STUDY OF PALM OIL AND GLUTAMINE AS FUNCTIONAL FOODS ON PEPTIDE TYROSINE TYROSINE AND GLUCAGON-LIKE PEPTIDE 1 IN OBESE PATIENTS FOLLOWING UP ON THE AMBULATORY HEALTH SERVICES OF A PUBLIC HOSPITAL

LUCIANA ARANTES SOARES [1]
ALEX AUGUSTO DA SILVA [2]
ALESSANDRA BAZAGA BAPTISTA [3]
THARSUS DIAS TAKEUTI [4]
JUVERSON ALVES TERRA-JÚNIOR [5]
EDUARDO CREMA [6]

ABSTRACT

Incretin-based therapies are an alternative for the treatment of type 2 diabetes and weight reduction. In this respect, functional foods such as palm oil and glutamine are dietary strategies for the stimulation of intestinal peptides. Objective: The aim of this study was to evaluate whether the palm oil capsules of ileal release (LI) and of glutamine (LI) result in increased secretion of glucagon-like peptide-1 (GLP-1) and ***Peptide*** tyrosine tyrosine (PYY). Method: Nineteen obese patients follow-up of the ***Ambulatory*** Health Services, received nutritional guidance and supplementation with ileal release capsules containing palm oil and glutamine. Result: Prospective analysis showed an increase in median GLP-1 levels between T0 (before treatment) and T2 (after 2 months of treatment) from 21.9 pmol/liter (2-93) to 25.7 pmol/liter (3-92.5) (p= 0.564). The baseline of peptide YY increased between T0 68.5 pg / mL (46.5 to 150) to 71 pg / mL (46-181) in T2 (p= 0.909). The significant level established for all analyses was 5% (p <0.05). Conclusion: The daily intake of palm oil capsules (LI) and of glutamine (LI) by a period of 2 months did not influence the secretion of GLP-1 and PYY in obese patients. However, weight maintenance was observed during the evaluated period. Further studies are needed for inferences in this population, to determine if functional foods such as palm oil and glutamine are associated with other specific health benefits.

KEYWORDS: Obesity. Health Services. Hospitals, Public. Functional Food. Incretins.

1- Pharmaceutical, Postdoctoral Fellow, Postgraduate Program in Health Sciences, UFTM, Uberaba-MG, Brazil
2- Assistant Professor, Division of Surgical Technique and Experimental Surgery, UFTM, Uberaba-MG, Brazil.
3- Nutritionist of Division of Surgical Technique and Experimental Surgery, Master, Program in Health Sciences, UFTM, Uberaba-MG, Brazil.
4- Biomedical scientist, Doctor, Program in Health Sciences, UFTM, Uberaba-MG, Brazil.
5- PhD, Assistant Professor, Division of Surgical Technique and Experimental Surgery, UFTM, Uberaba-MG, Brazil.
6- PhD, Full Professor, Division of Digestive Surgery, Universidade Federal do Triângulo Mineiro (UFTM), Uberaba-MG, Brazil.

INTRODUCTION

A possible therapeutic target for weight loss and control of type 2 diabetes is one of the hormone-producing organs of the body, the intestine (Soares & Crema, 2020). According to studies investigating the mechanism of satiety (Winkler *et al*., 2019), the release of intestinal peptides occurs according to the food content of the intestine and depends on the nutritional status of the individual. This release may contribute to the control of obesity and type 2 diabetes.

The population of overweight and obese people has increased worldwide over several decades and is a difficult problem to solve. As a consequence, the incidence of diseases such as type 2 diabetes has also increased (Amant *et al*., 2020). Intervention strategies designed to stimulate the secretion of intestinal peptides have been used for the control of type 2 diabetes and obesity (Rebelo *et al*., 2012). Several intestinal peptides are implicated in gastrointestinal tract signaling. The main peptides are glucagon-like peptide 1 (GLP-1) and peptide YY (PYY), which are associated with satiety, prolongation of gastric emptying, and increased insulin secretion and sensitivity (Camilleri, 2019).

Studies suggest that the consumption of functional foods such as palm oil and glutamine contributes to the control of metabolic events. Palm oil extracted from the oil palm tree *Elaeis guineensis* contains medium-chain fatty acids and has some proven therapeutic properties, such as antioxidant effect (Takeuti *et al*., 2014). Glutamine is an amino acid with several beneficial nutrition-related effects, including a reduction of infection risk, maintenance of intestinal integrity, and control of diabetes (Galera *et al*., 2010). Despite the known effects of palm oil and glutamine on diabetes control and weight reduction, coating with Eudragit® S100 may improve the results of future therapeutic application. The objective of this study was to evaluate the influence of ileal release

capsules containing palm oil and glutamine on the secretion of GLP-1 and PYY in obese patients before and after daily consumption of the capsules for 2 months following up on the Ambulatory Health Services at the University Hospital of the Federal University of Triângulo Mineiro (UFTM), Uberaba, Brazil.

METHODS

This prospective study was conducted between February 2013 and November 2015 on the Ambulatory Health Services at the University Hospital of the Federal University of Triângulo Mineiro (UFTM), Uberaba, Brazil.

The final sample consisted of 19 obese subjects who ingested palm oil and glutamine LI capsules for 2 months. The criteria for inclusion were obesity defined as a body mass index (BMI) of 30-35 kg/m^2. Exclusion criteria were metabolic surgery, history of pancreatic diseases, and age younger than 18 and older than 60 years. The participants were advised to ingest 1 palm oil LI capsule and 1 glutamine LI capsule three times per day before the main meals, totaling 6 capsules/day, for 2 months. The participants received written information about the study and signed the free informed consent form. The study was approved by the Ethics Committee of UFTM, Uberaba, Minas Gerais, Brazil (Protocol CEP/UFTM: 2341). The protocol was carried out following the recommendations of Resolution No. 196/96, which approves the regulatory norms for research involving human subjects.

PRODUCTION OF THE ILEAL RELEASE CAPSULES

The palm oil capsule (Sorocaps®, Sorocaba, São Paulo, Brazil) and the glutamine capsule (ErliCaps Envase Ltda.®, São Paulo, Brazil) contained 1 g of the active compound per unit. The LI coating was manipulated at the Laboratory of Pharmaceutical Technology, UFTM, Uberaba, Minas Gerais, Brazil, and contained 6.5% Eudragit® S100 (Evonik Industries). This coating was formulated to promote the release of the active compound in an alkaline environment at pH 6.8 (Soares & Crema, 2020).

ANALYTICAL METHOD

Fasting blood samples were collected from all participants before and 30 and 60 days after treatment with the palm oil and glutamine LI capsules. The blood sample was centrifuged immediately at 5,000 rpm and the serum obtained was stored in 1.5-mL sterile plastic tubes at -70°C. For GLP-1 measurement, the enzyme inhibitor DPP-4 (Millipore) was added to the blood sample at a proportion of 10 µL inhibitor per 1 mL of blood.

LABORATORY TESTS

GLP-1 and PYY was measured using sandwich enzyme-linked immunosorbent assay (ELISA) kits (Millipore®) at the Laboratory of Biochemistry, UFTM. Fructosamine and glucose were measured at the Laboratory of Clinical Pathology, University Hospital of UFTM. Glucose was determined by an enzymatic colorimetric method and fructosamine by an automated kinetic method.

STATISTICAL ANALYSIS

The results were analyzed using the GraphPad Prism® 5 and StatView® 4.57 programs. Normality of the variables was evaluated by the ***Shapiro-Wilk test. Normally distributed data are reported as the mean and standard deviation and were compared by the paired Student t-test and ANOVA. Data that were not normally distributed are reported as median, minimum and maximum and were compared by the nonparametric Wilcoxon and Friedman tests. Inferential analysis was performed at baseline (T0) and after 1 (T1) and (T2) 2 months of treatment. The results were considered significant when*** $p<0.05$.

RESULTS

Table 1 shows the physical characteristics of the patients at baseline and after 60 days of treatment. The body weight of the participants remained constant over the 60 days ($p = 0.22$). Among the 19 obese subjects submitted to the proposed protocol, 15 (78.94%) were female and 4 (21.05%) were male. The mean age was 40.84 years (± 11.55).

Table 1. Overall analysis of the baseline values (age, body mass index, fructosamine and glucose) of the patients and differences between means after 30 and 60 days

Parameter	Time	Mean ± SD	Overall difference between means		
			p T0/T1	p T0/T2	p T0/T1/T2

Age (years)	T0	40.84 (± 11.55)[a]			
BMI	T0	32.51 (± 2.54)[a]	0.21	0.22	0.26
	T1	32.37 (± 2.49)[a]			
	T2	32.22 (± 2.63)[a]			
Fructosamine (mg/dL)	T0	220 (191-254)[b]	0.85	0.45	0.88
	T1	216 (191-247)[b]			
	T2	215 (200-256)[b]			
Glucose (mg/dL)	T0	88.31 (± 13.29)[a]	0.50	0.21	0.37
	T1	86.14 (± 11.63)[a]			
	T2	84.07 (± 9.01)[a]			

BMI: body mass index. a = Mean (standard deviation). b = Median (upper-lower interquartile range).

p = significance.

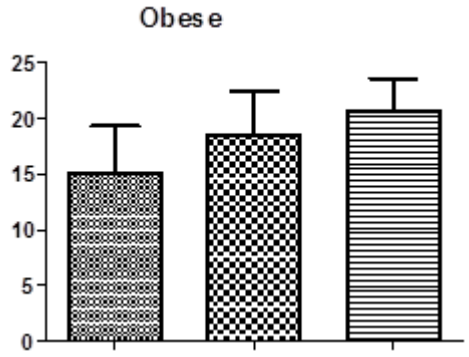
FRUCTOSAMINE AND GLUCOSE

A reduction of fructosamine and glucose was observed in the obese participants after 60 days of supplementation with LI palm oil and glutamine, but the difference was not statistically significant. Fructosamine levels were within the normal reference range at all time points analyzed: 220 mg/dL (191-254) at T0, 216 mg/dL (191-247) at T1, and 215 mg/dL (200-256) at T2 (Table 1).

GLUCAGON-LIKE PEPTIDE-1- GLP-1

In the participants, median GLP-1 increased from 21.9 pmol/liter (2.0- 93) at T0, 15.50 pmol/liter (2,1-99) at T1 to 25.7 pmol/liter (3- 92.5) at T2, but this increase was not significant (Graph 1).

Graph 1. Palm oil and glutamine capsules (LI) Effect on GLP-1 concentration

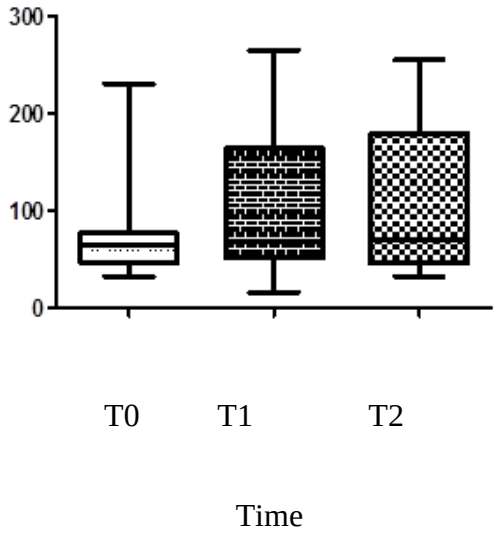


Graph 1. Effect of palm oil and glutamine ileal release capsules on GLP-1 concentration. 1) Graph showing median, minimum and maximum concentrations of GLP- 1 in the period before the intake of palm oil capsules (LI) and glutamine capsules (LI) in time (T0) and after the intake of palm oil (LI) and glutamine capsules (LI) in T1 and T2 (non-parametric Friedman test) = (0.564).

PEPTIDE TYROSINE TYROSINE -PYY

In the participants, median PYY increased from 68.5 picogram/milliliter (46.5-150) at T0, 68.5 picogram/milliliter (51-152) at T1 to 71 picogram/milliliter (46-181) at T2, but this increase was not significant (Graph 2).

Graph 2: Palm oil and glutamine capsules Effect (LI) on PYY concentration



2) Graph showing median, minimum and maximum PYY concentration in the period before the intake of palm oil and glutamine capsules (LI) in time (T0) and after ingestion of palm oil (LI) and glutamine capsules (LI) in T1 and T2 (non-parametric Friedman test) = (0.909).

DISCUSSION

This study evaluated the dietary ingestion of palm oil and glutamine LI capsules and their effects as coadjuvants in the treatment of obesity. The variables analyzed in this prospective clinical study were GLP-1, PYY, BMI, glucose and fructosamine. The palm oil and glutamine capsules were designed to release their content within about 1 hour after exposure to a pH of 6.8, corresponding to the ileal pH. The recommendation was to ingest the dose 1 hour before meals in order to promote satiety and to induce the subject to consume less food. This therapeutic approach was based on studies investigating the consumption of functional foods as a strategy for weight reduction. After oral administration of nutrients, a response directed towards the end of the ileum would probably trigger weight reduction and diabetes control because of an increase in intestinal peptides, causing a satietogenic effect. The undigested nutrients in the ileum make the individual feel satisfied. This signaling mechanism is known in the literature as ileal brake (Winkler *et al*., 2019). The presence of nutrients in the ileum stimulates the release of satiety hormones, which seems to be reduced in obese patients.

The outpatient health services constituted an organized system for the provision of health care at the Clinical Hospital of the Federal University of Triangulo Mineiro (UFTM) in Uberaba, Brazil. The health services offered were related to the clinical-care role and the development of actions focused on the care of obese, such as health education, pharmacotherapeutic follow-up and promotion of the rational use of medications, being performed by a team. Multidisciplinary team consisting of pharmacist, doctor, nutritionist and biomedic.

A median increase in GLP-1 from 21.9 pmol/L (2.0-93) at T0 to 25.7 pmol/L (3-92.5) at T2 was observed in the obese participants (graph 1), with the difference not being

significant. Soares et al. evaluated the effect of palm oil capsules of ileal release -LI and of glutamine -LI result in increased secretion of GLP-1 (Glucagon-like peptide-1) at of glucagon-1-like in type 2 diabetic patients. Although there was an increase in intestinal peptides in the analyzed groups, it had no statistical relevance (Soares et al., 2018).

A median increase in PYY 68.5 pg/ml (46.5-150) at T0, 68.5 pg/ml (51-152) at T1 to 71 pg/ml (46-181) at T2, but this increase was not significant (graph 2) in the obese participants. In obese individuals, changes have been reported in plasma levels of fasting peptides of the gastrointestinal and hypothalamic tract, as reduction of PYY (Li & Vederas, 2009). Takeuti *et al*. evaluated the effect of palm oil and glutamine on serum GLP-1, PYY and glucose levels in patients with type 2 diabetes mellitus submitted to metabolic surgery. A significant decrease in PYY was observed between the fasted state and 2 hours, suggesting that the peak in this peptide occurs immediately after administration of the oil (Takeuti *et al*., 2014). Robertson *et al.* administered diets containing a fraction of palm oil with other saturated fatty acids to healthy women and measured the serum levels of GLP-1 and PYY. The authors found a peak in the two hormones 30 min after administration of the diet (Robertson *et al*., 2002).

The pH in the duodenal portion ranges from 2.5 to 6.5 However, these values can vary minute-by-minute from 1 to 7 in the fasted state and from 3 to 7 after meals (Manadas *et al*., 2002). It is therefore unclear if the active compounds have reached the ileum intact.

It is not possible to know the concentration of palm oil and glutamine that reached the intestinal lumen because of inter- and intraindividual variations in gastric motility, volume of gastric/intestinal secretion, and profile of capsule opening. Theoretically, the dissolution of 10 capsules may trigger a better response of intestinal peptides (Meek *et al*., 2015) and the doses of palm oil and glutamine are likely to be a limiting factor in this case.

Analysis of fructosamine and glucose in the obese participants showed a reduction after 2 months of supplementation with LI palm oil and glutamine, but the difference was not significant. Serum fructosamine is measured by colorimetric assays and the normal reference range is 205 to 285 μmol/L (Feitosa & Andrade 2014).

Esser *et al*. evaluated the postprandial impact of shakes containing different compositions of saturated fatty acids (SFA; palm oil), monounsaturated fatty acids (MUFA; sunflower oil) or polyunsaturated fatty acids (PUFA; palm oil and D-Marinol D-40) in lean and obese men. Marinol D-40 is concentrated natural fish oil with a high content of docosahexaenoic acid (DHA) in glyceride form. The results showed that consumption of MUFA was associated with a more marked increase in triglycerides when compared to PUFA and SFA. The percentage of palmitic acid was 10% higher after ingestion of SFA, the percentage of oleic acid was 60% higher after ingestion of MUFA, and the percentage of DHA was 950% higher after ingestion of PUFA (Esser *et al*., 2013).

The present results showed no significant alteration in BMI. The aim of ingestion of palm oil and glutamine capsules proposed in this study was to serve as food coadjuvants. In this respect, a healthy, varied and balanced diet may improve the results obtained, enhancing the expected effect of the capsules. Dietary guidance was provided, but ingestion was controlled by the participant who was responsible for achieving the objectives of the diet program. An adequate diet and physical activity are relevant factors for the prevention and treatment of Metabolic Syndrome (Murici *et al*., 2015). Guimarães *et al*. investigated adherence to a group nutrition counseling program for subjects with excess weight and comorbidities. Two groups were studied: the intervention group received group nutrition counseling consisting of personalized care and participation in six group meetings to discuss healthy eating and physical activity using group dynamics and a

participatory method. The control group received personalized care in three outpatient visits. The study concluded that, despite the comprehensiveness of the content and the use of a participatory method in the intervention group, adherence was insufficient to significantly change the main dietary parameters studied (Guimarães *et al*., 2010).

Nutrients related to the control of obesity have been extensively investigated. Compounds that stimulate intestinal peptides have raised great interest in the medical, clinical and pharmaceutical area. Future studies that strictly control the research variables should be conducted, examining the dosage and long-term effects of these natural molecules on body physiology, which seems to be an important factor in the metabolic management of the human body.

CONCLUSION

The daily intake of palm oil capsules (LI) and of glutamine (LI) by a period of 2 months did not influence the secretion of GLP-1 and PYY in obese patients. However, weight maintenance was observed during the evaluated period. Further studies are needed for inferences in this population, to determine if functional foods such as palm oil and glutamine are associated with other specific health benefits.

FUNDING SOURCES: Supported by the National Council for Scientific and Technological Development (CNPq), the Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES).

There is no conflict of interest in this paper.

References

1- AMANAT S, et al. Exercise and Type 2 Diabetes. **Adv Exp Med Biol**. V.1228. p. 91-105.2020.

2- CAMILLERI M. Gastrointestinal hormones and regulation of gastric emptying. **Curr Opin Endocrinol Diabetes Obes**. V. 26. p. 3-10. 2019.

3- ESSER, D. *et al*. A high-fat SFA, MUFA, or n3 PUFA challenge affects the vascular response and initiates an activated state of cellular adherence in lean and obese middle-aged men. **J Nutr**.V.143. N. (6). p. 843-51. 2013.

4- FEITOSA, A. and ANDRADE, F. Avaliação da frutosamina como parâmetro de controle glicêmico na gestante diabética. **Arq Bras Endocrinol Metab**. 2014.v.58. n.7. p. 724-730. 2014.

5- GALERA, S.C. *et al.* The safety of oral use of l- glutamine in middle-aged and elderly individuals. **Nutrition**.v.26. n. 4. p. 375-381. 2010.

6- GUIMARÃES, N. *et al*. Adherence to a nutritional counseling program for adults with excesso weight and comorbidities. **Rev. Nutr. Campinas**. V. 23. N. 3. p. 323-333. 2010.

7- LI, J.W.H. and VEDERAS, J. C. Drug discovery and natural products: end of an era or an endless frontier? **Science**. v. 325. n. 5937. p. 161. 2009.

8- MANADAS, R.; PINA, M. E.; VEIGA, F. A dissolução in vitro na previsão da absorção oral de fármacos em formas farmacêuticas de liberação modificada. **Brazilian Journal of Pharmaceutical Sciences**.v.38. n.4.p 375-399. 2002.

9- MEEK, C. *et al*. Can encapsulated glutamine increase GLP-1 secretion, improve glucose tolerance, and reduce meal size in healthy volunteers? A randomised, placebo-controlled, cross-over trial. **Lancet**. v. 26. n. 385. p.68. 2015.

10- MURICI, G.R.; FRANCISCO, I.; RIBEIRO ALVES, M.A. Prevalência dos componentes associados a síndrome metabólica no Brasil e revisão crítica dos fatores dietéticos associados à prevenção e ao tratamento. **Revista Rede de Cuidados em Saúde**. V. 9. N.1. p.1-15. 2015.

11- REBELLO, C.J. *et al.* Efficacy of Olibra: a 12-week randomized controlled trial and a review of earlier studies. **J Diabetes Sci Technol**. v.6. n.3. p. 695-708. 2019.

12- ROBERTSON, M.D. *et al*. Acute ingestion of a meal rich in n-3 polyunsaturated fatty acids results in rapid gastric emptying in humans**. Am J Clin Nutr.** v. 76. n.1. p.232-8. 2002.

13- SOARES, L.A. *et al.* Influência da cápsula de liberação ileal de óleo de palma e de glutamina sobre peptídeos intestinais GLP-1 em pacientes com diabetes tipo 2. **Revista Brasileira de Obesidade, Nutrição e Emagrecimento**. v.12. n.73.2018. p. 615-623. 2018.

14- SOARES, L. A., & CREMA, E. Study of a delayed-release system for hard and soft capsules coated with eudragit® s100 acrylic polymers. **Acta Scientiarum. Health Sciences**, V.42. N. 1. p. 1-10. 2020.

15- TAKEUTI, T.D. *et al.* Effect of the ingestion of the palm oil and glutamine in serum levels of GLP-1, PYY and glycemia in diabetes mellitus type 2 patients submitted to metabolic surgery. **Arq Bras Cir Dig**. V. 30.n. 2. P. 51-55. 2014.

16- WINKLER G, HAJÓS P, KISS JT. [Glucagon-like peptide-1 (GLP1) and the gastrointestinal tract. GLP1 receptor agonists: overemphasized gastric, forgotten intestinal ("ileal brake") effect?]. **Orv Hetil.** V. 160. N.49. p. 1927- 1934. 2019.