ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 08, Issue, 05, pp.20487-20491, May, 2018

ORIGINAL RESEARCH ARTICLE OPEN ACCESS

EFFECTS OF 7.5% POLYDEXTROSE IN BLOOD LEVELS AND DETERMINATIONS OF BONE MINERALS IN WISTAR GASTRECTOMYMIZED RATS

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ARTICLE INFO

Article History:
Received 08th February, 2018
Received in revised form
12th March, 2018
Accepted 27th April, 2018
Published online 31st May, 2018

Key Words:
Calcium, Billroth II,
Gastrectomy, Functional Foods,
Prebiotic, Mice

ABSTRACT

The objective of this study was to evaluate the effects of polydextrose (PDX) supplementation on blood mineral levels and bone mineral composition in gastrectomizedwistarrats. Male Wistarrats (n = 40) with approximately 250 g underwent gastrectomysurgeryt o Billroth II with anterior truncalvagotomy, being distributed in two groups: gastrectomized (GXT) and false gastrectomized (SHAM). After seven post operative days were subdivided into four groups: SHAM Control (SHAM without polydextrose); SHAM PDX (SHAM with polydextrose); GXT Control (Gastrectomized without polydextrose); GXT PDX (Gastrectomized with polydextrose). Weight gain and diet in take were evaluated weekly. The animals were euthanized after 60 days. The animals in the SHAM control group had greater weight gain in comparison to the other groups. PDX maintained serumcalcium levels (calcium, phosphorus) in the groups supplemented with the prebiotic. Regarding phosphatase, the GXT PDX group presented higher averages, positively influencing the process of bonemineralization. The gastrectomy did not generate bone involvement in the studied groups GXT Control and GXT PDX and there was no difference with the groups SHAM Control and SHAM PDX. It can be concluded that PDX interferes positively in the maintenance of serum calcium and phosphorus levels with preservation of bonet issue.

Citation: Silvana Franco, Eliane Cesário Maluf, Daiana Novello and Rodrigo Gomes, 2018. "Effects of 7.5% polydextrose in blood levels and determinations of bone minerals in wistar gastrectomymized rats", *International Journal of Development Research*, 8, (05), 20487-20491.

INTRODUCTION

Partial or total gastrectomies are the main forms of treatment for gastric cancer and are indicated in practically all patients who are eligible for a non-complication-free surgical procedure (Toneto *et al*., 2012). In partial or total stomach gastrectomy, gastrointestinal transit resurfaced by anastomosis with the duodenum (Billroth I-BI) or jejunum (Billroth II-BII). Depending on the location of the tumor the extent of the stomach needs to be removed, with partial withdrawal of the stomach the tumors of antrum are treated (partial gastrectomy); (Toneto *et al*., 2012; Xiong, *et al*., 2013). Nutritional deficiencies are common in gastrectomized patients, since with the removal of each antrum there is a decrease in gastrin production and reduction of the stimulus for pepsin secretion, with loss of protein digestion, also a reduction of hydrochloric acid (HCl) secretion and intrinsic factor, with the appearance of iron and megalbolastic anemia. After gastric resection therapy, there may be a lower absorption of vitamin D and dietary calcium (Otha *et al*., 1995; Hara *et al*., 2000; Santos, 2009). In the intestine of mammals calcium is absorbed mainly in the small intestine, with maximum absorption in the duodenum along two pathways: one transcellular and the other paracellular, both are regulated by vitamin D (Bronner *et al*., 1986). However, Bouglé *et al*. (2002) in studies, the ingestion of PDX increased the calcium concentration in the bones of normal rats, which may be relevant to decrease the risk of osteoporosis (Hara *et al*., 2000; Santos *et al.,* 2009). PDX is a prebiotic, non-digestible, non-hydrolyzed in the upper small intestine being partially fermented in the large intestine, increasing the volume of fecal mass, reducing the intestinal transit time, smoothing and decreasing the pH of the faecal cake (Jie*et al*. 2000, Gijs *et al*., 2013, Röytiö, 2014). It is possible to identify functional components and / or ingredients of prebiotics that may positively influence calcium uptake in

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order to ensure a better bioavailability (Ohta *et al*., 1995; Saad, 2006). The objective of this study was to study the effect of supplementation of prebiotic PDX on blood mineral levels and bone mineral composition in gastrectomizedwistar rats in view of the range of properties of functional ingredients.

MATERIALS AND METHODS

The research was carried out in partnership between the Midwest StateUniversity (UNICENTRO), Guarapuava, Paraná, Brazil, Federal University of MatoGrosso do Sul (UFMS), Campo Grande, MatoGrosso, Brazil and the Federal University of Paraná (UFPR), Curitiba, Paraná, Brazil. The in vivo experiment was conducted at the Central Biotério of UFMS.

Animals for experimentation: A total of 40 rats (*Rattusnorvergicus, var: Albinus, Rodentia Mammalia*) were used, all of them male, weighing around 100g, from the colonies kept in the Central Biotério of UFMS.

Surgical procedure: The surgical procedure was performed by the researcher rigorously according to the principles of the specific surgical technique already standardized, as well as the anatomy of the animal (Waynforth, 1980).For surgery, the animals (mean weight 250g) were fasted for approximately 8 hours and previously anesthetized by the combination of Ketamine (80 ml / kg) and Xylazine (10 ml / kg) intraperitoneally. Anesthesia was certified through absence of neuromuscular reflex. During the first 5 postoperative days, the animals received intramuscular injection of non-steroidal anti-inflammatory 1 times a day.

Experimental design: After seven days of the surgical procedure, the animals were separated into four groups of ten animals. Group 1 (SHAM PDX): SHAM animals fed AIN-93M feed without addition of 7.5% polydextrose. Group 2 (SHAM Control): SHAM animals fed AIN-93M feed with addition of polydextrose. Group 3 (GXT PDX): Gastrectomized animals fed with AIN-93M ration without addition of 7.5% polydextrose. Group 4 (GXT Control): Gastrectomized animals fed with AIN-93M ration with addition of polydextrose.

Monitoring of diet consumption and weight gain of animals: During the experimental period (after surgery) control of dietary intake and weight gain of animals was performed twice a week. The measurement of dietary intake was performed considering the amount of diet offered in each box and subtracting the leftover from the diet. The result was divided by the number of mice housed in the respective boxes and number of days.

Collection of blood samples for serum dosages: At the end of the experiment the blood of the animal was collected by cardiac puncture. On the day before collection, the animals were housed in individual cages and submitted to 8 hours of fasting. The blood of the animals was collected by cardiac puncture after they had been anesthetized.

Calcium, Phosphorus, Alkaline Phosphatase, Total Proteins and Fractions: The contents of calcium (Ca), phosphorus (P), alkaline phosphatase and total proteins and serum fractions were determined by automated colorimetric method in a laboratory specialized in veterinary analysis. Diagno Vet Laboratório VeterinárioLtda, in the city of Campo Grande, MatoGrosso, Brazil.

Osteocalcin: The osteocalcin content was determined by the chemiluminescence method at Instituto Hermes PardiniLtda, in the city of Vespasiano, Minas Gerais, Brazil.

Bone determinations: The femur and tibia were exposed after euthanasia of the animals, cleaned and frozen. Afterwards, the bones were charred in Bunsen's beak and then calcined in muffle at 600 °C until clear ashes (approximately 12 h). The investigations of minerals in the bones were carried out in specialized biominerals laboratory in the city of Campinas, São Paulo, Brazil, which provides services in Instrumental Analytical Chemistry when the investigation of minerals, trace elements, toxic metals is necessary. The method used by said laboratory was Inductively Coupled Plasma Atomic Emission Spectrometry (ICAP 6300 - Thermo Scientific, Cambrige is ISO certificed).

Statistical analysis: The quantitative variables of the four groups of animals in the study were compared using the Analysis of Variance (ANOVA), when they presented normal distribution and, through the Kruskall-Wallis test, when they presented a non-normal distribution. All analyzes were performed using The Statistical Package for Social Sciences software version 22.2(SPSS, Chicago, IL, USA), with a significance level of 5%.

Ethical issues: The entire experiment was carried out in accordance with the principles and procedures described by the Brazilian College of Animal Experimentation and approved by the UFMS Ethics Committee on Animal Use under protocol nº. 640/2014, and the work is conducted within the international ethical standards that aim to eliminate all unnecessary suffering to the living being.

RESULTS AND DISCUSSION

Comparison between serum biochemical markers: Table 1 shows the serum biochemical parameters analyzed in the study, as well as the comparison of these parameters among the groups of the experimental design. Significant differences were observed between the groups in serum calcium concentration ($p = 0.002$), phosphorus ($p = 0.004$) and alkaline phosphatase ($p = 0.024$). For the calcium and phosphorus minerals, it was observed that the SHAM PDX and GXT PDX groups presented higher mean serum concentrations in relation to the GXT S/P group ($p<0.05$). The GXT S/P group had the lowest mean serum phosphorus level ($p = 0.004$). Regarding phosphatase, the GXT PDX group had a higher mean serum level in relation to the SHAM PDX group ($p = 0.024$). In relation to the other biochemical parameters there was no significant difference between the groups. In this study, in relation to serum levels, significant differences were observed between minerals in the four calcium groups ($p = 0.002$); ($p = 0.004$) and alkaline phosphatase ($p = 0.024$), showing that in relation to calcium and phosphorus the groups SHAM PDX and GXT PDX presented higher averages in relation to the GXT S/P group, suggesting the fact of PDX to maintain higher serum calcium levels in supplemented animals. Associated with this result, the level of alkaline phosphatase was higher in the group of GXT PDX animals, an enzyme involved in the mineralization of the bone matrix. Serum calcium levels with

Table 1. Description and comparis on of serious biochemical parameters between the study groups

Serumparameters	Studygroups				Value p*
	SHAM PDX n=9 Mean (SD)	SHAM S/P n=8 Mean (SD)	GXT PDX n=8 Mean (SD)	GXT S/P n=9 Mean (SD)	
Calcium (mg/dL)	8.16 (1.17)	6.80 (1.76)	7.20 (1.65)	5.15 (1.21)	0.002[a]
Phosphorus (mg/dL)	5.30 (1.09)	4.89 (1.42)	5.78 (0.81)	3.64 (1.10)	0.004[b]
Alkalinephosphatase (U/L)	31.93 (7.44)	33.87 (7.04)	46.12 (9.70)	38.11 (11.94)	0.024[c]
Osteocalcin (ng/mL)	31.93 (7.44)	36.17 (7.43)	29.04 (10.24)	29.99 (12.10)	0.463
Total protein (g/dL)	4.39 (0.74)	3.75 (1.07)	4.40 (1.05)	3.32 (0.92)	0.074
Albumin (g/dL)	2.86 (0.45)	2.56 (0.65)	2.83 (0.71)	2.09 (0.66)	0.057[d]
Globulin (g/dL)	1.52 (0.45)	1.19 (0.52)	1.56 (0.40)	1.23 (0.43)	0.239

Notes: DP = standard deviation; p = statisticalt est value; SHAM PDX = group of false animals operated on 7.5% polydextrose-based diet; SHAM S/P = group of false animals operated on with diet without polydextrose; GXT PDX = group of gastrectomized animals with 7.5% polydextrose-based diet; GXT S/P = group of animals gastrectomized with diet without polydextrose.

*Variables with normal distribution, analyzed by Analysis of Variance - ANOVA, level of significance of 5%.

In the Tukey Pos-Hoc Test, significance level of 5%, differences between the following groups were found: [a]SHAM PDX and G S/P (p=0,001), GXT PDX and G S/P (p=0,035); [b]SHAM PDX and GXT S/P (p=0,025), GXT PDX and G S/P (p=0,003); [c]SHAM PDX and GXT PDX (p=0,024); and[d] statistical trend the difference between the SHAM PDX e GXT S/P (p=0,076) e GXT PDX e GXT S/P (0,092).

Table 2. Description and comparison of the quantitative content of calcium and bonephosphorus between the study groups

Boneparameters	Studygroups				Value p*
	SHAM PDX n=9 Mean (SD)	SHAM S/P n=8 Mean (SD)	GXT PDX n=8 Mean (SD)	G S/P n=9 Mean (SD)	
Righttibia - meanweigh (g)	0.62 (0.10)	0.61 (0.04)	0.57 (0.10)	0.57 (0.09)	0.456
Rightfemur - meanweight (g)	0.78 (0.06)	0.81 (0.05)	0.76 (0.11)	0.74 (0.05)	0.358
Calcium(mg/g bone)	21.74 (1.24)	21.01 (1.07)	22.01 (1.17)	22.10 (0.97)	0.211
Phosphorus(mg/g bone)	11.78 (0.61)	11.71 (0.55)	11.99 (0.53)	12.13 (0.54)	0.393

Notes: g = grams; SD = standard deviation; p = statistical test value; SHAM PDX = group of false animals operatedon 7.5% polydextrose-based diet; SHAM S / P = group of false animals operated on with diet without polydextrose; GXT PDX = group of gastrectomized animals with 7.5% polydextrose-based diet; GXT S / P = group of animals gastrectomized with diet without polydextrose

*Variables with normal distribution, analyzed by Analysis of Variance - ANOVA, level of significance of 5%

the highest mean in the two groups supplemented with PDX suggest the beneficial effect of the prebiotic in preventing the reduction of serum levels of the mineral and subsequent mobilization of the same by the bone and may indicate that the levels in the blood are regulated. Soluble fibers are constantly being studied for their possible potential in improving bone health and in large part by the improvement in the absorption of minerals (Scholz-Ahrens, 2007; Weisstaub, 2013). Since bone is the main site of calcium storage in the body and undergoes adaptations regarding its constitution, it serves as a metabolic reserve of calcium and phosphorus, which can be mobilized during situations of homeostasis. Appropriate concentrations of calcium in the blood are critical to maintaining the structural integrity of the bone.

Bone mineral content: Table 2 presents the data of bone parameters analyzed in the study animals. No statistically significant differences were found between the groups of animals in the study regarding the weight of the right tibia (grams), weight of the right femur (grams), amount of calcium and phosphorus in the bone. In this study, the bone results suggest that PDX prebiotic supplementation had protective effects on bone mass, since there was no reduction in tibial and femur weight of GXT groups, and there was preservation in calcium and phosphorus content in GXT groups in relation to SHAM groups. OHTA *et al*. (1998) tested 7.5% FOS (Frutooligosaccharides) in diets for gastrectomized rats and found that Ca and P levels of the femur and tibia of rats increased. Takahara *et al.* (1999) evaluated the effects of 5% FOS on diets for rats and found an increase in femur and trabecular bone volumes of the femur and tibia, P content in the diaphysis and metaphysis, and Ca content in the epiphysis, diaphysis and metaphysis. These reports show the best mineralization of bones in animals fed with nondigestible oligosaccharides. One study demonstrated that FOS also stimulates transcellular absorption of Ca in the large intestine, as indicated by the higher concentration of calbidine-D9k, a Ca-carrying protein that plays an important role in the transport of intestinal Ca (Tungland, Meyer, 2002). Rats are good models for evaluating the benefits of prebiotic consumption because their shorter shelf-life allows for studies of long-term efficacy in relation to functional bone health outcomes (Coudry *et al*., 2003; Weaver *et al*., 2011). The ingestion of some types of prebiotics has been demonstrated in studies in adolescents and menopausal women, improving the absorption of calcium and bone mineral density (Roberfroid, 2010; Legette, 2012; Slavin, 2013). Suzuki and Hara (2004) have emphasized that oligosaccharides can increase the uptake of Ca also in the small intestine by means of the paracellular pathway, by direct stimulation on the epithelium of the small intestine. In in vitro studies using intestinal epithelium isolated from rats, these authors concluded that anhydrous difruthos III and IV (disaccharides), (FOS) and maltitol increased the paracellular absorption of Ca in the small intestine. In addition, the use of prebiotic agents, such as GOS (Galactooligosaccharides), FOS / inulin (Roberfroid *et al*., 2002; García-Vieyra *et al*., 2014) and polydextrose (Legette *et al*., 2012) have been associated with improvements in calcium absorption. Animal studies found that prebiotics had a dose-dependent effect on calcium absorption. In addition, intracellular fructanes (up to 20% of the diet) (Levrat *et al*., 1991) and lactulose (5% and 10%) (Brommage *et al*., 1993)

resulted in greater absorption as the dose increased. Despite encouraging dose-related results, these may vary depending on a variety of factors, including age of animals, experimental conditions, duration of treatment and methodology employed in the study. Longer-study studies measuring the dose of various combinations of prebiotics on bone density, strength and fracture appear to be complementary to understanding the mechanism of action of this type of soluble fiber.

Conclusion

The serum levels showed that there were differences between the groups in the minerals calcium and phosphorus, and the groups supplemented with polydextrose presented higher averages compared to those not supplemented. In addition, the study of bone mineral content showed that there was no difference between the experimental and control groups in relation to the weight of the tibia and right femur, nor mineral amount of calcium and phosphorus significant bone. Demonstrating preservation of these minerals analyzed in the gastrectomized group. The present research suggests that germ free animals are used for this surgical model and, with a longer experiment time of about 10 to 12 weeks. Sufficient time for recovery from surgical stress, healing, and weight stabilization.

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