



2nd INTERNATIONAL SYMPOSIUM of DOHaD and STRESS: METABOLISM, EXERCISE, STRESS and BEHAVIOR

In the last decades, a group of diseases has gained importance in human health, which are called, non-communicable diseases (NCD), among them its highlighted cardiovascular disease, diabetes and cancer. Recently, World Health Organization has estimated that this group of diseases has been doing more than 41 millions of victims every year, being between the main causes of death worldwide. The explanation that those diseases are exclusively caused by an inadequate lifestyle it's not sufficient to explain the rapid growth in the prevalence worldwide. A series of epidemiologic and experimental studies has shown evidences that these diseases may be caused by stressor events suffered by the subject during critical phases of his development (pre-conception, lactation, infancy and adolescence), programming his organism to the development of cardiometabolic diseases during adult life, being able to affect next generations.

In this sense, the DOHaD (Developmental Origins of Health and Disease) concept, search to clarify what are the susceptible phases to the programming of the organism, as well what are the insults that can initiate this phenom. The 2nd International Symposium of DOHaD and Stress (2ndISDS) approached the DOHaD concept, applied in different themes, such as: metabolism, exercise, behavior and reproduction.

The 2ndISDS promoted the interaction and change of experiences between researchers, professor and students of graduation and post-graduation that act in different areas (Biological Sciences, Biomedicine, Medicine, Biotechnology, Physical education, Nutrition, among others). The first edition of ISDS occurred in 2017 at Buenos Aires, Argentina, being it second edition made in the State University of Maringá, bringing together Brazilian lecturers from UNICAMP, USP, UFRGS, and researchers from Argentina, Mexico and USA worldwide recognized for their works regarding the themes of the symposium.

There was participation of 80 congressmen, distributed between researchers, professors, post-graduation and graduation students from different universities. The congressmen had the opportunity to present works approaching the themes of the symposium and discuss them with internationally renowned researchers. Thus, the 2ndISDS 2018 had come to Brazil with the mission of spread the DOHaD concept and integrate the academic society around discussions that bring advances to the researches in favor of health and life, in all his phases of development.

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ABSTRACTS

REPRODUCTION

INTERSEX FISH INDUCED BY EXPOSURE TO METFORMIN

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Background: The occurrence of intersex fish, where male reproductive tissues show evidence of feminization, has been found in freshwater systems around the world, indicating the potential for significant endocrine disruption across species in the ecosystem. Metformin is one of the most widely prescribed antidiabetic drugs in the world. Interactions between insulin signaling and steroidogenesis suggest potential endocrine-disrupting effects of metformin. The aim of this study was to determine whether a chronic exposure to metformin, in a level found environmentally, would cause detectable endocrine disruption to adults males of *Astyanax altiparanae*, a small characid fish widely distributed in South America. **Methods:** Fishes were divided into 2 tanks of 10 individuals. One tank were dosed with metformin at 100 ug/L and one tank control containing only dechlorinated water. After 30 days of exposure, all fishes were euthanized, and their gonads were fixed and submitted to histological analysis by light microscopy (LM). **Results:** 40% metformin-exposed male fishes had occurrence of intersexuality against none of the control fishes. Observation of histology slides showed the presence of perinucleolar oocytes (PO) and early vitellogenic oocytes (EV) scattered throughout testis. The development of fishes intersex suggests that within adult testicular tissue, relatively undifferentiated gonial stages are permanently present that can be induced to differentiate along a female pathway given a sufficient dose of metformin. **Conclusion:** The present study suggest that metformin acts as an endocrine disruptor. Other Studies are needed for a better understanding of the endocrine of metformin exposure and its effects on survival and reproductive success in the aquatic environment.

Key-words: Endocrine disruptors, Fishes, Gonads.

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PERIPUBERTAL EXPOSURE OF MALE RATS TO LOW DOSE OF MALATHION ALTERED HISTOPATHOLOGICAL PARAMETERS IN TESTIS AND TESTOSTERONE LEVEL BY INDUCING OXIDATIVE STRESS

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Background: Malathion is an organophosphate insecticide used to control insect in feed crops or *Aedes aegypti* control in the context of Zika virus outbreak. Thus, humans are exposed to it by mean of ingestion of contaminated food. The juvenile and peripubertal periods are critical for postnatal development of testis and more vulnerable to the action of toxic agents. The aim of the present study was to evaluate whether exposure to low doses of malathion during these periods can damage the testicular development. **Methods:** For this, 15 male *Wistar* rats (postnatal day (PND) 25) were assigned into 3 experimental groups and treated for 40 days. The animals were daily exposed to malathion 10 mg/kg b.w. (M10 group) or 50 mg/kg b.w. (M50 group) diluted in 0.9% saline via gavage. The control group received only the vehicle. At PND 65, the rats were anesthetized and euthanized. The blood was collected for determination of testosterone concentration. Testes were collected and submitted to histopathological analysis and evaluation of oxidative status. The data were compared using ANOVA followed by Dunnet's post-hoc test. Differences were considered significant for $p < 0.05$. Statistical analyses were performed using GraphPad Prism (version 7.00.159). **Results:** Histopathological analysis in testes revealed that M50 group showed significantly increase of abnormal seminiferous tubules when compared to the control group. The main alterations observed were the increase in vacuoles and immature germ cells in the lumen of tubules. On the other hand, no alterations were observed at M10 group. The results even showed that both malathion doses reduced Leydig and Sertoli cells number. Furthermore, the M50 group showed a reduction in plasma testosterone concentration and increased lipid peroxidation. **Conclusion:** Since seminiferous epithelium maintenance is testosterone dependent, and this androgen is produced by Leydig cells, we conclude that lipid peroxidation was the major mechanism by which malathion affected the testicular development in rats, even in low dose.

Key-words: male, development, toxicology, oxidative stress, histopathology



EXERCISE

FASTING AEROBIC EXERCISE SHOWS NO IMPROVEMENT ON BIOMETRICAL PARAMETERS AND GLUCOSE HOMEOSTASIS OF ADULT MALE RAT OVERFED IN EARLY LIFE COMPARED TO AEROBIC EXERCISE PERFORMED IN FED STATE

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Background: Early postnatal overfeeding leads to metabolic programming. Small litter rats present overweight, hyperphagia, hyperinsulinemia, high accumulation of white adipose tissue, peripheral resistance to insulin and hyperactivity of the parasympathetic nervous system. Exercise and fasting are alternatives used to prevent and treat overweight and obesity, as they increase energy expenditure and reduce caloric intake, respectively. Both factors raise the lipids oxidation and decrease body fat through elevated levels of lipolytic (adrenaline and cortisol) and anabolic (GH) hormones, in response to decreased glycemia and increased plasma glucagon concentration. We combined exercise and fasting and evaluated the effect on biometrical and biochemical parameters of rats programmed by overfeeding in early life. **Methods and Results:** Male Wistar litters were adjusted to 3 pups per cage on postnatal-day-3. At PN21, rats were assigned into 4 groups: SL (small litter); SL-F (small litter-fasting); SL-EX (small litter-exercise); SL-FEX (small litter-fasting exercise). The exercise protocol started at PN30, with training sessions performed until PN90, 3 times per week at 58-65% of the final workload achieved in effort test, repeated each 15 days to adjust training workload. The fasting protocol also started at PN30, lasting for 8 hours of daytime before training sessions for SL-FEX group and 3 times per week for SL-F group. Data was analyzed using two-way ANOVA, followed by Tukey's post-test, with $p < 0.05$ for significance. At PN90, SL-EX presented 8,59% and 15,97% of reduction in mesenteric and periepididymal fat pads ($p < 0.05$), respectively, and increase of 29,68% in gastrocnemius muscle weight ($p < 0.01$) in relation to SL. SL-FEX showed a reduction of 23,53% in gastrocnemius muscle weight ($p < 0.001$) and a raise of 23,24% in area under curve of ivGTT ($p < 0.05$) compared to SL-EX, however parameters such as body weight, retroperitoneal fat pad and brown adipose tissue weight showed no significant differences. SL-FEX and SL-F groups also showed no differences. **Conclusions:** Fasting does not potentiate the effect of aerobic exercise in adult male rats programmed by overfeeding; in addition, the exercise performed in fasting condition impairs glycemic homeostasis and blocks anabolic effect on the skeletal muscles.

Key-words: Fasting, exercise, overweight

PROTECTIVE EFFECT OF EARLY MODERATE INTENSITY EXERCISE ON FRUCTOSE-INDUCED METABOLIC SYNDROME IN ADULT RATS

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Background: Evidences in literature has shown that consumption of fructose increases the metabolic syndrome (MetS) prevalence worldwide. Among the factors that predispose to MetS emergence, inadequate diet and lack of physical exercise do important role. The aim of this study was: (1) evaluate whether fructose would programme adolescents rats to MetS and (2) whether moderate intensity exercise training during adolescence protects adult rat to metabolic dysfunctions programmed by fructose supplementation during adolescence. **Methods:** Four-week-old male Wistar rats were randomly divided into 4 groups: control-sedentary (C-SED), fructose-sedentary (F-EXE), control-exercised (C-EXE) and fructose-exercised (F-EXE), with a fructose enriched drink (10% w/v fructose in water) and a moderate intensity exercise for 4 weeks (concomitant with the fructose treatment). Food intake and body weight were measured weekly and the drink intake was measured each two days. At 120 days of age, glucose tolerance was evaluated using an intraperitoneal glucose tolerance test. Insulin levels were measured by radioimmunoassay and lipids profile was evaluated on plasma. Data were analyzed with two-way ANOVA and the Tukey post-test. **Results:** Wasn't found significantly differences in weight gain in all the groups. Regarding fat stores, the F-SED group showed increased stocks of retroperitoneal and periepididymal fat pad and the exercise were not able to protect the F-EXE group from the increase in stores of that fat. During the ipGTT, the exercise reduced significantly the blood glucose of F-EXE compared to F-SED group. The F-SED group showed an increased insulinaemia compared to C-SED and the exercise reduced significantly this insulinaemia in F-EXE compared to F-SED group ($P<0.05$). The F-SED increased total cholesterol and triglycerides significantly compared to C-SED group and the exercise decreased significantly these parameters in F-EXE compared to F-SED group ($P<0.05$). **Conclusion:** Our results showed that the 10% fructose supplementation during adolescence may program the metabolism to MetS in adulthood with development of insulin resistance, increased fat stores and dyslipidemia and and the short-term moderate exercise may protect the metabolism from development of these metabolic disturbances.

Key-words: Fructose, Metabolic Syndrome, Moderate Intensity Exercise

PROS AND CONS OF INSULIN ADMINISTRATION ON LIVER GLUCOSE METABOLISM IN STRENGTH-TRAINED HEALTHY MICE

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Background: Non-diabetic individuals use hormones like insulin to improve muscle strength and performance. However, as insulin also leads the liver and the adipose tissue to an anabolic state, its effects on trained non-diabetic Swiss mice were investigated. **Methods:** The mice were divided in sedentary treated with saline (SS) or insulin (SI) and trained treated with saline (TS) or insulin (TI). Training was made in vertical stair at 90% of the maximum load three times/week. Insulin (0.3 U/kg body weight) or saline were given intraperitoneally five times/week. After eight weeks tissue and blood were collected and *in situ* liver perfusion with glycerol+lactate or alanine+glutamine (4 mM each) was carried out. **Results:** The trained animals increased their muscle strength (+100%), decreased body weight gain (-11%), compared with the sedentary groups. There were not found changes on either lipid profile or fasting glycaemia, as well there was not significant difference on area under curve in insulin tolerance test (ITT). Liver glycogen was increased by insulin (SI +40% and TI +117%), as well as liver basal glucose release (TI +40%). Lactate and pyruvate release were reduced to a half by training. The greater gluconeogenesis from alanine+glutamine induced by training (TS +50%) was reversed by insulin (TI). **Conclusion:** Insulin administration had no additional effect on muscle strength and reversed some of the lipolytic and gluconeogenic effects of the resistance training. Therefore, insulin administration is not justified as adjuvant to the improvement of muscle strength.

Key-words: Gluconeogenesis; Liver metabolism; Resistance Training.

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RITALIN TREATMENT AT ADOLESCENCE IMPACTS THE METABOLISM BUT NOT THE ANXIETY BEHAVIOR OF ADULT MALE RAT OFFSPRING

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Background: Ritalin (methylphenidate) is a psychostimulant used in the treatment of Attention Deficit Hyperactivity Disorder, one of the most common behavioral disorders at adolescence. Methylphenidate inhibits the reuptake of dopamine mainly in the striatal nucleus and prefrontal cortex. Adolescence, as well as pregnancy and lactation, is considered a sensitive period of development, since neural connections, including dopaminergic system, are still being formed in the brain. Therefore, stressful insults in this phase can permanently modulate the development of systems, programming metabolic diseases and behavioral changes in adult life. We evaluated the effect of Ritalin treatment during adolescence on biometrical parameters, glucose metabolism and anxiety of adult male rat offspring. **Methods:** From weaning, Wistar male rats received Ritalin by gavage (Rit; 1 mg/kg/day) for 30 days, whereas control rats received saline (Sal; NaCl 0.9%) in the same volume. From 51 to 110 days-old both groups were untreated. At 51 and 110 days-old the experimental procedures were performed. **Results** During treatment, Rit animals presented 12% of reduction in food intake ($P < 0.01$), however there was no difference in body weight. At 51 days-old fat tissue stores were equal between groups and fasting insulinemia was decreased in 50% ($P < 0.05$). Glucose tolerance and insulin sensitivity assessed by ivGTT and K_{itt} showed no differences between groups. Animals presented ansiogenic-like effect ($P < 0.05$) as demonstrated by inhibitory avoidance in the elevated T-maze. After treatment, Rit group showed an increase of 23% in body weight ($P < 0.01$) and the final weight was 6% higher. Fat tissue stores were increased by approximately 20% ($P < 0.05$) in treated animals. ivGTT showed higher glucose levels in Rit group at 15 ($P < 0.05$), 30 ($P < 0.05$) and 45 minutes ($P < 0.01$) and Rit animals are insulin resistant, as demonstrated in K_{itt} ($P < 0.05$). At 110 days-old there were no difference between groups in elevated T-maze test. **Conclusions:** Ritalin treatment at adolescence programs male rats to overweight and metabolic alterations; however, no behavioral changes at adulthood.

Key-words: Ritalin, anxiety, overweight

CHRONIC VITAMIN D SUPPLEMENTATION AVOIDS HIPERINSULINEMIA AND INSULIN RESISTANCE IN HYPOTHALAMIC OBESE RATS.

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Background: Vitamin D (VD) deficiency has been described in obese population being related to metabolic disorders such as, insulin resistance (IR). Glutamate monosodium (MSG) treated rats present high visceral obesity, hyperinsulinemia, glucose intolerance and dyslipidemia in adulthood. Here we evaluated the effect of chronic VD supplementation on obesity and metabolic abnormalities induced by neonatal treatment with MSG. **Methods:** Wistar male rats were treated with MSG (4g/Kg) during first five days of life. Control (CON) received saline equimolar. At 30th the MSG and CON rats were randomly subdivided in VD supplemented (12µg/Kg) or non-supplemented (NS) constituting 4 experimental groups: CON-NS, CON-VD, MSG-NS and MSG-VD (n=10 rats/group). The VD was supplemented orally, 3 times/week, during 60 days. At 90th day of life rats were euthanized and biochemical and biometric parameters evaluated. HOMA-IR was calculated using basal insulin and glucose. Pancreas was used to histological analysis, islets isolation and protein expression. Data are mean ± SEM, in ANOVA with Tukey post-test (p<0.05). **Results:** MSG-NS rats presented higher adiposity (63.63%), glycemia (45.09%), hiperinsulinemia (833.33%) and HOMA-IR (1471.47%) in relation to CON-NS group (p<0.05). Moreover, MSG-NS rats had reduced number of pancreatic islets (43.66%) when compared to CON-NS group (p<0.05) without affecting glucose-induce insulin secretion (GIIS). CON-VD rats showed higher islets size (190.95%) when compared to islets from CON-NS group (p<0.05). MSG-VD rats showed smaller plasmatic insulin levels (86.73%) resulting in improvement of HOMA-IR in relation to MSG-NS group (p<0.05). The GIIS was not affected by VD supplementation in islets from MSG rats. However, the insulinotropic effect of cholinergic agonist carbachol (Cch; 10µM) was reduced (57.30%) in islets from MSG-VD, when compared to islets from MSG-NS group (p<0.05). This alteration in Cch response was not related to changes in protein expression such as, protein kinase-A (PKA) and muscarinic receptor 3 (MR3), and protein kinase-C (PKC). **Conclusion:** The chronic VD supplementation in MSG-obese rats avoids hiperinsulinemia and IR; an effect independent of changes in adiposity or GIIS. The reduced insulinotropic cholinergic effect found in pancreatic islets of MSG-VD rats could be involved in this process.

Key-words: Obesity, Pancreas, Metabolic syndrome.

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ORGANOGENESIS IN A DISH: HORMONE MODULATION OF VENTRAL PROSTATE DEVELOPMENT FROM UNDERNOURISHED ANIMALS

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Background: Maternal protein malnutrition (MPM) during gestational/lactational periods may lead to irreversible changes in the offspring by the fetal programming mechanism. It can create an imbalance in sexual hormone levels that directly influence the morphophysiology of androgen dependent structures, such as the prostate. In this study, we investigated the effects of MPM on the development of rat ventral prostate (VP) by examining hormonal modulation of epithelial cell proliferation and androgen receptor expression *in vitro*. **Methods:** Briefly, *Sprague Dawley* dams were distributed into NPD/control group (fed a normal protein diet containing 17% protein) or LPD/low protein diet group (fed a diet containing 6% protein) during gestation. After birth, all males were euthanized and the VP dissected. Some VP from NPD and LPD animals were submitted for morphological and molecular analysis. Other VPs were grown in organ culture medium for 7 days with/without 10nM testosterone. Four-hours before harvesting, 1nM of BrdU was added to the organ culture medium to evaluate epithelial cell proliferation rates. **Results:** LPD offspring showed reduction of plasma testosterone levels as well as lower body and glandular weights compared to NPD offspring at birth. LPD animals also presented smaller VP with lower number of main ducts and a lesser ductal branch complexity. Testosterone alone was not able to recover the difference between VP from LPD in relation to NPD offspring. The epithelial cell proliferation rate and the relative levels of stemness genes (*ABCG-2*, *NANOG*, *TROP-2*) were higher in the LPD animals. These results suggest that the proliferation and/or differentiation cell dynamic was disrupted, which favors basal cell phenotype (p63+) and limited the differentiation into secretory luminal cells (AR+). **Conclusion:** In summary, fetal programming by low protein diet promoted a delay in ventral prostate development. The proliferation/differentiation cell dynamic was altered, and testosterone alone was not able to restore the VP morphology, suggesting the important involvement of other hormones.

Key-words: prostate, low protein diet, fetal programming, organ culture, testosterone

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GESTATIONAL EXPOSURE TO PARACETAMOL ALTERS PROGENIE'S OLFACTORY DISCRIMINATION IN THE NEST SEEKING TEST

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Background: Paracetamol is an over-the-counter antipyretic and analgesic drug. It's considered safe to be used during pregnancy and is consumed by approximately 40% of pregnant women in Brazil. Epidemiological studies suggest paracetamol intake during pregnancy as a risk factor for neurodevelopmental disorders in the progeny, such as autism spectrum and attention-deficit hyperactive disorders. **Methods:** To evaluate if paracetamol could be a developmental neurotoxicant we gavaged pregnant Wistar rats with paracetamol (Tylenol®, 350mg/kg, n=10) or water (n=11) from gestational day 6 until delivery. We evaluated the behavior of 10-day-old progeny in the nest seeking test (NST). Only one male and one female pup from each litter were evaluated in this test (i.e., the experimental unity was the litter). Animals were individually placed in the center of a clear rectangular acrylic cage (40 x 20 x 18 cm) divided into 3 equal compartments by a permanent ink marker: a central arena and 2 side compartments, one containing nest bedding from the test pup's home cage and the same quantity of fresh clean bedding on the opposite side. Latency to crossing the line toward nest compartment with the forepaws and head was measured. Total duration of the test was 3 minutes. Normal distribution and homogeneity of variances were checked by Shapiro-Wilk and Levene's test, respectively. Log-transformed data were evaluated by two-way ANOVA (exposure and sex as factors). **Results:** Gestational exposure to paracetamol, independent of sex, increased the latency to reach nest bedding ($F=4.37$, $p<0.05$). **Conclusion:** Gestational exposure to paracetamol can alter normal neurodevelopment in rats as indicated by the disturbed olfactory discrimination observed in 10-day-old pups.

Key-words: acetaminophen, neurodevelopment, developmental neurotoxicity

Financial Support: CAPES

MEDIAL AMYGDALOID NUCLEUS MODULATES ANXIETY-LIKE BEHAVIOR TO THE EMOTIONAL STRESS IN RATS BY THE ANGIOTENSINERGIC NEUROTRANSMISSION

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Background: A limbic structures implicated in the control behavioral responses to the stress is the medial amygdaloid nucleus (MeA). Angiotensinergic mechanisms have been described as a mechanism involved in these to the stress. However, the involvement of angiotensin II (ANG II) and angiotensin 1-7 (ANG 1-7) receptors in anxiety-like behavior control in the MeA have never been investigated. Thus, the aim of this study was to investigate the involvement of AT₁, AT₂ and MAS receptors within the MeA in anxiety-like behavior evoked by an acute session of restraint stress in rats. **Methods:** Male Wistar rats (240g-260g) had cannula-guide bilaterally implanted into the MeA. The restraint stress was performed by placing the animals in a plastic cylindrical tube for 60 minutes. Independent group of animals received bilateral microinjections into the MeA of the selective AT₁ receptor antagonist losartan (1nmol/100nL), AT₂ receptor antagonist PD123319 (0.05nmol/100nL), Mas receptor antagonist A-779 (0.1nmol/100nL) or vehicle (saline, 100nL) 10 min before the onset of the restraint stress session. The animals of the naïve group remained in their boxes of houses throughout this process. The anxiety-like behavior were assessed in the elevated plus maze (EPM). **Results:** The One Way ANOVA indicated in time spend in open arms percent effect of treatment between columns ($F_{(4,30)}=5.25; p<0.01$). The *post-hoc test Tukey* showed the vehicle, PD123319 and A-779 groups decrease time spend in open arms ($p<0.05$), and losartan treatment reverted this effect ($p>0.05$). The same effects of treatment between columns was showed in open arms entries percent ($F_{(4,30)}=4.72; p<0.05$). The analysis *post-hoc* revealed vehicle and PD123319 groups decrease the open arms entries percent ($p<0.05$) and losartan treatment reverted this effect ($p>0.05$). No was observed effects of treatment between columns in time spend in center ($F_{(4,34)}=0.57; p>0.05$) and closed arms entries ($F_{(4,33)}=0.75; p>0.05$). **Conclusion:** Our results indicate that AT₁ receptor participates of anxiety-like behavior control in MeA to the emotional stress.

Key-words: Medial Amygdaloid nucleus, Restraint Stress, Angiotensinergic Neurotransmission.

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