfR1 was predominantly expressed in the apical membrane of syncitiotrophoblast, but Fpn was predominantly found in villous stroma.
Conclusions: Our results support the prescription of iron supplements during pregnancy and provide evidence on the relationship between biochemical parameters of maternal iron state. No significant relationships were found between TfR1 or Fpn expression and maternal and neonatal iron status although the Fpn significantly correlated with the neonatal ferritin.
Key-words: Iron transport, Iron receptors, Maternal Anemia and ferropenia, Serum ferritin, Term placenta.

Hypertension, possible early marker of endothelial dysfunction in Chilean children

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Background/Aims: Previous studies have demonstrated the relationship of histidine on the differentiation of some cell types, such as hepatocytes. One of the main characteristics of tumors, such as melanoma, is cell without differentiation. It is known that this type of cancer produces large amounts of histamine, having a direct relationship with histaminergic. The purpose of our work is study the effects of a histidine supplementation on the differentiation and tumor progression. Histidine is converted to histamine by histidine decarboxylase, and its presence in high pathophysiological process of cancer suggests a role of importance.
Methods: Melanoma B16F10 cells were cultured, subsequently performed in vitro cell viability MTT method. Cells were treated with histidine, cimetidine or the association of histidine/cimetidine, and evaluated 24, 48 and 72hours, after treatment. The in vivo assay, cells were injected subcutaneously into C57BL/6 mice. The animals were divided into 4 groups of 8 animals (4 males and 4 females aged between 8 and 10 weeks), as follows: control, histidine, cimetidine and association histidine/cimetidine. After 10 days of implantation of the cells, the animals were treated orally for 12 days with the drugs at doses of 21.4mg/kg histidine, 343.3mg/kg of cimetidine association between both 21.4mg/kg histidine/343.3mg/kg of cimetidine and assessed for tumor growth.
Results: The dose-dependent response of histidine, cimetidine and the association histidine/cimetidine melanoma cells in vitro showed that the concentrations of 0.01 to 10μM histidine limited cell viability while overdosing 20μm stimulated growth after 72h of treatment. The concentrations of cimetidine study led to inhibition of cell proliferation. The effects of histidine and cimetidine and combination on tumor growth in vivo showed that all treatments have limited he growth of tumors until the sixth day, but the end of the ten day treatment with cimetidine only, histidine/cimetidine combination showed limitation in the final volume of the tumor.
Conclusions: Histidine plays an important role in tumor progression and stimulating or inhibitory depending on the dosage/day of treatment. Cimetidine, and association cimetidine/histidine inhibited tumor growth. Future experiments will help to elucidate the mechanism related to these events.
Key-words: Metabolic syndrome, flow mediated dilation, endothelial dysfunction, children

Aerobic training normalizes pro-inflammatory cytokines in intrauterine growth restricted rats: possible involvement of Toll Like Receptor-9 and circulating mitochondrial DNA

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Background/Aims: The aim was to evaluate the effects of intrauterine growth restriction (IUGR) and aerobic training (AT) on blood pressure (BP), heart expression of Toll like receptor 9 (TLR9) and circulating levels of mitochondrial DNA (mitDNA) and pro-inflammatory cytokines.
Methods: Pregnant Wistar Rats were submitted to ad libitum or 50% of ad libitum diet throughout gestation. At birth, pups were weighted. Adult Male offspring of both groups were assigned to Sedentary Control (SC), Sedentary Restricted (SR), Trained Control (TC) and Trained Restricted (TR). After AT (10 weeks, 5 days/week, 1h/day, 50-60% of maximum exercise capacity) indirect BP was evaluated, plasma collected and heart excised. Circulating mitDNA and bacterial DNA (bDNA) was quantified by NADH dehydrogenase 1 and 16-S genes, respectively using quantitative real-time PCR. Plasma TNF-α and MCP-1 were measured by Multiplex. Cardiac expression of TLR9 was evaluated by western blot. Heart hypertrophy index was assessed (left ventricle (LV mg)/length tibia (cm)). Results are shown as mean±SEM, Student's t test or two-way ANOVA were used, P<0.05, all of the experimental protocol were approved by the Ethics Committee from UNIFESP (nº 836880).
Results: IUGR was assessed by birth weight (Control: 6.8±0.18g vs. Restricted: 4.1±0.1g). AT restored BP (SC: 111±2mmHg; SR: 120±7mmHg; TC: 109±3mmHg; TR: 110±2mmHg) and circulating mitDNA levels in TR (SC: 1; SR: 2.49±0.70; TC: 0.93±0.27; TR: 0.71±0.24). Groups did not show levels of bDNA. Plasma TNF-α (SC: 13.2±0.7pg/mL; SR: 17.8±1.5pg/mL; TC: 14.6±1.0pg/mL; TR: 13.5±1.0pg/mL) and MCP-1 (SC: 142.2±6.8pg/mL; SR: 173.5±5.5pg/mL; TC: 134.8±9.4pg/mL; TR: 133.0±6.8pg/mL) concentration were also normalized in TR group. In addition, AT reduced the high cardiac expression of TLR9 in restricted group (SC: 1; SR: 1.54±0.13; TC: 0.93±0.14; TR: 0.69±0.07). Heart hypertrophy was similar among groups. ***Conclusion:*** AT was effective in restoring BP in IUGR rats. AT normalized elevated mitDNA and cardiac expression of TLR9 receptor in restricted group. These effects can contribute to reduced levels of TNF-α and MCP-1 in TR group.
Key-words: Intrauterine growth restriction, aerobic training, inflammation.
Financial Support: FAPESP, CNPq.

Inulin supplementation effects on the intestinal motility and myenteric innervation of mice with high-fat diet-induced obesity

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Background/Aims: High-fat diets (HFDs) promote obesity and associated conditions, including gastrointestinal disorders. The use of prebiotics in the diet can attenuate the effects of chronic inflammation caused by HFDs, improving the functions of the intestinal barrier. This study evaluated the effects of a HFD and inulin supplementation on biochemical blood parameters and the myenteric innervation in the distal colon of mice.
Methods: Male Swiss mice (42 days) were distributed into groups of six animals, treated for 17 weeks: group C, fed standard AIN 93G diet; group Ci, fed AIN 93G diet supplemented with inulin (9:1); group H, fed HFD (59% kcal from fat); and group Hi, fed HFD supplemented with inulin (9:1). Stool collection, intestinal and colonic motility and food consumption were assessed. Blood samples were collected for biochemical analysis and the distal colon was collected for neuronal immunofluorescence techniques (myosin-V antibody).
Results: Group H had an increase in body weight and abdominal fat, with no differences between animals supplemented with inulin and their controls. The HFD increased the total cholesterol in group H, and HFD with inulin reduced these values to normal levels in group Hi. The levels of blood glucose, triglycerides and total protein, food intake, distal colon area and intestinal motility showed no significant difference between groups. Stool water content and colonic motility decreased in the HFD-treated group, while inulin supplementation in the group Hi was able to reverse these parameters. Supporting these results, analysis of myenteric neural density (myosin-V-immunoreactive neurons) showed 21% neuronal loss in the group H, with no differences between the groups supplemented with inulin and their controls.
Conclusions: The HFD caused obesity, neuronal loss, decreased colonic motility and increased total blood cholesterol. Although neuronal loss was not reduced, inulin supplementation was able to improve some of these parameters.
Key-words: Myenteric plexus, myosin-V neurons, distal colon

Maternal high-fat diet and its influence on the endocannabinoid system of male and female offspring rats at weaning

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Background/Aims: We have shown that maternal high-fat diet (HFD) programs early obesity, hyperleptinemia and central leptin resistance in male offspring rats. Endocannabinoid system (ECS) is known to control energy homeostasis promot-

ing energy intake and lipid metabolism control in the adipose tissue. Here, we evaluated the effect of a maternal high-fat diet on ECS in the adipose tissue of the offspring at weaning.

Methods: Female Wistar rats were divided into two groups: control group (C), which received a standard diet (9% of the calories as fat), and a high-fat group (HF), which received a HFD (28% of the calories as fat) during 8 weeks before mating and throughout gestation and lactation. At weaning (21 days), male and female offspring were euthanized, and blood and retroperitoneal adipose tissue (RWAT) was collected. The endocannabinoid receptors (CB1 and CB2) and monoacylglycerol lipase (MAGL, a key enzyme involved in the hydrolysis of the endocannabinoids) content was measured by western blotting. Leptin serum concentration was measured by radioimmunoassay.

Results: Male and female HF offspring had increased body weight (+31% and +27%, respectively; p<0.05), retroperitoneal adipose tissue weight (4 fold; p<0.05) and serum leptin (+53% and +84%, respectively; p<0.05). CB2 receptor was decreased in the RWAT of female (–18.3%; p<0.05) with no changes in male offspring (–13.6%). MAGL content in the RWAT was increased in male (+40.26%; p<0.05) with no statistical significance in female (+20.7%).

Conclusions: Maternal HFD induces early obesity and hyperleptinemia in both male and female offspring. CB2 receptor in the RWAT is decreased only in female offspring. We speculate that the endocannabinoid system is over-activated in HF offspring and this might contribute to the obese phenotype observed in these rats.

Key-words: Maternal high-fat diet, endocannabinoid, metabolic programming

Financial support: National Council for Scientific and Technological Development (CNPq), Coordination for the Enhancement of Higher Education Personnel (CAPES), and State of Rio de Janeiro Carlos Chagas Filho Research Foundation (FAPERJ).

Effects of environmental enrichment on behavior in a rat model of hyperleptinemia neonatal programming.

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Background/Aims: Even though leptin anxiolytic effect, neonatal leptin treatment during the first ten days of lactation (PN) programs in greater anxiety and resistance to the leptin at the adult progeny. Enriched environment (EE) reduces anxiety and increases leptin sensitivity. This study shows the temporal analysis of the neonatal leptin exposition effects and verifies if EE reverts, in adult rats that were programmed, anxiety and search for novelty.

Methods: Ninety litters of Wistar rats were used. Divided into 2 experimental groups: leptin (L: 8µg/100g/kg/once a day s.c.) or saline (C: 50µl) both from PN1 to PN10. From PN 30 until PN 45, groups were randomly assigned to the nonenriched housing or enriched housing (EE). The EE consisted of cage, which contained stimulus designed to induce exploratory behavior, such as toys, toys that makes sound, and stimulus designed to provide the animals with free access to exercise, such as tunnels, running wheels, stairs and ramps. We analyzed the anxiety levels before and after the EE (PN 21, 30, 45 and 150). The animals were submitted to elevated plus maze test (EPM) and Hole Board (HB) over a period of 10 minutes and the time spent and % of time spent in open arm (Time OA and %Time OA) and the number and % of the number of open arm entries (Entries OA and %Entries OA) were recorded and used as anxiety measures.

Results: At PN 21, was observed a significantly increased (+1000x; T test: P<0.001) in the Time OA in the L group (78191±13301s) when compared by C group (6700±2841s) and increased in the Entries OA (+380%; T test: P>0.001; L: 6.75 ± 1.37) and C group (1.40 ± 0.67). At PN 30, L rats (28±8.5%) showed an increased in the %Time OA (+438%; T test: P<0.05) when compared to C ones (5.2±2.3%). Curiously, at PN 45 the EE was not able to reduce anxiety in the C animals but had already a modest ansiolytic effect (+141% in Time OA; ANOVA: P>0.05) in the programmed L group and also in %Time OA (+119%; ANOVA: P>0.05). This effect is accentuated at PN 150, where EE had a higher ansiolytic effect in L group observed by an increased in Time OA (+656%; ANOVA: P>0.001) and %Entries OA (+154%; ANOVA: P<0.001, for example), notwithstanding a modest ansiolytic effect observed by an increased Time OA (+8.7%; ANOVA: P>0.05) in the C group.

Conclusion: The leptin anxiolytic effect is lost during growth. However, EE restores this effect, because L-EE animals reduce anxiety at EPM and PN150 at HB reduces search for novelty.

Key-words: Anxiety, metabolic program, leptin

Financial support: FAPERJ, CNPq, CAPES and SR2-UERJ

Role of birth weight on cardiac autonomic modulation of child and adolescent

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Background/Aims: Disturbance in the pattern of intrauterine growth are associated with the development of cardiovascular diseases in adulthood. It is evident that changes in the autonomic nervous system may be one of the mechanisms responsible for the increased risk for cardiovascular events. However, the influence of intrauterine growth autonomic control in children and adolescents is not known. Evaluate the relationship between intrauterine growth, characterized by birth weight, and cardiac autonomic modulation (CAM) in children and adolescents.

Methods: 71 children and adolescents were divided in 4 groups according to birth weight quartile (Q1< 2677g, Q2: 2677g-3100g, Q3:3100-3400g e Q4>3400g) and matched for level of physical activity and anthropometry. The CAM was assessed by heart rate variability (HRV) from the indices of time domain, Standart desviation of intervals R-R (SDNN),

Root meand square of successive differences (RMSSD) and proportion of diference intervals R-R (pNN50, and frequency domain, low frequency (LF), High frequency (HF), and low/high ratio. HR was continuously measured, by heart rate monitor (Polar RS800CX), for 10 minutes at rest in the supine position with spontaneous breathing. Results are expressed as mean ± standard error.

Results: Compared to the time domain, we found that children belonging to quartiles Q1 and Q4 showed less pNN50 in relation to third quartile ($p<0.01$). The HF and HF in index were significantly lower quartiles Q1 and Q4 relative to Q3 quartile (HF: $p<0.01$; HF: ln $p<0.01$ and $p=0.01$ and $p<0.01$ respectively).

Conclusion: Our results show that children with low (quartile Q1) and high (quartile Q4) birth weight have impaired vagal cardiac autonomic modulation, which can contribute early for the increased risk of cardiovascular events in these individuals.

Key-words: Birth weight, cardiac autonomic modulation and children

Newborn vascular tissue from maternal obesity shows insulin resistance: potential role of endoplasmic reticulum stress

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Background/Aims: Maternal obesity (MO) has been associated with the development of insulin resistance (IR) in offspring, being more prone to develop metabolic syndrome later in life. Evidence shows that endoplasmic reticulum (ER) stress is related with development of IR in obese individuals. However, the link between MO, ER stress and IR has not been elucidated. Here we evaluated whether insulin response is altered in neonatal tissues from MO and whether ER stress is involved.

Methods: Primary cultures of human umbilical vein endothelial cells (HUVEC) were isolated from normal (HUVEC-N) or MO (HUVEC-OB) pregnancies, attending to obstetrics service at the Clinical Hospital of Pontificia Universidad Católica de Chile. Using western blot analysis, we evaluated phosphorylated and total protein levels of IRS-1, Akt, p42/44 MAPK and eNOS in cells exposed or not to insulin in presence or absence of tunicamicyn (inducer of ER stress) or tauroursodeoxycholic acid (TUDCA, Er stress blocker). Isolated rings of umbilical veins (UV) were used to evaluate vasodilatation capacity by myography.

Results: The exposure (0-60 min) of HUVEC-N to physiological levels of insulin (1nM) showed a quickly and maintained increase of p~Akt (~12 fold) and p~p44/42mapk (~20 fold). Conversely, HUVEC-OB showed a reduced and delayed p~Akt (~5 fold) and p44/42mapk (~8 fold) in response to insulin. Further, treatment for 24h with tunicamycin induced IR in HUVEC-N, but treatment with TUDCA reversed the IR in HUVEC-OB. We found an increase in the inhibitory phosphorylation of IRS-1 (~2.5 fold) and a reduced phosphorylation (~0.4 fold) and total protein (~0.6 fold) of eNOS, compared to HUVEC-N. Additionally, unlike the normal samples, UV rings from MO did not show relaxation in response to insulin.

Conclusions: In this study we have shown evidence that MO promotes neonatal IR in umbilical cord vein and HUVEC involving ER stress.

Key-words: Birth weight, cardiac autonomic modulation and children

Low birth weight is associated with impairment of memory related to food in adolescents

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Background/Aims: Individuals with intrauterine growth restriction (IUGR) have typical food preferences and it could explain in part their increased risk for developing metabolic disorders during life course. The aim of this study was to investigate if the impairment of memory related to food could be related with their unbalanced feeding behavior.

Methods: This study was conducted in adolescents who have studied near the Hospital de Clínicas of Porto Alegre (HCPA). They could choose foods offered in a snack bar. Before eating it, a photograph of the snack was taken. Between 1 to 9 months after, it was shown 4 photos of snacks and the question: "If you eat now, which of the following snacks would you choose?" They did not know, but 1 of the photos was of their own snack eaten. Feeding behavior was assessed by 24h Dietary Recall and Food Frequency Questionnaire. Fetal growth was based on the birth weight ratio (BWR) and those in the lower tertile of the BWR distribution were considered with IUGR. Student's t test and Pearson's Chi-square test were performed.

Results: 49 individuals were evaluated (21 men, 17.50±2.35 years old) and 15 (31%) had IUGR. IUGR group consumed less calories from fat (969±103kcal) in comparison to normal birth weight group (1413±176kcal; $P=0.04$). IUGR individuals recognize with less frequency their own snack (18% remembered) in comparison to normal birth weight group (82% remembered; $P=0.01$).

Conclusions: Our data evidence that disturbances in early life have consequences in feeding behavior in later life. Individuals with IUGR have a different feeding intake and the impairment in memory related to food may be the cause of this distinct pattern. Analysis of the cognitive profile are being assessed to improve our understanding of the current results.

Key-words: RCIU, feeding behavior, memory related to food.

Financial support: CNPq and FIPE/HCPA and approved by the Ethics Commission of HCPA (nº 12-0254).

High-sugar but isocaloric diet promotes obesity associated with nonalcoholic steatohepatitis: biochemical and histological aspects

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Background/Aims: Obesity is a direct risk factor for several metabolic disorders like nonalcoholic fatty liver disease (NAFLD), which may evolves to nonalcoholic steatohepatitis (NASH). This study aimed to characterize biochemical and histological changes in the liver of mice fed a 25% sugar isocaloric diet.
Methods: 30 days-old male mice were fed for 90 days with regular chow (CTR) or high-sugar isocaloric diet (HS). Throughout the period, body weight gain and food intake were taken weekly. At day 120, Lee index (LI) and glucose tolerance test (GTT) were performed. Blood samples were collected to determination of serum lipid profile, TyG index and serum leptin. White and brown adipose tissues fat pad, as well as, liver histology and hepatic lipid profile were also analyzed. Results were expressed as mean±SEM and analyzed by Student's t-test for $p<0.05$.
Results: Body weight gain and LI of HS were 14% and 8% higher than in CTR. HS animals were glucose intolerant and showed hepatic insulin resistance, as depicted from GTT and TyG index data, respectively. HS also presented higher serum levels of glucose (33%), leptin (87%), triglycerides (59%), and total cholesterol (22%), paralleling to increased white and brown adipose tissues fat pads. In the liver, total fat (54%), triglycerides (158%), and cholesterol (104%) were all augmented as compared to CTR. Histological analysis showed HS group presented 37% of steatosis and 50% of steatohepatitis, while no alteration was found in CTR.
Conclusions: The results show that intake of a 25% high-sugar diet promotes precocious nonalcoholic steatohepatitis, which might be related to development of hypertriglyceridemia and hepatic insulin resistance in mice.
Key-words: Obesity; high-sugar diet; nonalcoholic steatohepatitis.
Financial support: FAPEMA, CAPES and UFMA.

Glycosylation patterns of transferrin receptor in villous placentas in severe preeclampsia and gestational anemia

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Background/Aims: The transferrin receptor 1 (TfR1) is a glycoprotein involved in the iron uptake mediated by the syncytiotrophoblast. An increase of TfR1 expression has been observed in gestational anemia but it is reduced in preeclampsia. Thus, we hypothesized that glycosylation participates in the proper expression of TfR1 in the syncytiotrophoblast cell membrane.
Methods: We determined the glycosylation profile of whole villous placenta and immunoprecipitated TfR1 obtained from early-onset severely preeclamptic women, pregnant women with gestational anemia and uncomplicated gestation. Lectin blot analysis was performed to determine glycan patterns associated with *N*- and O-glycoproteins using these lectins: *Datura stramonium agglutinin* (DSA), *Maackia amurensis agglutinin* (MAA), Galanthus nivalis agglutinin (GNA), Sambucus nigra agglutinin (SNA) and Peanut agglutinin (PNA), for Gal-GlcNAc, sialic acid α2–3, mannose, sialic acid α2–6 and Gal-GalNAc glycotopes respectively.
Results: α2–3 N- and O-linked sialic acid, mannose and Gal-GlcNAc was over-expressed in villous obtained from severe preeclamptic placentas. Preliminary results suggest an overexpression of Gal-GlcNAc and mannose glycans in TfR1 from severely preeclamptic women. α2–6 sialic acid is absent from placental TfR1.
Conclusions: Changes in the glycosylation patterns determined by lectin blot could be associated with the reduced expression of TfR1 in preeclampsia, a situation that could contribute to intrauterine growth restriction presented in most of these pregnancies. Moreover, differences in the glycotopes tested could be associated with the expression and function of placental glycoproteins in preeclampsia.
Key-words: Preeclampsia, glycosylation, transferrin-receptor.

How can maternal height help to get a better fetal growth in Chile?

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Background/aims: We have previously demonstrated in Chile the joint association of pre-gestational body mass index (BMI-PG) and weight gain during pregnancy (WG-P) on birth weight (BW) categories < 3000g and ≥ 4000g. We aimed to analyze in the same data base the influence of maternal height categories on BW and birth length (BL).
Methods: 11,466 healthy pregnant women were included in a prospective cohort study until term, i.e. 39–41 weeks of gestation, in a maternity hospital of Santiago, Chile. Mean BW and BL < 50cm were presented in the different maternal height categories. Statistically significant differences were calculated using chi-square test and t-test for distributions and mean values, respectively.
Results: Differences of mean BW values were statistically significant when comparing maternal height categories (<152cm; 152–160cm; and >160cm): 3255g, 3354g, and 3456g. Differences of proportions of BL <50cm were statistically significant when comparing maternal height categories (<152cm; 152–160cm; and >160cm): 57.0%; 45.6%; and 35.8%.
Conclusions: Maternal height was found as a clear determinant of BW and BL. Efforts should be made to increase BL

at each generation of newborns and therefore reach higher maternal height during adulthood. On the other hand, early menarche has been related to obesity during adolescence and therefore reducing expected height gain in girls. Efforts should be done to avoid excessive weight gain during adolescence in Latin American girls.

Key-words: Nutrition during pregnancy, maternal height, birth weight, birth length

How can maternal body mass index help to get a better fetal growth in Chile?

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Background/aims: We aimed to determine the influence of two local body mass index (BMI) standards on fetal growth assessed by birth length (BL).
Methods: Both the total sample of 28,897 pregnant women and a sub-sample that excluded maternal conditions and complications resulting in 11,465 healthy pregnant women (HPW) were included in a prospective cohort study in a maternity hospital of Santiago, Chile. They were nutritionally classified using two BMI-PG standards for nutritional classification during pregnancy: the Rosso and Mardones (RM) chart and the Atalah et al (AEA) chart. Women using each of the two classifications at the beginning of pregnancy were compared in their proportions delivering BL< 50cm. Those proportions in each nutritional status category were compared using a Mc Nemar Chi-squared test.
Results: Differences in the distribution with BL< 50cm were all significant between women nutritionally classified by RM chart and by the AEA chart, both for the total sample and the subsample of HPW. In the total sample the distributions of cases falling in the nutritional categories of underweight, normal, overweight and obese women, were the following: for RM: 17.4%, 36.4%, 18.3% and 27.9%, meanwhile for AEA were the following: 10.2%, 51.1%, 27.4% and 11.3%. In the subsample of HPW the distributions of cases falling in the nutritional categories of underweight, normal, overweight and obese women, were the following: for RM: 22.2%, 35.7%, 18.8% and 23.2%, meanwhile for AEA were the following: 13.3%, 53.0% 25.3% and 8.4%.
Conclusions: There was a significant tendency for a misclassification of BL< 50cm cases by the AEA standard. Both in the total sample and the subsample of HPW, the AEA standard showed that the normal nutritional status category during pregnancy concentrated more of those cases than the RM chart in the same category.
Key-words: Nutrition during pregnancy, body mass index, birth length.

Adult mortality in the Dominican Republic: is there an association with the early origins of disease?

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Background/aims: Dominican Republic has a general mortality of 6.25 per 1,000 (2013). Mortality by cause has not yet been reported for this country. Year 2010 has the most accurate reporting of deaths certificates regarding sex and age; just 5.6% did not present them. However, the only available report of causes of death from the death certificates is from year 2004; just 7.2% were ill defined causes of death. This study aims to report adult mortality rates by cause in year 2010 using the distribution of causes of death observed in year 2004 in the Metropolitan Region which has the lowest figures for under reporting (apparently none). It was also aimed to calculate their mortality rates for 2010 in their first leading causes and compare its mortality structure, expected to be from the developing world, with that structure studied by Barker (IJE 2006; 35: 886-7).
Methods: Mortality rates by cause of death were calculated for years 2004 and 2010 using the causes of death distribution of 2004. Coronary heart disease, considered as the most important cause of death linked to the early origins of disease in Barker's work, was equivalent to acute myocardial infarction.
Results: Mortality by the first leading causes of death in the Metropolitan Region had the following rates: a) Acute myocardial infarction: 1/1,000; b) Diabetes Mellitus: 0.5/1,000; c) Traffic accidents: 0.4/1,000; d) Cardiac insufficiency: 0.3/1,000; e) Stroke: 0.3/1,000. Myocardial infarction in the UK reached in 2002 a similar rate that in the Dominican Republic although it was reduced by one half in year 2010 (BMJ 2012. 344. DOI: 10.1136/bmj.d8059).
Conclusions: This mortality structure, which is not concentrated in infectious diseases as in less developed countries, would allow associations to the perinatal factors as studied by Barker and be part of the early origins of disease reserch.
Key-words: Causes of death, myocardial infarction, developing world.

The type of feeding at 3 months is related to meat intake at pre-school age: preobe study

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Background/aims: We aimed to identify the different relationships between the infant feeding type in the first 6 months of life, total protein intake and meat intake at pre-school age.
Methods: 203 children participating in the PREOBE project

(www.ClinicalTrials.gov NCT01634464) were studied in the present analysis. Feeding type was enquired to the mothers at 3 and 6 months of life in the offspring, and it was classified in 3 groups: Exclusive breastfeeding (EBF) n=90, infant formula feeding (IFF) n=45, and mixed feeding (MF) n=68, when the babies received both types of feeding. Dietary assessment in children aged 3.6±0.42 (no group differences p<0.07) was performed using 3-day food diaries, protein intake g/Kg/day and the percentage of total energy value (%TEV) from meat, were analysed using the DIAL software for assessing diets and food calculations. ANOVA test and Bonferroni post-hoc correction was performed for data analysis using IBM SPSS Statistics 21.0.

Results: It was seen that at 3 and 6 months, 44% and 17% received EBF; 22% and 52% received IFF; and 34% and 31% received MF respectively. The protein Recommended Dietary Allowance (RDA) for children between 1-3y is 1.05g/Kg/day and 4-8y is 0.95g/Kg/day. There were not statistically significant differences between the total protein intake at pre-school age (EBF 3.5±0.7; IFF 4.0±0.7 and MF 3.7±1.1g/Kg/day) and feeding type received at 3 mo (p=0.435) and 6 mo (p=0.839). However, the children who received IFF at 3 mo, showed higher %TEV intake from meat (18.9±2.2%) at 3.6y versus those who received EBF (12.5±4.1%) or MF (12.1±4.5%) (p=0.001), although this association was not demonstrated with the type of feeding received at 6 mo. Nevertheless, BMI z-score at 3.6y did not show significant differences between groups 0.62±0.75 (p=0.340).

Conclusions: The intake of protein in all children is more than the RDA according to the Dietary Reference Intakes, regardless of the type of feeding received in the first 6 mo of life. The higher energy intake from meat at pre-school age seems to be related with infant formula feeding during the first months of life. This work suggests a potential protective role of human milk against the development of obesity, which could be related to the programming of taste and eating behaviour, owing to low protein and saturated fat of meat. The contrary effect is shown in children who were fed with infant formula.

Key-words: breastfeeding; infant formula feeding; offspring

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Soft-drinks and childhood obesity in Northwest Argentina

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Background/aims: childhood obesity is an increasing problem in Northwest Argentina, even among the low income population. In 2013 the prevalence of obesity was 17.6% among children 0-6 years of age, beneficiaries of state-funded maternal-child health programs. It is therefore important to identify nutritional risk factors. We analyzed the association between the consumption of beverages and childhood obesity.

Methods: The study was conducted in the provinces of Jujuy, Santiago del Estero, Tucumán and Catamarca. In each capital city we randomly selected primary health clinics proportionally to the existing numbers. In 16 clinics we surveyed 1107 mothers of children 0-6 years of age. We collected mother and child anthropometric data, and information about children's food and beverage consumption in the previous day. For this report we included children 2 to 6 years of age (N=640).

Results: Consumption of natural or artificial juices, flavored water, tea, coffee or chocolate milk were not associated with obesity. A greater percentage of children who consumed soft-drinks were obese (>5 glasses 20.9%; 1-5 glasses 16.1%; none 9.8%; p=0026). On the other hand, a smaller percentage of children who drank water were obese (> 5 glasses 5.9%; 1-5 glasses 15.1%; none 20.5%; p=0.001). In multivariate logistic regression models the likelyhood of being obese was 2 to 4 times higher among children who consumed soft-drinks (>5 glasses OR=4.0, 95% CI 1.3-12.9; 1-5 glasses OR=2.2, 95% CI 1.1-4.1). Children who drank 5 or more glasses of water had less than half the likelyhood of being obese (>5 glasses OR=0.4, 95% CI 0.2-0.8; 1-5 glasses OR=0.5, 95% CI 0.2-1.3).

Conclusions: The results contribute to the evidence linking soft-drink consumption and obesity by showing a dose-effect at early ages. Results also highlight the importance of implementing warning labels, sugar content and advertising regulation policies, as well as risk communication strategies.

Key-words: Childhood obesity, soft-drinks, Argentina

4TH INTERNATIONAL SYMPOSIUM ON METABOLIC PROGRAMMING AND STRESS AND THE 1ST MEETING OF IBERO-AMERICAN CHAPTER OF DOHAD SOCIETY

POSTERS

Musical intervention in mother-infant pairs moderates the effects of IUGR on palatable food preferences during childhood in girls.

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Background/Aims: Children born after intrauterine growth restriction (IUGR), especially girls, show an increased intake of palatable foods in several developmental stages, which likely contribute to their increased risk for obesity over the life course. Our group has been showing that alterations in the brain reward system may be involved. Recently, neuroimaging studies suggest that musical exposure activates this brain region. Our objective was to evaluate the impact of an intervention in mother/infant pairs (exposure to music classes) on feeding behavior during childhood with regards to birth weight.

Methods: Controlled longitudinal study evaluating 56 children exposed (structured musical intervention between 2005 and 2007) or not (populational communitarian age-matched sample) to the musical intervention in anthropometric and nutritional outcomes. A series of general linear model (GLMs) adjusted for socioeconomic status (SES) and maternal education were performed to evaluate the interaction between music exposure, birth weight and sex on the consumption of different types of foods using a food frequency questionnaire.

Results: There was an interaction between the birth weight, sex and exposure to the musical intervention with the consumption of sugar during childhood (Wald=7.87, df=2, p=0.02). In which girls, non-exposed girls eat more sugar as the birth weight decreases (B=-8.673, p<0.0001) without effect on the girls exposed (B=3.352, p=0.15) or in boys (exposed B=2.870, p=0.44 and non-exposed B=3.706, p=0.236). There were no other effects suggesting that the finding is specific for sweet foods.

Conclusions: the data suggest that an early intervention in mother/infant pairs may moderate the effects of IUGR on palatable foods' preference in girls.

Key-words: IUGR, musical intervention, palatable food.
Financial support: FIPE/HCPA

Liver glucose metabolism in post-absortive rats is altered by food restriction since birth

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Background: WInvoluntary food restriction impairs growth in humans and rodents. As blood glucose control remains a priority, the liver glucose production (LGP) through glycogenolysis and gluconeogenesis in rats under food restriction since birth was investigated.

Methods: Male Wistar rats were raised in 6-puppies litters and fed freely after weaning (group GC) or were subjected to food restriction (group GR) by increasing litter size (12 puppies) and decreasing food supply to 50% after weaning. At 50 days of age, after overnight fasting, the animals had their liver perfused in situ with buffer (basal perfusion) or buffer containing alanine (ALA, 10 mM) or lactate (LAC, 5 mM) or glucagon (GLU, 1 nM) or adrenaline (ADR, 1 μM) (stimulated perfusion). The areas under the curve (AUC) for glucose production (μmol x glucose x g-1 of liver) were compared through t-test at the significance level of 5%.

Results: The body weight of the GC was 120–230g, while that the age-matched GR was 90–140g. The basal LGP was markedly greater (p<0.01) in the GR (11.76±3.80; n=19) than in the GC (0.89±0.25; n=26). The LGP in the presence of ALA and GLU was significantly lower (p<0.05) in the GR than in the GC (ALA 0.83±0.17 in the GC; 0.45±0.17 in the GR; n=5–8) (GLU 0.27±0.02 in the GC; null in the GR; n=4–6). The LGP in the presence of ADR was exceedingly greater (p<0.01) in the GR (GC 0.92±0.71; GR 62.99±13.09; n=4–6), and the LGP in the presence of LAC did not differ between the groups (GC 4.27±1.23; GR 3.83±1.73; n=5–6; p>0.05). Numerical data are the means±SDs of the AUCs.

Conclusions: The peculiar pattern of LGP in food-restricted rats (high basal LGP and intense ADR-stimulated glycogenolysis) may have important consequences to blood glucose control, especially during challenging instances, such as physical exercise and hypoglycemia. It remains to be determined if this pattern is a manifestation of metabolic programming.

Key-words: Metabolic program, larger litter, food restriction, gluconeogenesis

Availability of intracellular camp regulates activation of liver glycogen breakdown in high-fat diet fed mice.

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Background: Liver glycogen catabolism was evaluated in male Swiss mice fed a high-fat diet rich in saturated fatty acids (HFD) or normal fat diet (NFD) during one week.
Methods: Liver glycogenolysis (LG) and liver glucose production (LGP) were measured either under basal or stimulated conditions (infusion of glycogenolitic agents). Thus, isolated perfused livers from HFD and NFD mice were infused with glucagon, epinephrine, phenylephrine, isoproterenol, adenosine-3'-5'-cyclic monophosphate (cAMP), N6, 2'-O-dibutyryl-cAMP (DB-cAMP), 8-bromoadenosine-cAMP (8-Br-cAMP) or N6-monobutyryl-cAMP (N6-MB-cAMP). Moreover, glycemia and liver glycogen content were measured.
Results: The HFD diet significantly increased inguinal and periepididymal fat deposits. Glycemia, liver glycogen content and basal rate of LGP and LG were not influenced by the HFD. However, LGP and LG were lower ($p<0.05$) in HFD mice during the infusions of glucagon (1 nM), epinephrine (20 μM) or phenylephrine (20 μM). In contrast, the activation of LGP and LG during the infusion of isoproterenol (20 μM) were not different (HFD vs. NFD). Because glucagon showed the most prominent response, the effect of cAMP, its intracellular mediator, on LGP and LG was investigated. cAMP (150 μM) showed lower activation of LGP and LG in the HFD group. However, the activation of LGP and LG were not influenced by HFD whether DB-cAMP (3 μM), 8-Br-cAMP (3 μM) or N6-MB-cAMP (3 μM) were used.
Conclusions: Considering that glucagon and cAMP, but not cAMP analogues, showed lower effects on LGP, we can suggest that the process of inactivation of cAMP overcomes its formation in livers of HFD mice. Since the activation of LGP and LG depends on the intracellular availability of cAMP, it can be concluded that cAMP played a pivotal role on the activation of LG in high-fat diet fed mice.
Key-words: Metabolic program, larger litter, food restriction, gluconeogenesi

Genetic expression of *Adipoq* and *Tnf* adipokines in monosodium glutamate-treated obese rats submitted to exercise

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Background: Rats treated with monosodium L-glutamate (MSG) in the first days after birth develop obesity in adult life and present insulin resistance, glucose intolerance, dyslipidemia and cardiovascular dysfunction, which are typical symptoms of metabolic syndrome carriers. Regular physical activity is an important tool in the obesity control and associated comorbidities, improving inflammatory condition typical of an obese individual. The aim of this study was to evaluate the genetic expression of adipokines in the adipocytes of MSG-treated obese rats submitted to swim training.
Methods: Obesity was induced by neonatal MSG administration. Exercised rats (MSG and control, CON) were subjected to swim training for 30 min for 10 weeks, whereas their respective controls remained sedentary (called SED rats). Total RNA was obtained from sections of the mesenteric adipose tissue of the rats. mRNA levels of adiponectin (*Adipoq*) and tumor necrosis factor alpha (*Tnf*) adipokines were quantified by quantitative Real-Time Polymerase Chain Reaction (qRT-PCR).
Results: The MSG treatment in the first days of life allowed the installation of obesity in rats. There was no statistical difference in the expression of Adipoq between MSG-SED and CON-SED groups. In the exercise-trained control group, the expression of Adipoq was 6-fold higher compared to the MSG-CON ($P<0.05$), which was not observed in the MSG-obese rats. *Tnf* expression significantly increased approximately 4 times in the MSG-SED group in comparison with the CON-SED group. Nevertheless, in both groups, exercise did not alter the expression of *Tnf* in the mesenteric adipose tissue.
Conclusions: The regular physical activity was not capable to correct the expression of proinflammatory adipokines in MSG-obese rat adipocytes. Other factors or adipokines cannot be discarded. More studies are necessary to determine which adipokines have altered expression by physical activity and the resulting effect in energy homeostasis.
Key-words: Mesenteric fat, qRT-PCR, swim training.

Associations of prenatal growth with maternal anthropometry in Uruguay.

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Background/aims: There is no information available on the joint association of pre-gestational body mass index (BMI-PG) and weight gain during pregnancy (WG-P) on birth weight categories < 3000g and ≥ 4000g in Uruguay. To determine the prevalence of the different categories of BMI-PG and WG-P and to study their associations with birth weight categories < 3000g or intrauterine growth retarded (IUGR) and ≥ 4000g or macrosomic newborns in a national sample of mothers and their offspring.
Methods: The 23,832 pregnant women were included in a prospective cohort study, being nutritionally classified using BMI-PG according to the Institute of Medicine norms of the USA. WG-P was also classified using a recommendation from Denmark. Independent and combined associations of BMI-PG

and WG-P with birth weight categories <3000g and ≥4000g were analyzed using relative risk (RR).

Results: RR values for IUGR and macrosomic newborn were all significant in their independent associations with BMI-PG and WG-P. High risk of IUGR was observed in pregnant women with low BMI-PG meanwhile high risk of macrosomia was observed in pregnant women with high BMI-PG (overweight or obese). All those associations were also observed in the combined associations of BMI-PG and WG-P.

Conclusions: There was an independent and combined effect of the maternal parameters on perinatal outcomes. It is suggested to compare these results, which used BMI-PG from the USA and WG-P categories from Denmark, with other BMI-PG and WG-P categories.

Key-words: Nutrition during pregnancy, birth weight, body mass index, weight gain.

Height, body mass index and inappropriate gestational weight gain as risk adverse intrauterine growth in Chile and Uruguay

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Background/Aims: The newborn risks (NR) arising from an adverse intrauterine growth of women from Chile and Uruguay are unknown. NR given by maternal height, body mass index (BMI) and the inappropriate gestational weight gain (GWG) are compared between Chile and Uruguay.

Methods: Comparative study of two prospective data cohorts consisting of 11,466 pregnant women from Chile and 23,832 from Uruguay and their respective NRs. The pre-gestational nutritional status according to the BMI-pre-gestational (BMI-PG) has been classified in accordance with the World Health Organization (WHO) criteria. The GWG has been classified according to the Institute of Medicine from the National Academy of Science of the United States (IOM). The association between the intrauterine growth retardation (IUGR), the macrosomia and the low height with the BMI-PG criteria, women´s height and GWG has been studied. The risk of having an adverse result with a binary regression showing the Odd Ratios (OR) has been determined.

Results: When the NRs of both studies were compared, it was noted that with a similar height category, BMI-PG and GWG, more cases of IUGR and <50cm long were found in the Uruguayan infants. The Uruguayan infants presented a greater risk of IUGR and doubled the chances of birth length <50cm long in comparison with the Chileans infants. More cases of macrosomia were found in the latter.

Conclusions: Given the positive and statistically significant association between the intrauterine growth and the women´s anthropometry over the perinatal results, assessing the BMI from the beginning as well as the GWG is of utmost importance in order to give an appropriate guidance to women. Besides, investigating the causes why pregnant women in Uruguay develop a greater risk of IUGR under similar anthropometric categories is herein suggested.

Key-words: Nutrition during pregnancy, birth weight, body mass index, gestational weight gain, and birth length <50cm.

Perinatal mortality, weight-height evaluation and neuropsychomotor development of 6-month old children belonging to a birth cohort in the western region of São Paulo, Brazil

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Background/Aims: A great number of studies suggest the association between environmental aggressions suffered in utero or in early childhood and the emergence of chronic diseases throughout life. The aim of this project is to determine the mortality rate and describe the weight, height and neuropsychomotor development of 6-month old children belonging to a birth cohort in the western region of São Paulo/SP – Brazil, which includes patients from 5 Basic Health Units.

Methods: The perinatal mortality was calculated in the Rio Pequeno and Raposo Tavares neighborhoods using statistics from official government databases from 2012 and standardized according to the number of pregnant women, resulting in the cohort's Standard Mortality Ratio (SMR). Data on children's weight, height and body mass index were obtained at routine visits, and the Z-score on the indexes of weight/age, height/age, weight/height and BMI/age were calculated, according to the curves proposed by the WHO in 2006. Evaluation of cognitive, language and motor development was obtained through Bayley Scales of Infant Development (BSID).

Results: The SMR of the cohort was 2.36 times higher than the reference neighborhoods, with CI = 1.05–4.86, according to the Wald index. The height/age (M = –0.7381) and weight/age means (M = –0.2709) found are inferior to those expected by the WHO. As for the neuropsychomotor development, there were 7 children below or at the 5th percentile on cognitive (1.36%), 15 on language (2.93%) and 40 on motor development (7.84%).

Conclusions: The high SMR observed suggests adverse conditions during pregnancy. The low height for this age group may be fruit of intrauterine deprivation or of nutritional, environmental or inadequate health conditions in the child's first few months of life. Lastly, lowest score and greatest standard deviation were found on the motor component, indicating that it is also rapidly influenced by environmental factors.

Key-words: Perinatal mortality, weight-height infant, neuropsychomotor Bayley.

Do parental harmful life-style factors and their socio-economic status affect children's growth in Venezuela?

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Background/Aims: Maternal smoking and alcohol consumption during pregnancy have been studied as risk factors that affect newborn's health. Less is known about the potential effects of parental harmful habits on older children's growth. Our aim was to measure to which extent these lifestyle behaviors compromise children's growth living in disadvantaged conditions ***Methods:*** Anthropometric measures were obtained in 2081 children between 7 to 12 years old attending public and private schools in Venezuela. Height and BMI were converted to z-scores according to WHO international growth reference. Socio-demographic and lifestyle factors were obtained through a validated semi-structured questionnaire.
Results: Children whose mother were smokers had a significant lower height z-score (mean difference: 0.27; 95% CI: 0 – 0.55; p=0.05), compared to those who were not. This result was maintained when corrected for social class and height of the mother, but not when corrected for target height. Z-scores for height are significantly related to social class of the mother, but only when corrected for target height. The mean BMI z-score decreased significantly with social class, and this effect remains significant when corrected for BMI and smoking status of the mother. Although there is a significant association between smoking and alcohol use in mothers, these results are not affected by additionally adding this factor in the models.
Conclusion: It appears that the relation between height or BMI, and smoking, alcohol intake and social status is complex. Height z-scores are related to social class when corrected for target height, and to smoking when not, but target height itself is associated with neither of these factors, and maternal smoking is not significantly associated with social class. Possibly, the relation between height and current maternal smoking habits express how living in disadvantaged conditions can influence normal growth in exposed children, irrespective of social class. Further research is needed.
Key-words: Parental harmful habits, smoking, alcohol intake, school age children, Venezuela

Involvement of leptin in defective inflammatory response in culture of lung endothelial cells stimulated by LPS from intrauterine undernourished rats.

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Background/aims: Intrauterine undernourishment can induce a range of fetal adaptations, which can lead to permanent alterations in adult life, such as reduced inflammatory response. The vascular endothelium is closely related with the circulatory control, and plays an important role in cellular and molecular events which occur during immune system reactions and tissue injuries. Leptin, a hormone mainly synthesized by adipose tissue, is involved in various biological systems, acting in the food intake control and energy metabolism; in addition, it modulates immune response, hematopoiesis and lymphopoiesis. To evaluate the expression of long-form leptin receptor in rat pulmonary endothelial cells intrauterine malnutrition and its role in the production of lipid mediators.
Methods: Pulmonary endothelial cells were obtained from intrauterine malnourished rats or nourished rats, at 12 weeks age. These cells were stimulated with leptin (10ng/mL) or LPS (1µg/mL) or leptin plus LPS. Six hours after the stimulation, the production of inflammatory mediators (PGE2 and LTB4) and western blots analysis of leptin receptor were performed. All the procedures used in this study were approved and are in accordance with the rules established by Ethics Committee of UNIFESP.
Results: Western blot assay showed that expression of long-form leptin receptor is decreased (63%) in the primary cultures of endothelial cells derived from intrauterine malnourished rats. Leptin alone did not induce any alteration on the levels of the inflammatory mediator evaluated, whereas LPS increased the PGE2 (250%) and LTB4 (29%) levels. Only in endothelial cells from nourished rats, leptin enhanced lipid mediators production induced by LPS (PGE2, 28% and LTB4, 18%). Interestingly, the same was not observed in endothelial cells from intrauterine malnourished rats.
Conclusion: Our preliminary results suggest that intrauterine malnutrition dowregulates leptin receptor expression and modulate lipid mediators production in primary culture of pulmonary endothelial cells stimulated by LPS.
Key-words: Intrauterine undernourishment, leptin, primary cell culture, endothelial cells, inflammation
Financial support: FAPESP (2010/01404-0, 2012/51104-8), CNPq

The effects of fusarium oxysporum metabolites on myeloperoxidase activity

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Background/Aims: The metabolites of the fungus F. *oxysporum* cause inflammation in the skin and this study aimed to quantify the inflammatory response through biochemical analysis of the enzyme myeloperoxidase (MPO).

Methods: After approval by the Ethics Committee from State University of Maringa (UEM; nº 080-2010), 70 male Wistar rats (n=5), underwent manual epilation of the back, near of the front legs, under anesthesia (Ketamine and Xylazine mixture; 0.1ml/100g body weight). The control saline solution and the crude extract of the fungus were applied intradermally in the treated animals. At 1, 3, 6, 12, 24, 36 and 48hours after the application, the rats were sacrificed. Then the activity of MPO was assessed by homogenate supernatant of the skin sections from both experimental groups (treated and control). The MPO activity was determined by measuring the absorbance (optical density; OD at 450nm) and for this we used an ELISA reader. Data were subjected to analysis of variance one-way ANOVA.

Results: There was an increase of MPO activity when the skin of healthy rats was exposed to the crude extract of F. *oxysporum* fungus at the times 3, 6, 12 and 24hours, when compared to its control ($p<0.05$). However, in the times 1, 36 and 48hours there was no statistical difference when compared with their respective controls.

Conclusions: The activity of the enzyme MPO is used as an indicator of the presence of polymorphonuclear leukocytes. Therefore, it is concluded that the crude extract of F. *oxsporum* have high rates of leukocyte migration in times 3, 6, 12 and 24hours. Nonetheless, at 1, 36 and 48hours this migration was dramatically reduced. Therefore, it is assumed that the crude extract of this fungus has a proinflammatory role with rapid inflammatory effect in epithelial tissue.

Key-words: Fusarium oxysporum, metabolites, myeloperoxidase activity

Low-protein diet during suckling phase disturbs lipid-profile as that high-fat diet does in adult rat offspring

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Background/Aims: Metabolic syndrome has been associated with nutritional disturbances in early life, in which lipid profile is one of the pivotal marker. We studied the effect of high-fat diet on the lipid profile of adult rats that were malprogrammed by low-protein diet during the suckling phase.

Methods: Lactating dams were fed a low-protein diet (4%) during the first 2 weeks and a normal-protein diet (23%) during the last week of lactation. At these times, control dams were fed a normal-protein diet. At weaning (21-day-old) male rat offspring were fed a control diet until the age 60th when half of both animal groups were fed a high-fat diet (35%, NP/HF and LP/HF groups) until 90-day-old; while the other half of rats were fed a normal-fat diet (7%, NP/NF and LP/HF groups). Body weight and food intake were evaluated every two days. At 90-day-old, all rats were euthanized, fat pad were weighted and blood samples collected to assess lipid profile, fasting glucose and insulin.

Results: LP/NF were leaner when compared with NP/NF rats ($p<0.01$). However, high-fat diet increased body weight (+22.4%, NP/HF and +16.3% LP/HF), retroperitoneal (+158%, NP/HF and +124%, LP/HF) and periepididymal (+142%, NP/HF and +123%, LP/HF; $p<0.01$) fat pads, even though the magnificence of these was extremely greater in the NP/FH rats. Even normoglycemic, LP/NF rats were hypoinsulinemic (–43%; $p<0.01$) and high-fat diet increased drastically these parameters ($p<0.001$). Lipid profile was altered both by low-protein and high-fat diets. Total-cholesterol (+36%, NP/HF and +24%, LP/HF), HDL-cholesterol (–43%, LP/NF), VLDL-cholesterol (+30%, NP/HF), LDL-cholesterol (+47%, NP/HF and +109%, LP/NF), triglycerides (+25%, NP/HF), Castelli-index-I (+17%, NP/HF and +101%, LP/NF) and Castelli-index-II (+31%, NP/HF and +260%, LP/NF).

Conclusions: Low-protein diet during lactation imprints the metabolism of adult rat offspring to display dyslipidemia, which seems to be not amplified by a high-fat diet regimen.

Key-words: Protein restriction, metabolic programming, obesity, metabolic syndrome

Financial support: CNPq, CAPES

Long-term effects of Buscopan treatment on insulin secretion in postnatal overfeeding rats

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Background/Aims: Insulin plays a key role on animal development. Hypoinsulinemia and hyperinsulinemia during development may cause metabolic diseases later in life. Postnatal overfeeding rats display overweight, increased plasma insulin and impaired glucose/insulin homeostasis. We investigated whether neonatal treatment with the muscarinic antagonist, buscopan, is able to improve the insulin/glucose homeostasis in early overfeeding rat.

Methods: At birth, offspring rats from litters with 9 (B-NL) and 3 (B-SL) pups were intraperitoneally injected with buscopan [0.5 mg/kg body weight (BW)] during the 12 first days of life. Control pup rats (S-NL and S-SL) were injected with saline solution. At 90-day-old, biochemical parameters, glucose/insulin homeostasis and pancreatic islet function were evaluated.

Results: B-SL group displayed smaller BW and hypophagia than S-SL group ($P<0.05$). The B-NL group presented mild hyperglycemia; however, there was no effect on the glucose tolerance. In the SL rats, blood insulin levels was reduced, and glucose-induced insulin secretion, in isolated pancreatic islets were decreased by buscopan treatment ($P<0.05$).

Conclusion: Obesity programmed by reducing litter size is attenuated by early treatment with buscopan that is suggested to be associated with lower insulin secretion.

Key-words: Metabolic programming, insulin, buscopan.

Funding: CNPq/CAPES/FA

Malnutrition in adulthood does not cause metabolic programming

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Background/aims: Stressor insults during critical period of development can predispose offspring to became obese and with the metabolic syndrome as adult. We propose to study whether low-protein diet offered at adulthood is able to program metabolic disruptions in rats.
Methods: Wistar male rats were fed a low-protein diet (4%, LP group) and control rats were fed a normal-protein diet (23%, NP group) from 90-to-120-day-old. At 120-day-old all groups were fed a normal-protein diet until 180-day-old, when body composition, fat pad accumulation, glucose-insulin homeostasis and pancreatic islets function were evaluated. Data were analyzed by Student t-test.
Results: Throughout the low-protein diet treatment the bw gain of LP rat was almost unchanged; At 180-day-old LP rats displayed a slight lean phenotype ($P<0.05$) and a higher catch up growth ($P<0.001$). Beside of this, fat pad accumulation, glycemia and insulinemia in fasting and glucose tolerance test condition, as well as pancreatic islets insulinotropic response to glucose and acetylcholine from LP rats were not statistically different, when compared with NP ones ($P>0.05$).
Conclusions: Our results suggest that low-protein diet insult in adulthood does not program long-term consequences on metabolism. Therefore, our data contribute to identify the sensitive periods of life that predisposes for metabolic disorders in response to nutritional insults.
Key-words: Low-protein diet, adulthood, metabolic programming
Funding: CNPq/CAPES/FA

Myeloperoxidase, inflamation and cardiac risk in tobacco growers

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Background/aims: Pesticides are widely used around the world for pest control and guarantee increased productivity. They are harmful to insects and weeds, but also toxic to humans. Pesticides are related to alterations of the immune system, are neurotoxic, and involved with alterations in endocrine system, besides interfering with metabolism. Exposure to pesticides is monitored by measurement of biomarkers, especially cholinesterase, and through changes in laboratory tests. The most commonly used pesticides are those belonging to the class of organophosphates, whose mechanism of action is irreversible inhibition of cholinesterase. This study aimed to evaluate the activity of serum myeloperoxidase in individuals chronically exposed to pesticides, in addition to other laboratory markers.
Methods: This study included 79 volunteers, divided into 3 groups: 1) EDP: direct exposure to pesticides; 2) EIP: indirect exposure to pesticides, and 3) C: control, volunteers not exposed to pesticides. Laboratory tests for glucose, total cholesterol and fractions, triglycerides, myeloperoxidase (MPO), alpha-1-acid glycoprotein (AGP), high sensitive C-reactive protein (Hs-CRP), cholinesterase and total antioxidant capacity of serum were performed.
Results: High levels of MPO, AGP and glucose were found for the EDP and EIP groups. The Hs-CRP showed a direct correlation with the values of AGP, but was elevated only for the EIP group. Significant reduction in total antioxidant capacity of serum was found in groups EDP and EIP. The cholinesterase values showed significantly higher activity in the EDP group.
Conclusion: Chronic exposure to pesticides can lead to an adaptive increase in cholinesterase activity resulting in decreased anti-inflammatory action of acetylcholine, with increased levels of myeloperoxidase and other markers of inflammation.
Key-words: Pesticides, lipoproteins, inflammation, mieloperoxidase, cardiac risk.

Cross-fostering in suckling male rats can attenuate metabolic dysfunctions in adulthood

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Background/aims: Maternal obesity is associated with lifelong obesity and metabolic disturbances. Nevertheless, changes in lactation period can modify the offspring development. To identify the effect of altered milk composition environment on offspring metabolism in adulthood we used cross-fostering during lactation in male rats.
Methods: Neonate female Wistar rats were subcutaneously injected during the first 5 days of life with monosodium L-glutamate (MSG) at a dose of 4 mg/g body mass. Control animals received a saline solution. At adult age, control and obese female rats were mated. The pups of control mothers were denominated CONF2, pups of obese mothers MSGF2, cross-fostered obese pups with control mothers CR-MSGF2 and cross-fostered control pups with obese mothers CR-CONF2. Milk composition was evaluated. At 120-day-old we evaluated fat tissue accumulation, body weight, plasma glucose, insulin and leptin levels, intravenous glucose tolerant test and insulin secretion from isolated pancreatic islets.
Results: Breast milk composition in obese mother's exhibit higher total cholesterol, triglycerides, glucose, insulin and leptin content. MSGF2 rats displayed higher body weight and fat pad storage as related to CONF2. The

MSGF2 rats presented hyperinsulinemia and hyperleptinemia upon fasting. Pancreatic islets from MSGF2 rats released more insulin upon glucose stimulation. Interestingly, the adoption by control mothers restores the fat pad mass, insulinemia, leptinemia, and insulin secretion from isolated islets in CR-MSGF2. The CR-CONF2 rats impairs the insulin secretion stimulated by glucose, but not presented differences in others parameters compared to control ones.

Conclusions: Cross-fostering attenuated long-term effects of maternal obesity in offspring. Our data suggest that alterations in breast milk composition can mitigate the obesity effects and may prevent the programming of adult diseases.

Key-words: Cross-fostering, obesity, metabolic dysfunctions.

Low-protein diet at later-gestation or early-lactation disturbs offspring's metabolism as different phenotypes

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Background/aims: Nutritional disturbances early in life are drastically implicated on the metabolic syndrome. Both gestation and lactation are sensitive-windows to program metabolic diseases in later life. Here we evaluated the effect of maternal low-protein diet in two different stages of life (later gestation and early lactation) on body composition and metabolic profile of adult rat offspring.

Methods: A low-protein diet (4%) was offered to female Wistar rats at (last 1/3 of gestation, LPG-group) or (first 2/3 of lactation, LPL-group) while normal-protein (23%) was offered to control dams (NP-group) throughout this period. At 90-day-old, male offspring were used to evaluate the body composition (body weight (BW) and retroperitoneal fat pad). The fasting values of glucose, insulin and corticosterone, and the lipid profile were evaluated.

Results: While adult LPL-rat offspring displayed a lean phenotype, lower (BW, –17% and retroperitoneal fat pad, –31%; p<0.001); the LPG-rat offspring showed an obese phenotype, higher (BW, +5% and retroperitoneal fat pad, +64%; p<0.01), when compared with NP-rats. The values of corticosterone were higher in both animals model (LPL-rat, +105% and LPG-rats, +35%; p<0.001) in relation to NP-rats. While the LPL-rats were normoglycemic even hypoinsulinemic (–37%; p<0.001), the LPG-rats were hyperglycemic and hyperinsulinemic (+21% and +31%, respectively; p<0.05). Interestingly, when compared with NP-rats, the lipid profile was not changed in LPG-rat; however, it was severely altered in LPL-rats (Triglycerides, +59%; VLDL-cholesterol, +59%; LDL-cholesterol, +257% and HDL-cholesterol, –52%; p<0.0)1). On this line, the Castelli indexes I and II were higher in LPL-rats (+134% and 286%, respectively; p<0.001) than NP ones.

Conclusions: While low-protein diet during later-gestation programmed an overweight phenotype associated with insulin resistance, this treatment at early-lactation imprinted a lean phenotype associated with high insulin sensitivity and high risk to cardiovascular diseases onset in later life.

Key-words: Intrauterine protein restriction, insulin resistance, obesity, undernutrition, metabolic syndrome

Financial support: CNPq, CAPES

Maternal low-intensity physical exercise blocks small litter-induced obesity in offspring

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Background/aims: Chronic diseases are the main public health problem in the world. Insults in the fetus during critical periods of development can lead to adaptations in the structure and physiology of the fetal body, and thereby increase the risk of diseases later in life. We aimed to evaluate the effect of low intensity exercise training in female rats on blocking obesity induced by overfeeding in early life in male offspring.

Methods: Female Wistar rats underwent low intensity exercise training in treadmill (30% VO2max); 3x/week during pregnancy and lactation. On the same time, the controls were remained sedentary. To guarantee the offspring overfeeding, pups with three days-old were randomly standardized to 3 pups/dam, preferentially male (small litters-SL) or 9 pups/dam (normal litters-NL). Offspring from both mother groups were divided in exercised and sedentary (NLSM, SLSM, NLEM and SLEM). Body weight (BW) was evaluated weekly from birth to 90 days-old. Ninety one-day-old rats from all groups underwent an overnight fasting, and after that were submitted to the intravenous glucose tolerance test (ivGTT), for the determination of glucose and insulin serum concentrations. In the day after the ivGTT, the rats were anesthetized and then euthanized; both retroperitoneal fat pads were isolated and weighted.

Results: The SLSM animals showed an significant increase in the area under BW curve (AUC), retroperitoneal fat pad, AUC of the ivGTT both for glycemia and insulinemia, with the magnitude of the increase around 11%, 22%, 14% and 29% (p<0.05) respectively compared to the NLSM animals. However this difference was not observed in the offspring of exercised mothers, both NLEM and SLEM was similar when compared to the control group. However, the AUC of the ivGTT for glycemia and insulinemia from NLEM showed significantly reduction (–2.3% and –23%, respectively) than NLSM animals (p<0.05). NLEM and SLEM offspring showed a decrease in BW, retroperitoneal fat pad, glycemia and insulinemia, when compared to SLMS ones (p<0.05).

Conclusions: Maternal moderate exercise training in perinatal life can block obesity in offspring submitted to early overfeeding.

Key-words: Low physical exercise intensity, metabolic programming, small litter

Financial support: CNPq, CAPES and Fundação Araucária

Most prevalent systemic diseases of patients examined in the Diagnostic Unit of a Dentistry School: perspectives for an early origins study

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Background/aims: NThe presence of systemic diseases in patients attending a dental checkup is now common. A retrospective study was designed to determine the prevalence of various systemic diseases and their association with periodontal diseases in a population of patients who were examined in Santiago, Chile.
Methods: This study collected information recorded for 647 patients, 289 male and 358 female at the Diagnostic Unit of the School of Dentistry, Pontificia Universidad Católica de Chile, during the first half of 2014. All signed informed consent. The information was recorded, tabulated and subjected to descriptive statistical analysis.
Results: The clinical features of the study showed that 39.1% of the total population reported systemic illness, females 46.4% and males 29.8%. The percentage of sick patients according to their ages increased progressively. Between 15 and 25 years, only the 13.9% claims to be suffering from some chronic disease. Between 36 and 45 years old, the percentage of patients with chronically illness rises to 22.4%; at the end of life, the percentage was 100%. The most frequent diseases were hypertension and diabetes mellitus. Periodontal disease is a common chronic oral inflammatory disease, characterized by destruction of soft tissue and bond, this pathology is most frequent in adults, nearly 70% of the population and increases with the passage of the years. Periodontal disease was a positive association between diabetes and hypertension.
Conclusions: This study shows that the dentist has many patients with chronic diseases. We hope to further study the association of those pathologies to fetal growth in that population and explore the early origins of health and disease hypothesis.
Key-words: Oral health, hypertension, diabetes mellitus, periodontal disease.

Evaluation of the cytotoxic and cytostatic effects of glibenclamide in human lymphocytes in vitro

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Background/aims: Glibenclamide is an oral hypoglycemic drug commonly prescribed for the treatment of type 2 diabetes mellitus, whose anti-tumor activity has been recently described in several human cancer cells. The cytotoxic and cytostatic potentials of glibenclamide were evaluated using human lymphocytes cultures.
Methods: The cytotoxic effect of glibenclamide in therapeutic plasma (0.6 μM) and higher concentrations (10 μM, 20 μM, 40 μM, 80 μM, 120 μM, 240 μM and 480 μM) was assessed by the mitotic index (MI). In addition, the cytostatic effect of glibenclamide was evaluated by the cytokinesis-block proliferation index (CBPI) at 0.6 μM, 10 μM, 100 μM, 240 μM and 480 μM concentrations.
Results: Glibenclamide failed to alter the MI and the CBPI rates at therapeutically plasma concentration. On the other hand, glibenclamide at the 40 μM and higher concentrations produced MI rates that were significantly different from the negative control. The CBPI was significantly different from the negative control when glibenclamide was used at 480 μM. Results demonstrated the cytotoxic and cytostatic effects of glibenclamide in human lymphocytes at 40 μM and 480 μM respectively.
Conclusions: Since glibenclamide has been proposed as a potential anticancer agent either alone or in combination with standard chemotherapeutic drugs, data in the current study demonstrate that glibenclamide is an anti-mitotic and anti-proliferation agent. This fact encourages further investigations on the use of this antidiabetic agent as a chemotherapeutic drug.
Key-words: Glibenclamide, mitotic index, cytokinesis block proliferation index, lymphocytes.

Permeation on skin from free insulin and complexed with cyclodextrins in the process of excisionals wound-healing in rats

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Background/aims: GThe skin lesions are a serious public health problem due to the high level of disability and deformity caused in the individuals, and because of the long time and the cost that healing demands. It has been reported that insulin stimulates the re-epithelialization, proliferation and migration of keratinocytes. The aim of this study was to evaluate the healing effect of two pharmaceutical forms, a gel containing insulin alone and another containing insulin complexed with HPβCD (HPβCD-I) in skin permeation of excisional wounds in rats.
Methods: 48 Wistar male rats, at 50-days-old were used. Rats underwent a manual hair removal from dorsum and forelegs, under anesthesia. After that, two boundaries were performed using a metal marker to delimitate 1cm2 for the known area, parallel to each other. The wounds on the right (Control) were treated with the base gel and those on the left side were treated with insulin with HPβCD gel or gel-I. The wounds were treated daily with topical application of approximately 1g of the proposed formulations. After the periods of 4, 7, 10 and 14 days the rats (n=5 for each time and for each group) were

sacrificed. After that, the heparinized blood was used for determination of serum insulin levels.

Results: The gel HPβCD-I increased serum insulin levels in the bloodstream from the first until the fourteenth day of treatment, while those that were treated with insulin gel, displayed the same increment only at the tenth day of treatment.

Conclusion: The gel HPβCD-I increased the bioavailability of insulin in tissue, favoring the healing process, indicating that this formulation may promote tissue repair as well as acute and chronic wounds, the wounds of the second and third intention of difficult healing process.

Key-words: Insulin, cyclodextrin, healing, permeation.

Neonatal metformin treatment protects against rat obesity onset

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Background/aims: Metformin is an oral antidiabetic most prescribed worldwide. Postnatal early overnutrition can be induced by litter size reduction, which is an appropriate experimental model to study short- and long-term consequences later in life. As metformin is used for the treatment of diseases correlated with metabolic syndrome, the aim of this study was to evaluate if intraperitoneal treatment with metformin can block/attenuate obesity onset induced by litter size reduction.

Methods: Wistar rats were divided in four groups: control saline (CS), control metformin (CM), small litter saline (SLS) and small litter metformin (SLM). Three days after birth, litter sizes were adjusted to 9 (CS and CM) or 3 (SLS and SLM) pups per dam. From the 1st to the 12th day the animals of groups CM and SLM received intraperitoneal injection of metformin at a dose of 100mg/kg body weight (bw)/day. CS and SLS groups received intraperitoneal injections of saline. At day 21, litters were weaned and the weight gain and food consumption was evaluated until 90-day-old. We analyzed the nasoanal length, fat pad stores, fasting blood glucose and insulin levels.

Results: Treatment with metformin did not alter food intake and bw gain to rats from control litters; while, the SLS animals showed increased food consumption and bw compared CS and CM groups, which was reversed by treatment. The SLS animals had higher blood glucose and insulin compared to the CS and CM groups; however, metformin treatment normalized glucose levels of those animals. Remarkable that metformin treatment did not alter the glycemic and insulinemic levels in rats from control litters. The animals SLS had higher nasoanal length regarding group of normal litters, which was reversed by treatment with metformin.

Conclusion: Perinatal metformin treatment attenuates the obesity onset induced by litter size reduction.

Key-words: Metformin, obesity, metabolic syndrome, glycemia, insulinemia

Funding: CAPES, CNPq, Sutopar and Fundação Araucária

Low intensity exercise alters offspring adiposity at 21-day-old from mothers fed cafeteria diet.

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Background/aims: Studies regarding the fetal programming of obesity have shown that maternal nutrition during pregnancy may promote significant changes related to fetal development and metabolic programming. However, the influence of exercise during this period as a protector attenuator of the deleterious effects caused by maternal malnutrition remain unknown. Thus, we investigated the effects of a light physical activity program on the damage caused by the cafeteria diet during gestation and lactation on changes in several parameters related to adiposity in male pups, 21 days old.

Methods: To this end, we used male rats from female rats, 70 days old. At the beginning of pregnancy and throughout the whole period of exercise and lactation the female rats received the cafeteria diet. After birth, the following groups were randomly formed: Sedentary Control Pups (FSC), Trained Control Pups (FTC), Sedentary Cafeteria Pups (FSCA) and Trained Cafeteria Pups (FTCA). At weaning, 21 full days, pups were euthanized and several adipose tissue fat pads were collected and weighed. The subcutaneous and periepididymal pads were treated with collagenase and adipocytes isolated. Brown adipose tissue (BAT) histological analysis was performed in order to verify changes in the adipocyte area lipid droplet area.

Results: The data obtained in our experimental model indicate a related imprinting characteristic of adiposity, such as changes in body weight of offspring for the diet accompanied by weight changes in fat pads, which also suffered the influence of physical exercise. Furthermore, the diameter of periepididymal adipocytes increased significantly in FSCA. On the other hand, FSCA and FTCA subcutaneous adipocytes showed an increase. In BAT, adipocyte and lipid droplet areas were higher in FSCA and FTCA. In summary, we conclude exercise performed by mothers during pregnancy only decreased diameter in periepididymal pad and did not revert the deleterious effects of cafeteria diet on brown adipose tissue morphology.

Key-words: Exercise; diet; metabolic programming; adipose tissue.

Cross-gavage of fecal microbiota during lactation leads to different outcomes in adulthood depending on the phenotype of the mother

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Background/aims: One of the factors linked to obesity are alterations in the gut microbiota composition. We aimed to study the effect of gavage of fecal microbiota (FM) taken from obese and lean mothers during lactation at adulthood.
Methods: Wistar rats (F0) were breed and had their litter reduced to 3 pups per dam (small litter, F1SL), females and males. At 90 days old, the F1SL male and female, from different litters, were breed and had their litter reduced (F2SL). The control group was standardized at 9 pups per dam (normal litter, NL). Some litters of those groups received gavage of diluted feces from the dams in saline solution (1g/kg of body weight, BW), creating two more groups. The NL group received gavage from the feces of the F1SL mother, NLOMC (normal litter obese microbiota), and the F2SL group received gavage from feces taken by NL dams, F2SLLMC (F2 small litter lean microbiota). The BW and food intake was evaluated 3 days per week from the weaning until 90 days-old. At 91 day-old, rats from all groups underwent an overnight fasting, and after that, were submitted to the intravenous glucose tolerance test (*ivGTT*), for the determination of glucose concentrations. In the following day, the rats were anesthetized and euthanized and the fat pads removed and weighted.
Results: The treatment leads to an increase in the area under the curve (AUC) of BW and final body weight in all groups, compared to NL ($p<0.05$), but only the NLOMC group showed an increase in the relative food intake. The same pattern was observed in the retroperitoneal and periepididymal fat pads, compared to NL ($p<0.05$). Regarding the ivGTT, the NLOMC and F2SL groups showed glucose intolerance, and the early treatment in the however F2SLLMC group shows blocked the glucose intolerance. The number of animal was (n=9–15 rats from at least 3 different litters per group).
Conclusions: The early gavage of FM from obese (F1SL) mothers leads to obesity and glucose intolerance, but the gavage of FM from lean mothers was not able on blocking the installation of obesity. More studies are needed to better understand the mechanisms behind the observed dichotomy.
Key-words: Microbiota, obesity, small litter

Female Wistar rats develop obesity after gavage of fecal microbiota from small litter mothers during lactation

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Background/aims: One of the factors linked to obesity are alterations in the gut microbiota composition. We aimed to study the effect of gavage of fecal microbiota (FM) on female rats during lactation at adulthood.
Methods: Wistar rats were breed and their offspring had the litter size standardized to 9 pups per dam (control, CTL). Some litters received from the 10th day of life until the 25th day of life gavage of diluted feces from obese small litter (SL) females in saline solution (1g/kg of body weight, BW), creating the group MC (microbiota). The BW and food intake were evaluated 3 days per week from the weaning until 90 days-old. At 91-day-old, rats from all groups underwent an overnight fasting and after that, were submitted to the intravenous glucose tolerance test (ivGTT), for the determination of glucose and insulin serum concentrations. In the following day, the rats were anesthetized and euthanized, and the fat pads removed and weighted.
Results: The early gavage did not change the area under the curve (AUC) of BW in the MC group, but leads to an increase in the AUC of absolute and relative food intake, 20% and 21.6%, respectively ($p<0.05$). This was also observed in the final BW and body length, 6% and 3.78%, respectively ($p<0.05$). The same pattern occurred in the fat deposition, with all fat pads showing an increase in the MC group, with the lowest magnitude of increase at 36.18% and the biggest at 64.84% ($p<0.05$). Regarding the ivGTT, the MC group showed glucose intolerance and were hyperinsulinemic in fasting condition when compared to CTL ($p<0.05$). The number of animals was (n=9–15 rats, from at least 3 different litters per group).
Conclusions: The early gavage of FM from obese mothers leads to obesity and glucose intolerance. More studies are needed to better understand the mechanisms behind this effect.
Key-words: Microbiota, obesity, female rats

Low protein diet during puberty programs to hypertension

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Background/aims: Clinical and experimental studies have suggested that protein restriction during critical phases of life may lead to cardiometabolic syndrome, which may depend on central nervous system changes. Recently, we have shown that puberty is, as well, a susceptible phase to develop obesity and glucose metabolism dysfunction, which depends on autonomic dysfunction. In this context we hypothesised that low protein diet at puberty may lead to hypertension dependent on hyper activation of sympathetic nervous system.
Methods: Adolescent Wistar rats (30 to 60 day-old) were

exposed to a low protein (LP) diet (4% of protein). Control animals had access to normal commercial (NP) chow (23% of protein). Basal blood pressure and pulse interval were recorded in 120-day-old rats. Vascular and cardiac sympathetic and cardiac parasympathetic activity was estimated via spectral analyses and baroreflex sensitivity was evaluated with the sequence method. Student t-test was used to compare groups, with the GraphPad Prism software version 6.01.

Results: LP diet increased in 27% retroperitoneal fat deposits, compared with rats exposed to NP diet (p<0.05). LP animals showed significant greater basal blood pressure levels compared NP animals (108 vs 96 mmHg, respectively, p<0.01). Pulse interval was similar between groups. Vascular and cardiac sympathetic activity was, respectively, 42% and 86% greater in LP animals (p<0.05). Cardiac parasympathetic activity was 38% reduced in LP compared with NP rats (p<0.05). Baroreflex sensitivity was 26% lower in LP compared with NP animals (p<0.05).

Conclusions: LP diet exposition during puberty programs to hypertension later in life, which may depend on baroreflex and cardiovascular autonomic dysfunction.

Key-words: Cardiovascular programing, hypertension, low protein diet, autonomic nervous system.

Myeloperoxidase, inflammation and cardiac risk in pregnant women

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Background/aims: Pregnancy is a condition in which is important to evaluate laboratory parameters for clinical purposes. So, new biomarkers may improve this monitoring of pregnant women. The aim of this study was to evaluate the utility of serum myeloperoxidase and current metabolic and inflammatory parameters for monitoring pregnant women each trimester.

Methods: A total of 88 voluntary participated in this research. Some biochemical parameters such as uric acid, total cholesterol, LDL-cholesterol, triglycerides, HDL-cholesterol, albumin, total protein, glucose, glycosylated hemoglobin (HbA1c) and total antioxidant capacity (TAC) were performed. Leucocyte counting, serum myeloperoxidase, ultrasensitive CRP (us-CRP) and serum alpha-1-acid glycoprotein were analyzed in different trimesters of pregnancy. Data were analyzed using SPSS program.

Results: Significant laboratory changes in different trimesters of pregnancy were observed. The total cholesterol (at 1st trimester, 173.2±31.9mg/dL; 2nd trimester, 227.7±45.5mg/dL; 3rd trimester, 244.4±42.6mg/dL, and control group (CG), 184.6±29.6mg/dL); LDL-c (1st trimester, 93.8±22.6mg/dL; 2nd trimester, 128.9±37.6mg/dL; 3rd trimester, 139.4±33.5mg/dL, and GC 103.8±24.6mg/dL); triglycerides (1st trimester, 105.6±51.3mg/dL; 2nd trimester, 193.9±84.3mg/dL; 3rd trimester, 217.9±71.1mg/dL; CG, 91.5±32.0mg/dL); albumin (1st trimester, 4.0±0.2 mg/dL; 2nd trimester 3.7±0.2mg/dL; 3rd trimester, 3.5±0.2mg/dL; CG, 4.1±0.2mg/dL); TAC (1st trimester, 46.6±4.3%; 2nd trimester, 45.7±3.7%; 3rd trimester, 43.8±2.45%; CG 59.9±1.9%); us-CRP (1st trimester, 4.6±5.5mg/L; 2nd trimester, 7.3±4.9mg/L; 3rd trimester, 7.0±6.8mg/L; CG, 3.0±2.8mg/L), and leukocytes count (1st trimester, 7540±2057cell/mL; 2nd trimester 8605±1582cell/mL; 3rd trimester, 10398±2990cell/mL; CG, 7500±2038cell/mL). No statistical changes were observed on MPO, glucose, HbA1c, HDL-cholesterol and uric acid compared to the control group.

Conclusion: The occurrence of metabolic changes in pregnancy is well known, but this study show possible systemic inflammation occurrence in those women. Significant increased us-CRP levels were found in all quarters, what may indicate possible risk of cardiac events. However, prospective studies are needed to assess the association between pregnancy, cardiovascular risk and MPO.

Key-words: Pregnancy, us-PCR, MPO, inflammation, cardiac risk.

Exercise training performed by parents modulates offspring adiposity

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Background/aims: Childhood obesity is on the verge of becoming an epidemic, which origins are linked to environmental and genetic factors. In that regard, parents play an important role in the development and metabolic programming of their offspring. On the other hand, exercise attenuates the deleterious effects of obesity reducing adiposity of genitors and offspring as well. Hence, the aim of the study was to determine the effect of exercise training performed by parents on the development of offspring adiposity. ***Methods:*** For such purpose, 16 male and 16 female mice were divided into sedentary and trained (aerobic treadmill exercise) groups. After 6 weeks of protocol animals were bred and following birth male and female offspring groups randomly assigned: OS (offspring from sedentary parents), OT (offspring from trained parents), OST (offspring from sedentary male and trained female) and OTS (offspring from trained male and sedentary female). After weaning, the animals were euthanized and several fat depots collected. Either One-way or Kruskal–Wallis were used in data analysis.

Results: Lee index was lower in male OT when compared to the other groups and in female OT when compared to OST and OTS (p<0.05). Female OT mesenteric fat pad weight was lighter when compared to the other groups (p<0.05). Male OT total visceral fat weight was lighter in relation to OST and OTS and female OT total visceral only in comparison to OST (p<0.05).

Conclusions: In summary, aerobic exercise performed by genitors contributes to a reduction of total visceral adiposity in both male and female offspring.

Key-words: Epigenetics, exercise, offspring, adipose tissue.

Analyses of macrosomic newborns

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Background/aims: We aimed to describe the possible association between maternal age and birth weight in macrosomic newborns who were delivered during January 2011 at the Instituto Nacional Materno Perinatal (INMP).
Methods: There were 1237 deliveries during January 2011 at the INMP: 539 were cesarean sections and 698 vaginal deliveries. 124 deliveries resulted in macrosomic newborns (10%). The possible associations of maternal age with both birth weight and birth length were analyzed using a box-and-whiskers plot stratified by 5 maternal age groups. The so-called locally weighted scatter plot smooth (lowess) was used to show the possible linear association of maternal age with birth weight. A regression line was adjusted to confirm or deny that possibility.
Results: Macrosomic births were 10% in the total sample with 124 cases. Results of the mean values for birth weight, birth length and gestacional age were the following by maternal age group: Total sample: (N = 124): 4241, 52, 40; ≤ 19 years old (N = 11): 4276, 51, 40; 20-24 years old (N = 29): 4231, 52, 40; 25-29 years old (N = 33): 4206, 52, 40; 30-34 years old (N = 29): 4278, 52, 40; ≥ 35 years old (N = 22): 4239, 52, 40. None of the above mentioned tests found statistically significant differences.
Conclusions: No differences were observed by maternal age.
Key-words: Maternal age, birth weight, fetal macrosomía.

Low frequency and moderate exercise training reduces adiposity in early overfeeding rats and improve interscapular brown adipose tissue thermogenisis.

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Background/aims: Early overfeeding programs to obesity leading to metabolic syndrome in later life. Recently our group demonstrated that rats induced this condition by litter size reduction impaired thermogenic function of interscapular brown adipose tissue (IBAT). In this study, we investigate the effects of exercise training at moderate intensity and low frequency over IBAT thermogenic function of rats raised in Small Litters (SL).
Methods: At 2-days-old Wistar rats litters were adjusted to 9 pups (normal litters- NL) and 3 pups per dam for SL group. At 21-day-old all offspring were weaned and randomly separated in exercised and sedentary (NL SED, NL EXE, SL SED, and SL EXE). At 30-day-old the exercise protocol started with an incremental test to measure the VO2max capacity, after that, the sessions were performed 3 times per week, at 60% of the final workload achieved in tests. Test was performed each 15 days to adjust the training workload. At 80-day-old rats from 4 groups were submitted to a final VO2max test. At 81-day-old animals underwent a surgery to implant a temperature transponder under IBAT. After 6 days, the IBAT temperature was measured at light/dark periods during 4 days. At 100-day-old animals were euthanized. Retroperitoneal, perigonadal and IBAT fat pads was dissected and weighed.
Results: SL rats shows increased body fat pad deposition (retroperitonealNL SED vsSL SED, p< 0,01; perigonadal NL SED vsSL SED, p< 0,001; and IBAT NL SED vs SL SED, p<0,01). Exercise attenuate fat pad deposition in SL groups (retroperitoneal SL SED vs SL EXE, p<0,05; perigonadal SL SED vsSL EXE, p<0,05; IBAT SL SED vsSL EXE, p<0,001) and improve IBAT activity in NL and SL in lights-on period (NL SED vs NL EXE, p<0,05; SL SED vs SL EXE, p<0,01) but not in lights-off.
Conclusion: Low frequency and moderate exercise attenuate obesity in SL rats and improve thermogenic activity in both groups.
Key-words: Moderate intensity exercise, low frequency, small litter, interscapular brown adipose tissue.

Short term moderate exercise provides long-lasting protective effects against metabolic dysfunction induced by high fat diet in rats

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Background: Sedentary lifestyle and high-fat feeding are risk factors for increase metabolic dysfunctions and consequently developing diabetes and obesity. This study determined whether moderate exercise training could prevent the metabolism changes induced by a high-fat diet (HFD).
Methods: Wistar rats (60-day-old) were submitted to a treadmill moderate exercise program, three times a week, for 30 days. A test of maximum effort was performed at 58, 75 and 94 days of age, for confirm the intensity of the exercise. After, animals were exposed to a HFD 30% of lard during 30 days. Control animals had access to normal commercial chow. Body weight and food intake were measured once a week. At 120-day-old, animals were submitted to intravenous glucose tolerance test (ivGTT) for measure of glucose and insulin. Under anaesthesia (thiopental) rats were sacrificed and visceral and brown fat pads and soleus muscle were removed and weighed. Data were submitted to two-way ANOVA and Tukey post test using GraphPad Prism software.
Results: The intensity of exercise was 49.5%, 66.2% and 68.4% of the maximal workload, characterizing moderate exercise. At 90 days-old, trained animals achieved higher workloads than the sedentary, the effort test lasting for longer indicating greater resistance of these animals (p<0.001). Body weight gain of HFD rats was prevented by early exercise; however, it was

not observed difference regarding diet intake. HFD increase the visceral pads in animals and the exercise protect this increase in EXE-HFD comparing to SED-HFD animals (p<0.001). HFD increase mass of brown tissue in SED-HFD animals (P<0.001) and previous exercise, blocked this increase (p<0.001). Exercise increase the mass of soleos muscle in EXE-NFD animals comparing to SED-NFD animals (p<0.01), but not in HFD animals. HFD animals showed glucose intolerance and fast and feeding hyperinsulinemia; however, it was not observed in rats that were previously trained (p<0.01).

Conclusion: Previous moderate exercise in early adulthood attenuates the metabolism impaired induced by HFD late in adulthood; however, how long this protective effect holding it is to be test.

Key-words: Moderate exercise, glucose metabolism, insulin metabolism.

Chronic suphonylurea treatment attenuates the Walker 256 tumor growth

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Background/aims: Sulfonylurea glibenclamide is an antidiabetic agent that blocks ATP-dependent potassium channels (KATP), which allow pancreatic beta-cell depolarized and started secreting insulin. Glibenclamide is widely used in the treatment of type 2 diabetes mellitus. Beyond its antidiabetic effect it has been observed in diabetic population low incidence of certain types of cancer; however, the mechanism is under debate: glibenclamide throughout improving the metabolism and/or directly inhibiting cell proliferation and increasing apoptosis of tumor. In the current work we aimed to test possible anti-cancer effect of glibenclamide in pre-diabetic rats.

Methods: Hyperinsulinemic Wistar rats were obtained by neonatal treatment with monosodium L-glutamate (MSG). Rats, control and MSG groups were daily treated, from weaning to 100-day-old, with glibenclamide (2mg/Kg of body weight). After glibenclamide treatment, one batch of animals from Control and MSG groups were grafted with Walker 256 tumor cells, a rat cell line obtained from breast cancer. After 14 days grafted rats were euthanized. Tumor growth, cachexia, fasting glycemia and insulinemia were evaluated.

Results: Glibenclamide was able to attenuate tumor growth by 27% in control and MSG-rats. The cachexia was 19.5% and 13.6% in control and MSG groups, respectively; while glibenclamide treatment decreased it by 16.0% and 12.6% in both groups. There was no difference in fasting glycemia among control group glibenclamide-treated or -untreated, however, the control group treated with glibenclamide inoculated with tumor cells showed 21% decrease in plasma glucose concentration compared to untreated control group. The MSG-rats treated with glibenclamide showed 18% decrease in plasma glucose, but there was no difference in tumor-bearing MSG-rats treated or untreated with glibenclamide. Fasting insulinemia was increased 2-fold in MSG animals and the glibenclamide treatment decreased 53%; while, there was no difference in control animals. Tumor transplantation decreased the fasting plasma insulin levels in control and MSG groups; however, there was a 5-fold reduction in control rats and a 15-fold in MSG rats. Tumor-grafted control and MSG animals treated with glibenclamide showed a decrease of 8-fold in the fasting insulinemia.

Key-words: Glibenclamide, Walker 256 tumor, metabolic syndrome, MSG, pre-diabetic rats

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Hypercorticosteronemia effect on autonomic nervous system imbalance in obese rats

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Background/aims: Obesity is particularly characterized by neuroendocrine changes leading to metabolic dysfunctions such as hyperglycemia, insulin resistance, hyperinsulinemia and dyslipidemia. The factors involved in this syndrome are not been fully elucidated. However, there are evidences of hypothalamic-pituitary-adrenal cortex axis involvement. Thus, the aim of this study was to investigate, in obese rats, the involvement of the hormone corticosterone on the autonomic imbalance and the development of peripheral insulin resistance and hyperinsulinemia.

Methods: Neonate male Wistar rats were subcutaneously injected, during the first 5 days of life, with monosodium L-glutamate (MSG), at a dose of 4g/kg body weight. Controls animals received equimolar saline solution. At 90-day-old, MSG-treated and untreated were submitted to bilateral adrenalectomy. At 100-day-old, electrical autonomic nerves activity was recorded and the animals were underwent to euthanasia to evaluate the following parameters: plasma glucose, insulin and corticosterone levels. Retroperitoneal, epididymal and brown fat pads were removed and weighed.

Results: MSG rats showed high fat pad storages, hyperactivity of parasympathetic tonus, fasting hyperinsulinemia and hypercorticosteronemia. Adrenalectomized rats shown a decreased retroperitoneal, epididymal and brown fat pad tissues. Bilateral adrenalectomy abolished the fasting hyperinsulinemia and insulin resistance. Parasympathetic activity was restored after adrenalectomy surgery.

Conclusions: The hypercorticosteronism observed in obesity provoke disruptions in neuroendocrine pathways. Decrease in corticosterone levels can restores autonomic nervous system imbalance and diminish obesity and their consequences.

Key-words: Monosodium glutamate, bilateral adrenalectomy, autonomic nervous system imbalance.

Chronic isoflavone treatment effect on metabolism of MSG-obese rats

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Background/aims: Isoflavones are polyphenolic compounds found mainly soy and its derivatives, are known as phytoestrogens for presenting structural and functional similarities with estrogen hormone. Nowadays, is currently the importance a balanced diet in maintaining health and preventing the risk of developing diseases. Studies indicate that isoflavones has a beneficial effect in mitigating changes caused by metabolic syndrome. The goal of this study was to investigate the effect of chronic treatment with isoflavone on obese rats metabolism.
Methods: Neonatal rats were submitted for five consecutive days to subcutaneous injections of monosodium glutamate (MSG) at a dose of 4g/kg body weight (bw). Control animals received injections of equimolar saline solution. At 60-day-old control and MSG animals were separated into 4 groups (n=10/group): CTL- control untreated rats; CTL-ISO, controls treated with isoflavone (600 mg/ kg/ bw/ day); MSG untreated rats; MSG-ISO, treated with isoflavone (600 mg / kg / bw / day). The isoflavones was administered by gavage for 30 successive days. At 90-day- old, all groups were underwent the intravenous glucose tolerance test. Fasting glucose, insulin and lipid (HDL, triglycerides, total cholesterol) plasma concentrations was measured. Fat pad stores was removed and weighted.
Results: MSG rats exhibit an increase in retroperitoneal and epididymal fat pad when compared to CTL animals. Hyperinsulinemia and glucose intolerance were also observed in MSG animals. There was no differences in CTL-ISO animals. MSG-ISO rats showed a decrease in fat pad deposits and restored insulin, glucose, HDL, total cholesterol and triglycerides plasma levels.
Conclusion: Isoflavones have healthful benefits in obesity and have a positive influence on plasma cholesterol. We suggested that isoflavone treatment is a potential alternative therapy in obesity prevention and treatment.
Key-words: Monosodium glutamate, isoflavone, metabolic syndrome

Oxidative stress in the liver subcellular fractions from rats with Freund's adjuvant arthritis

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Background/aims: Adjuvant-induced arthritis is an experimental immunopathology in rats that is often used as a model for studying autoimmune chronic inflammation. In these animals oxidative stress is quite pronounced in the articular inflammation sites. The purpose of the present study was to evaluate oxidative stress in the liver of arthritic rats where morphological and metabolic alterations have been reported to occur.
Methods: Oxidative injury parameters, levels and production of reactive oxygen species (ROS) and antioxidant parameters were measured in the total liver homogenate and in subcellular fractions, namely cytosol, mitochondria and peroxisomes.
Results: Arthritic rats presented higher levels of ROS than controls in the total homogenate (46% higher) and in all subcellular fractions 51%, 38% and 55% higher for mitochondria, peroxisome and cytosol, respectively). Arthritic rats also presented higher levels of protein carbonyl groups in the total homogenate (75%) and in all subcellular fractions (189%, 227% and 260%, respectively, for mitochondria, peroxisomes and cytosol). Arthritic rats also presented higher levels of NO markers in the peroxisomes (112%) and in the cytosol (35%). The catalase activity of all cell compartments was strongly diminished (between 77 and 87%) by arthritis and glutathione peroxidase activities were diminished in the mitochondria (33.7%) and cytosol (41%). The GSH content was diminished by arthritis in all cellular compartments (50 to 59% diminution).
Conclusion: The results reveal that the liver of rats with adjuvant-induced arthritis present a pronounced oxidative stress and that, in consequence, injury to lipids and proteins is highly significant. The higher ROS content of the liver of arthritic rats seems to be the consequence of both a stimulated pro-oxidant system and a deficient antioxidant defense with a predominance of the latter as indicated by the strongly diminished activities of catalase and glutathione peroxidase.
Key-words: Chronic inflammation, adjuvant-induced arthritis, cachexia, oxidative estate, oxygen reactive species.
Financial support: CNPq.

Oxidative state and oxidative metabolism in the brain of rats with adjuvant-induced arthritis

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Background/aims: Adjuvant-induced arthritis is an immunopathology in rats often used for studying autoimmune chronic inflammation. In these animals oxidative stress is quite pronounced in the articular inflammation sites and systemically. The purpose of the present study was to evaluate oxidative stress and energy metabolism in the brain of arthritic rats, where morphological alterations have been reported to occur.
Methods: Oxidative injury parameters, levels and production of reactive oxygen species (ROS) and antioxidant parameters were measured in the total liver homogenate and in subcel-

lular fractions, namely cytosol and mitochondria.
Results: Arthritic rats presented higher levels of reactive oxygen species (ROS) than controls in the total homogenate (25% higher) and mitochondria (+55%). The same occurs with nitrite plus nitrate content, a nitric oxide (NO▪) marker, that increased in the mitochondria (+27%) and cytosol (+14%). Arthritic rats also presented higher levels of protein carbonyl groups in the total homogenate (+43%), mitochondria (+69%) and cytosol (+145%). The mitochondrial transmembrane potential was higher in arthritis. The mitochondrial cytochrome C oxidase activity was reduced 55% in the arthritis. The transmembrane potential was 16% higher in the arthritis. The pro-oxidant enzyme xanthine oxidase was 150%, 110% and 283% higher, respectively, in the homogenate, mitochondria and cytosol of arthritic animals. The same occurred with the calcium-independent NO-synthase activity that was higher in the homogenate (90%) and cytosol (122%). The catalase activity in all fractions was diminished (between 30 and 40%) by arthritis.
Conclusion: The results allow to conclude that the brain of arthritic rats presents pronounced oxidative stress which seems to be the consequence of both a deficient antioxidant defense and a stimulated pro-oxidant system with a predominance of the latter. This imbalanced situation probably contributes to the brain symptoms of the arthritis disease.
Key-words: Chronic inflammation, adjuvant-induced arthritis, brain oxidative state, reactive oxygen and nitrogen species
Financial support: CNPq.

Effects of copaiba oil on oxidative status in the plasma and livers of rats with adjuvant-induced arthritis

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Background/aims: Copaiba oil is an oleoresin which has been used as folk medicine as anti-inflammatory and antioxidant. Adjuvant-induced arthritis is an experimental immunopathology in rats that shares many similarities with the rheumatoid arthritis and it is often used as a model for studying autoimmune chronic inflammation. The present study was planned to evaluate the action of copaiba oil (Copaifera reticulata) on the oxidative state of plasma and liver of rats with adjuvant-induced arthritis.
Methods: The arthritis was induced with Freund's adjuvant and the rats were treated with saline or 1.26 mL/Kg cobaiba oil via oral during 20 days. After this period, the rats were anesthetized, the blood collected from cava vein, the liver removed and clamped in liquid nitrogen. The blood was centrifuged and the plasma was used to measured the thiol groups, total antioxidant capacity (TAC) and albumin. The liver samples were homogenized with phosphate buffer (pH 7.4) and used to measure glutathione (GSH), reactive oxygen species (ROS) and the activities of catalase, glutathione peroxidase, glutathione reductase and superoxide dismutase (SOD).
Results: Arthritic rats presented lower TAC (-35%), albumin (-32%) and thiols (-63%) in the plasma than the controls. The treatment of arthritic rats was not enough to improve these parameters. The ROS content was 75% higher and the GSH levels were 77% lower in the liver of arthritic rats. Similarly, the activity of liver catalase, glutathione reductase and glutathione peroxidase were, respectively 81, 27 and 31% lower in the arthritic condition. The treatment of arthritic rats with copaiba oil improved the levels of GSH and the activity of catalase and glutathione reductase.
Conclusion: The results reveal that the plasma and liver of rats with adjuvant-induced arthritis present a pronounced oxidative stress and that, the treatment of rats with copaiba oil improved the liver oxidative status.
Key-words: Chronic inflammation, adjuvant-induced arthritis, brain oxidative state, reactive oxygen and nitrogen species
Financial support: CNPq.

Aveloz reduces *ITPR1* expression in larynx carcinoma cultures

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Background/aims: Some plants had been used in the treatment of cancer and one of these has attracted scientific interest, the *Euphorbia tirucalli* (aveloz), used in the treatment of asthma, ulcers, warts and has active principles with activities scientifically proven as antimutagenic, anti-inflammatory and anticancer. We evaluate the influence of the antitumoral fraction of the latex the aveloz of the larynx squamous cell carcinoma (Hep-2), on the morphology, cell proliferation and gene expression.
Methods: The Hep-2 cells were cultivated in complete medium (MEM 10%) and treated with aveloz for 1, 3, 5 and 7 days. After statistically analyzing the proliferation of the tested cells, we cultivated the cells again for RNA extraction and used the Rapid Subtractive Hybridization (*RaSH)* technique to identify genes with altered expression. The genes found using the *RaSH* technique were analyzed by Gene Ontology (GO) using Ingenuity Systems©. The five genes found to have differential expression were validated by real-time quantitative PCR.
Results: Though treatment with aveloz did not change the cell morphology in comparison to control samples, cell growth was significantly decreased. The RaSH showed change in the expression of some genes, including *ANXA1, TCEA1*,

NGFRAP1, ITPR1 and *CD55*, which are associated with the inflammatory response, transcriptional regulation, apoptosis, calcium ion transport regulation and complement system, respectively. The aveloz treatment down-regulated ITPR1 gene, validated by real-time quantitative PCR. ***Conclusion:*** The data indicate the involvement of the aveloz in the altered expression of genes involved in tumorigenic processes, which could potentially be applied as a therapeutic indicator of larynx cancer.

Key-words: Aveloz, cell culture, Rapid Subtractive Hybridization (RaSH), gene expression, real-time quantitative PCR

Financial support: FAPESP, CNPq.

TOPICS IN INTERNAL MEDICINE
ORIGINAL ARTICLE
SYSTEMIC LUPUS ERYTHEMATOSUS WITH AND WITHOUT SEROSITIS
LÚPUS ERITEMATOSO SISTÊMICO COM E SEM SEROSITE

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Key words: Lupus erythematosus systemic; Serositis; Pleuritis; Pericarditis
Descritores: Lupus eritematoso sistêmico, Serosite, Pleurite, Pericardite

Abstract

Background: Serositis is a common feature among the wide range of manifestations in patients with systemic lupus erythematosus. About 16% of individuals with SLE present pleuritis and pericarditis, but they rarely cause ventilatory or circulatory repercussions. The inflammation of the serous membranes, including the pericardium, pleura, and peritoneum can lead to pain, fluid accumulation, adherences and even fibrosis.

Objective: To know the prevalence of serositis in SLE patients of the local population and to relate them to other clinical manifestations and serological profile of SLE.

Methods: This is a retrospective study of the last 10 years (2003-2013), with analysis of 412 records of com Lupus erythematosus patients identified with serositis, from the outpatient Rheumatology from HUEC. The comparison was performed between clinical data and autoantibody profile in patients with and without serositis. All patients of both genders and of any age that have at least four qualifiers for the disease of the American College of Rheumatology and sufficient to judge the appearance of serositis data criteria were included.

Results: Serositis was found in 20.6% (85/412 patients) and 43.5% (37/85 patients) presenting only pleuritis, 20% (17/85 patients) presenting pericarditis and only 36.5% (31/85 patients) presented both: pericarditis and pleuritis. In univariate analysis, no differences were found in the prevalence of discoid lesions, aphthous ulcers, Raynaud, psychosis, glomerulonephritis, malar rash, photosensitivity, arthritis, oral ulcers, leucopenia, lymphopenia, and hemolytic anemia (p = ns). The serositis population showed a higher prevalence of seizures (p = 0.03), antids-DNA (p = 0.01) and anti-Sm (p = 0.04).

Conclusion: SLE patients who present antids-DNA and anti-Sm has higher chance for serositis than others, and more chance of showing convulsions. **Endocrinol diabetes clin exp 2014; 1756 -1759.**

Resumo

Justificativa: A serosite é uma característica comum dentre a ampla gama de manifestações em pacientes com LES. Cerca de 16% dos indivíduos com LES apresenta pleurite e/ou pericardite, mas raramente o derrame causa repercussões ventilatórias ou circulatórias. A inflamação das membranas serosas, que incluem pericárdio, pleura e peritônio, pode levar a dor, acúmulo de fluidos, aderência e até mesmo fibrose.

Objetivo: Conhecer a prevalência das serosites nos pacientes com LES da população local e relacionar a presença de serosite com as demais manifestações clínicas e com o perfil sorológico do LES.

Metodologia: Trata-se de estudo retrospectivo, dos últimos 10 anos (2003-2013), com análise de 412 prontuários de pacientes com Lupus eritematoso sistêmico, identificados com serosite, do ambulatório de Reumatologia do HUEC. Foi realizada a comparação de dados clínicos e de perfil de autoanticorpos entre os pacientes com e sem serosite. Foram incluídos todos os pacientes de ambos os sexos e de qualquer idade que possuíam pelo menos quatro dos critérios classificatórios para a doença do Colégio Americano de Reumatologia e dados suficientes para julgar o aparecimento de serosites.

Resultados: Serosite foi encontrada em 20,6% (85/412 pacientes), sendo 43,5% (37/85 pacientes) apresentando só pleurite, 20% (17/85 pacientes) apresentando só pericardite e 36,5% (31/85 pacientes) apresentando pericardite e pleurite. Na análise univariada não se encontraram diferenças quanto à prevalência de lesão discóide, aftas, Raynaud, psicose, glomerulonefrite, rash malar, fotossensibilidade, artrite, úlceras orais, leucopenia, linfopenia e anemia hemolítica (p=ns). A população com serosite apresentou maior prevalência de convulsões (p=0,03), antids-DNA (p=0,01) e anti-Sm (p=0,04).

Conclusão: Pacientes com LES, que apresentem antids-DNA e anti-Sm tem maior chance de apresentar serosite do que os demais, e também pacientes com serosite, tem mais chance de apresentarem convulsões com a evolução da doença.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease of unknown origin in which cells and tissue are damaged by autoantibodies and immune complexes (1). This is a chronic disease whose the most striking feature, from clinical and pathological points of view, is the development of inflammatory reactions in various tissues and organs (2). Although both genders may be affected by SLE it is observed a ratio of 9 females (mainly in the reproductive age) to 1 male. Children and elderly people may be affected more rarely. The disease tends to be more common and more severe in black people; Chineses and some Asians also show a higher incidence (1). Genetic, environmental and hormonal factors are involved in the immune system imbalance, which produces autoantibodies against nuclear proteins, some of which participate in tissue injury (3). Familial history is present in 10-12% of cases; HLA-DR2 and HLA-DR3 increase the relative risk of acquiring SLE (1,3). Also several viruses have been implicated as possible etiologic agents, but nothing has yet been proved (1). The disease progresses in bursts of activity interspersed with periods of remission and can present quite pleomorphic clinical and laboratory manifestations (1).

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Autoantibodies are an important feature of SLE and may provide clues to the clinical variants found. Although a very large number of autoantibodies have been described in SLE, only anti-double-stranded DNA (dsDNA), Smith (Sm) and phospholipids (PL) autoantibodies are part of the classification criteria defined by the American College of Rheumatology, although it should be noted that the anti-PL (aPL) are auto-antibodies not specific for SLE. Similarly, other nuclear and cytoplasmic major antigenic targets, including various ribonuclear proteins (RNP), proteins binding to ribonucleic acids ,while prevalent in SLE, are not specific to the disease (4,5).

The involvement of diverse organs may occur simultaneously or sequentially. The frequency of these affections varies. Skin, joints, kidneys, central nervous system, lungs, heart and serosa are some of the most frequently involved systems. Serositis is recognized as one of the 11 criteria of the American College of Rheumatology (ACR) criteria for classification of SLE. Serositis refers to inflammation of the serous membranes including the pericardium, pleura, and peritoneum, leading to pain, fluid accumulation, tissue adhesion and even fibrosis (4). It is a common feature in patients with SLE but rarely brings ventilatory or circulatory repercussions. Pleuritis appears in about 16% of individuals; peritoneal serositis presenting with ascites (called lupus peritonitis) is an especially rare manifestation (3)

In this study, the objective was to determine the prevalence of serositis in SLE patients from the local population and to relate them to the presence of other clinical manifestations and serological findings of SLE.

Methods

This study was approved by the Ethics Committee of *Sociedade Evangélica de Curitiba* under number 235 535. This is a retrospective study including patients from the last 10 years (2003-2013) from the outpatient rheumatologic Unit of *Hospital Evangélico de Curitiba* and analyzed 412 records of patients with SLE. We included patients of both genders and of any age that completed at least four classification criteria of the American College of Rheumatology for this illness (6) and had data to judge the appearance of serositis.

The records were submitted to a protocol for information extraction that included demographic data (patient age, gender, duration of disease, race, and smoking addiction); data on clinical profile (discoid lesion, oral ulcers, malar rash, Raynaud's pleuritis, pericarditis, peritonitis, seizures, psychosis, arthritis, hemolytic anemia, leukopenia, thrombocytopenia and glomerulonephritis); and data on the presence of autoantibodies (anti-DNA, anti-Ro, anti-La, anti-Sm, anti-RNP, aCl-IgG or anticardiolipin, aCl-IgM, LAC or lupus anticoagulant and rheumatoid factor).

For the analysis and the study charts were divided into 2 groups: (1) with lupus serositis and (2) with and without lupus serositis and comparison of clinical and autoantibodies profile in patients with and without serositis was performed.

Data were analyzed in frequency and contingency tables. Fisher's and chi-square tests were used for association of nominal data and Mann-Whitney and unpaired Student t tests for numerical data. The significance adopted was 5%.

RESULTS

Description of studied patients sample

In the 412 patients, 29 were men and 383 women. The age of patients ranged from 16 to 79 years, with a median value of 38 and IQR (interquartile range) from 26.0 to 79.0. The duration of the disease varied from 0.5 to 36 years with a median of 5.0 and the IQR from 1.0 to 11.0. Regarding ethnicity, 44.5% were African Americans and 55.5% were Caucasian. With respect to smoking, 32% of patients reported being smokers or have made use of cigarettes at some point in life.

The study of clinical profile showed that 16.5% had discoid lesions. Oral ulcers were present in 76%; 55.7% have had malar rash. Raynaud was present in 9.4%; hemolytic anemia in 6.4%, leukopenia in 28.4%, thrombocytopenia in 23.3%, glomerulonephritis in 40.2%.

Serositis was found in 20.6% (85/412 patients) and 43.5% (37/85 patients) presenting only pleuritis, 20% (17/85 patients) presenting pericarditis and only 36.5% (31/85 patients) presenting pericarditis and pleuritis. Figure1.

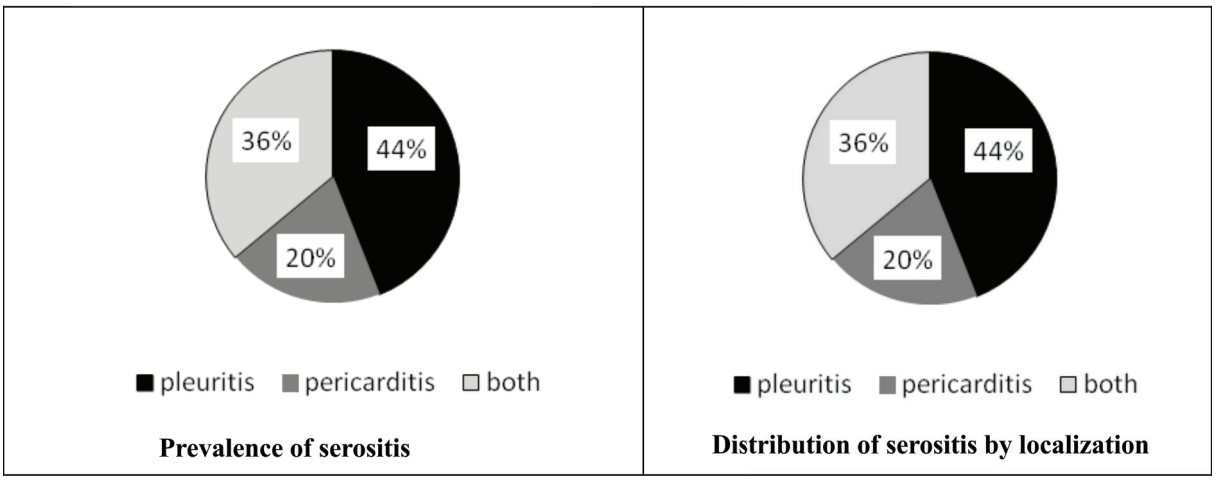
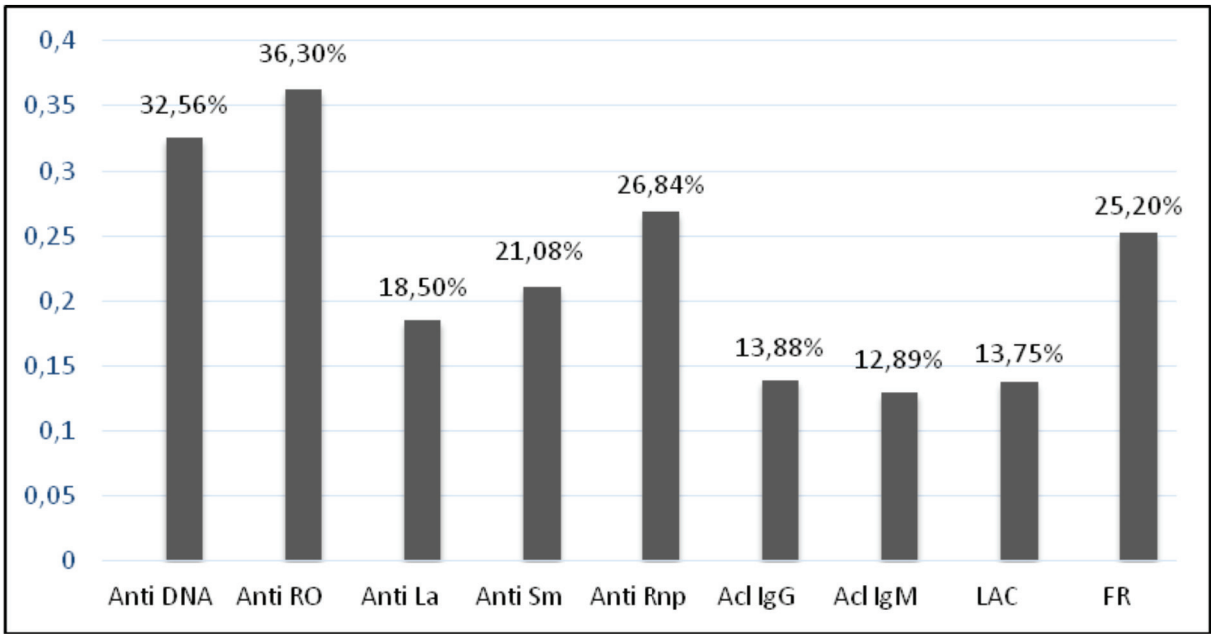


Figure 1- serositis prevalence and distribution in the studied population.

Serological profile can be seen in figure 2.



Comparative analysis among patients with and without serositis

In the table 1 there is a parallel of the population with and without serositis. It is possible to note that those with serositis had a higher prevalence of seizures, anti ds-DNA and anti-Sm.

Table 1- comparison of clinical data, and demographic and serological data of systemic lupus erythematous patients with and without serositis

	WITH serositis N=85	Without serositis N=327	P
Age (years)	16- 73,0 Median 38,0 IQR= 26,0-46,0	16-79,0 Median 38; IQR= 27,0-47,0	0,83*
Disease duration (years)	0,5-36 Median 5,0 IQR = 2,0-11,5	0,5- 32,0 Median 6,0 IQR= 1,0-11,0	0,98-*
Gender	8 males/77 females	19 males/308 females	0,23 – **
Etnia	Caucasians- 29 Afrodescendants – 16	Caucasians -101 Afrodescendants-56	0,98- **
Tobacco exposure	22/80 – 27,5	102/310 -32,9%	0,35- **
Discoid lesion	8/75 – 10,6%	51/350 – 16,7%	0,19- **
Oral ulcers	36/80 – 45%	142/308 – 46,1%	1,00- **
Photosensitivity	56/79 – 70,8%	244/319 – 76,4%	0,30- **
Rash malar	43/75 – 57,3%	166/308 – 53,8%	0,85 – **
Raynaud	38/75 – 50,6%	153/312 – 49,03%	0,80 – **
Convulsions	14/83 – 16,8%	29/322 – 9,0%	0,03- **
Psychosis	6/83 – 7,2%	12/319 – 3,7%	0,17 – **
Arthritis	54/84 – 64,2%	188/322 – 58,6%	0,35 – **
Hemolitic anemia	5/83 – 6,02%	21/316 – 6,6%	1,00 – §
Leukopenia	21/82 – 25,6%	94/318 – 29,5%	0,48 – **
Thrombocytopenia	20/83 – 24,09%	73/313 – 23,3%	0,88 – **
Glomerulonefritis	42/85 – 49,4%	131/327 – 40,4%	0,11 –**
Anti- dna	35/79 -44,3%	90/299 – 30,1%	0,01 – **
Anti- ro	30/77 – 38,9%	106/294 – 36,0%	0,63 – **
Anti- la	13/76 – 17,1%	56/291 – 19,2%	0,67 –**
Anti- sm	22/73 – 30,1%	55/286 -19,2%	0,04 – **
Anti – rnp	16/71 – 22,5%	73/261 – 27,9%	0,35 – **
Anticardiolipin igg	10/78 – 12,8%	43/299 -14,3%	0,72 – **
Anticardiolipin ig m	9/68 – 11,6%	40/299 – 13,3%	0,69 – **
Lupus anticoagulant	8/65 – 12,3%	39/274 – 14,2%	0,68 –**
Rheumatoid factor	21/74 – 28,3%	70/283 -24,7%	0,52 – **

**= Man Whitney test ; **=chi square test ; §= Fisher test*

DISCUSSION

The first aim of our study was to know the prevalence of serositis (pleuritis and pericarditis) in SLE patients from the local population. This is important since the Brazilian population is highly mixed from the ethnic point of view and does not follow the standard demarcated classification from other countries. As SLE manifestations have genetic influence, to know local data becomes essential to the knowledge of this disease in our country. It was found that 21% of patients had serositis; of these, 43.5% had only pleuritis, 20% had only pericarditis and 36.5% had pericarditis and pleuritis. A study in Hong Kong, by Man et al (4), with 310 patients, found a prevalence of 12% (37/310 patients) of serositis among those studied. In this study occurred 69 episodes of serositis and 26% were only pericarditis; only pleuritis in 44%; and 30% were peritonitis. In 35% of patients, two or three manifestations of serositis were present. Man et al data (4) is similar to those obtained in our study, especially in the case of isolated pleurisy.

In a case report followed by literature review done by Junior Pott et al (7), it is described that serositis occurs in approximately 16% of patients with SLE: pleuritis and pericarditis are more common, and more rarely, appearing peritoneal involvement. In our work we have not identified cases of peritoneal involvement.

The second aim of our study was to correlate the presence of serositis with other clinical manifestations of SLE. To recognize associations between manifestations of lupus or their connection with autoantibodies allows the clinician who attends this type of patients to predict future events and to act preventively. In the present paper, statistical significance was found only in the relation of serositis with seizures (p=0.03), with a prevalence of 16.8% of seizures in patients with serositis and 9% in patients without serositis. The same study cited above, carried out by Man et al (4) had a similar finding, wherein the neurological involvement was individually associated with serositis with an OR= 2.82 (95% CI 1.88, 4.23). Yet in the same study there was statistical significance between serositis and discoid rash [OR= 0.93 (95% CI 0:57, 1:53)], hematological involvement [OR= 2:05 (95% CI 1:38, 3.04)], anda negative relationship with photosensitivity [OR 0.73 (95% CI 0:51, 1:03)], which was not demonstrated in our research. Other authors such as Li et al (8) found serositis in association with hemolytic anemia (p=0.02) and lymphadenopathy (p=0.04), which also was not seen at present. Still others have found that malar rash was less frequent in patients with serositis (p = 0.03) (5).

The third aim of our study was to correlate the presence of serositis with serological profiles of SLE. Statistical significance between serositis and anti-DNA (p=0.01) and anti-Sm (p=0.04) was found. Among patients with serositis, 44.3% showed presence of anti-dsDNA and 30.1% of anti-Sm against 30.1% and 19.2%, respectively, in the group without serositis. The association between anti-Sm and serositis was also evidenced both by the study of Li et al (8) as in the study by Wang et al (9).

Some authors believe that there are three groups of SLE patients serologically distinct: group 1 - with anti-dsDNA; group 2 - with anti-Sm/anti-RNP/antiphospholipid and group 3 - with anti-Ro/anti-La. These authors noted that patients in group 1 (anti-dsDNA) have more kidney disorders but lower prevalence of other clinical manifestations. On the other hand, groups 2 and 3, have less renal disorders but higher prevalence of other manifestations. Exceptions are hematological involvement and serositis that overlaps between these two extremes (8). In another study, comparing the frequency of six manifestations, including nephritis, serositis, musculoskeletal, hematologic and CNS symptoms was observed that the presence of serositis correlated with the group Sm/RNP (p = 0.0022) (5). Previously the relationship between anti-Sm antibodies and serositis had only been observed in children (10). The findings of this study confirm the association with anti-Sm.

CONCLUSIONS

In the present study we conclude that:

- Patients with SLE in our population who have serositis (pleuritis and pericarditis) have more chances of having seizures;
- Serositis is more common in patients with positive anti-dsDNA and anti-Sm.

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