ISSN (electrónico): 1699-5198 - ISSN (papel): 0212-1611 - CODEN NUHOEQ S.V.R. 318

ARÁN

Nutrición Hospitalaria

Trabajo Original

Otros

Effects of 3-day fasting on secretory IgA concentration in the saliva and lymphotoxin alpha expression in healthy volunteers: a proof of concept study

Efecto de 3 días de ayuno sobre la concentración de IgA secretora en la saliva y la expresión de linfotoxina alfa en voluntarios sanos: un estudio de prueba de concepto

Gabriela Quiroz-Olguín[1,2], Gabriela Gutiérrez-Salmeán[2], Angélica I. Borja-Magno[3], Adriana Flores-López[1], Martha Guevara-Cruz[3], Francisco Enrique Gómez[3], Aurora E. Serralde-Zúñiga[1]

[1]Clinical Nutrition Service. Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán; [2]Centro de Investigación en Ciencias de la Salud. Faculty of Health Sciences. Universidad Anáhuac; [3]Department of Nutrition Physiology. Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán. Ciudad de México, Mexico

Abstract

Background: animal studies have shown that enteral stimuli play an important role in modulating gut-associated lymphoid tissue thus fasting may exert detrimental effects on such.

Objective: the main objectives of this study were: *1)* to evaluate the effect of 3-day fasting on secretory immunoglobulin A (S-IgA) in parotid saliva; and *2)* to determine the levels of lymphotoxin (LT) transcription in peripheral blood mononuclear cells (PBMNC) from healthy volunteers as a proxy for studying GALT health.

Methods: these adult volunteers had fasted for 3 days as part of a cultural ritual. Eleven volunteers (seven men and four women) with a median age of 43 (40-56) were included. Parotid saliva and blood samples were collected on day 0 (no fasting) and 3 days after fasting. Parotid saliva was obtained using a modified on-Crittenden device, and S-IgA was quantified using ELISA. Total RNA was isolated from peripheral blood mononuclear cells, and the level of lymphotoxin (LT) mRNA expression was determined by RT-PCR. Lipopolysaccharide (LPS), IL-10 (interleukin), tumor necrosis factor (TNF)α, body composition and other metabolic indicators were measured.

Results: median BMI was 27.3 kg/m^2 (24.9-29.1). After 3 days of fasting, there were no significant differences in S-IgA concentrations ($p = 0.657$), LT expression ($p = 0.063$), LPS ($p = 0.182$), nor IL-10 ($p = 0.110$). We found a statistical difference in TNFα levels (13.5 vs 11.3 pg/mL; $p = 0.005$) between the 0 and 3-day samples; TNFα decreased after fasting.

Conclusion: further studies are needed to clarify the role of LT in the production of S-IgA and its relationship with nutritional status and inflammatory factors.

Keywords:

Fasting. Immunoglobulin A. Gut-associated lymphoid tissue. Mucosal immunity. Inflammation. Lymphotoxin alpha.

Received: 30/03/2024 • Accepted: 01/11/2024

Conflicto de intereses: los autores declaran no tener conflicto de interés.

Inteligencia artificial: los autores declaran no haber usado inteligencia artificial (IA) ni ninguna herramienta que use IA para la redacción del artículo.

Quiroz-Olguín G, Gutiérrez-Salmeán G, Borja-Magno AI, Flores-López A, Guevara-Cruz M, Gómez FE, Serralde-Zúñiga AE. Effects of 3-day fasting on secretory IgA concentration in the saliva and lymphotoxin alpha expression in healthy volunteers: a proof of concept study. Nutr Hosp 2025;42(1):131-136

DOI: http://dx.doi.org/10.20960/nh.05249

Correspondence:

Aurora E. Serralde-Zúñiga. Clinical Nutrition Service. Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán. Vasco de Quiróga 15, Col Belisario Domínguez, Sección XVI. Tlalpan 14080 Ciudad de México, Mexico
e-mail: aurora.serraldez@incmnsz.mx

Resumen

Introducción: estudios en animales han demostrado que el estímulo enteral desempeña un papel importante en la modulación del tejido linfoide asociado al intestino.

Objetivo: evaluar el efecto del ayuno de 3 días sobre la inmunoglobulina A secretora (S-IgA) en saliva de parótida y determinar el estado del GALT mediante los niveles de transcripción de linfotoxina (LT) en células mononucleares de sangre periférica (PBMNC) de voluntarios sanos.

Métodos: los voluntarios ayunaron durante 3 días como parte de un ritual cultural. Se incluyeron once voluntarios (siete hombres y cuatro muje-res) con una edad media de 43 años (40-56). Se obtuvieron muestras de saliva de parótida y sangre en el día 0 (sin ayuno) y 3 días después del ayuno. La saliva parotídea se obtuvo utilizando un dispositivo Crittenden modificado y la S-IgA se cuantificó mediante ELISA. Se aisló ARN total de PBMNC de sangre periférica y se determinó mediante RT-PCR el nivel de expresión de ARNm de LT. Se midieron lipopolisacáridos (LPS), IL-10, factor de necrosis tumoral (TNF)α, composición corporal y otros indicadores metabólicos.

Resultados: después de 3 días de ayuno, no hubo diferencias significativas en las concentraciones de S-IgA ($p = 0,657$), expresión de LT ($p = 0,063$), LPS ($p = 0,182$) o IL-10 ($p = 0,110$). La concentración de TNFα (13,5 *vs.* 11,3 pg/mL; $p = 0,005$) entre las muestras al día 0 y 3, disminuyó después del ayuno.

Conclusión: se necesitan más estudios para aclarar el papel de la LT en la producción de S-IgA y su relación con el estado nutricional y los factores inflamatorios.

Palabras clave:

Ayuno. Immunoglobulina A. Tejido linfoide asociado al intestino. Inmunidad de mucosas. Inflamación. Linfotoxina alfa.

INTRODUCTION

Mucosal-associated lymphoid tissue (MALT) is the first line of defense against pathogens, in which secretory immunoglobulin A (S-IgA) plays an important role (1). Animal studies have demonstrated a relationship between feeding (route of feeding) and fasting S-IgA levels (2-5). Modulation of the immune response by gut-associated lymphoid tissue (GALT) is based on S-IgA and lymphotoxin (LT) production. LT, a member of the tumor necrosis factor superfamily, is located on the surface of T and B cells as a heterotrimer composed of one alpha and two beta chains (LTa1b2). In addition to regulating the growth and function of lymphocytes, LT is important for developing secondary lymphoids (intestinal Peyer's patches) and intestinal immune responses. Preclinical studies have shown that feeding through the enteral route maintains the concentration of S-IgA, whereas total parenteral nutrition (TPN) significantly reduces its concentration and decreases the GALT size (5-16).

The most significant changes associated with the lack of enteral stimulation occur in the lamina propria; a decrease in S-IgA production and transportation to the intestinal lumen has been reported. Moreover, there is a decrease in the number of Th2 cells, which further reduces the levels of the interleukins (IL) IL-4 and IL-10, two cytokines necessary for S-IgA synthesis (17). In addition, there was a shift in the microbiome to gram-negative strains with an increase in circulating lipopolysaccharide (LPS), leading to an exacerbated pro-inflammatory response and impacting GALT function and development (16,18) (Fig. 1).

However, there are only a few reports in humans because of the complexity of GALT samples. GALT levels did not vary in healthy adults who volunteered to receive TPN for two weeks; biopsies from the proximal jejunum did not show changes in intraepithelial lymphocytes (IELs) or immunoglobulins (IgA, IgM, and IgG); nevertheless, the nutritional status was not assessed (19).

To our knowledge, no study has assessed the changes in S-IgA concentrations and LT expression in healthy individuals after acute fasting. Therefore, our study aimed to document whether S-IgA in parotid saliva and serum inflammation markers, including LPS, IL-10 and TNFα concentrations and LT expression, were affected in healthy volunteers who fasted for 72 hours.

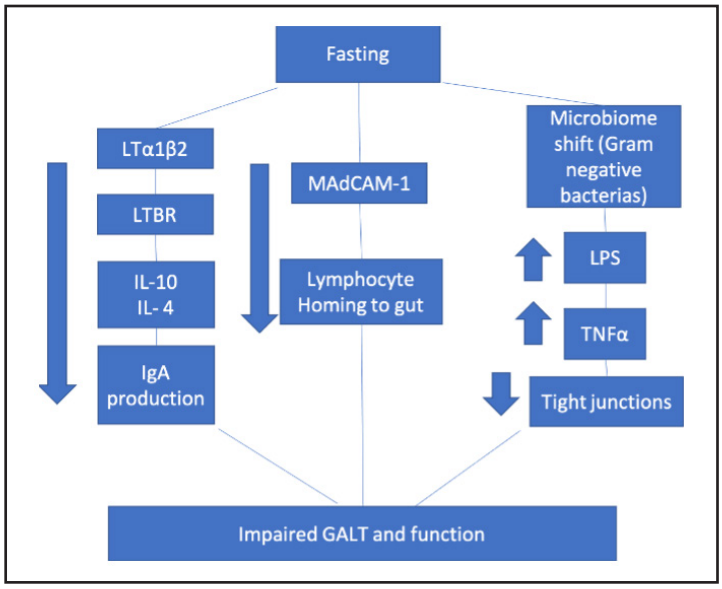

Figure 1.

Changes produced during fasting and their effect on the development of the GALT.

METHODS

We performed a proof-of-concept study in July 2019 on a group of people who fasted for three days as part of a prehispanic ritual according to their cultural belief that fasting purifies their souls. The fasting includes no water or food for three days and the subjects were camping in a mountain. This study was conducted in accordance with the Declaration of Helsinki, and all procedures involving human subjects were approved by the Committee of Biomedical Research on Humans. Written informed consent was obtained from all the subjects. Anthropometric measurements, body composition, and handgrip strength were recorded. Subsequently, we collected parotid saliva and peripheral blood samples at the baseline (T0, no fasting) and on day 3 (T3, 72 h of fasting).

ANTHROPOMETRICS, BODY COMPOSITION AND HANDGRIP STRENGTH

The weights of the subjects were determined using a digital scale (ADE model BA 1301, ADE, Hamburg, Germany) with a sensitivity of 0.1 kg; measurements were taken with the subject standing, with feet slightly apart, barefooted, and after evacuating/urinating.

The heights of the subjects were measured with the subjects standing with their heels together, arms on their sides, legs straight, shoulders relaxed, and the head in the Frankfort horizontal plane. This measurement (cm) was performed using an ultrasonic measuring rod (Inbody model PUSH stadiometer, Inbody, Korea).

Handgrip strength of the dominant hand was assessed with a digital Camry dynamometer, model Eh 101, with the subject sitting comfortably with the shoulder adducted, forearm neutrally rotated, elbow flexed to 90°, and forearm and wrist in a neutral position. They were instructed to perform three consecutive contractions one minute apart, and the mean value was calculated.

Body composition (fat mass, lean mass, and phase angle) was determined using multifrequency BIA equipment (InBody S10; InBody Co., Ltd., Seoul, Korea) using the standard technique (20).

S-IgA AND PROTEIN QUANTIFICATION

The stimulated parotid saliva samples were obtained using a modified Unk On-Crittenden device (21). The device was placed over the Stensen's duct using a slight vacuum created by a rubber bulb. The parotid gland was stimulated by placing three drops of 10 % citric acid solution into the mouth, and clear parotid saliva was collected and stored at -20 °C until assayed. Total S-IgA concentration (mg/dL) was determined using an enzyme-linked immunosorbent assay (ELISA) (22), and protein content was determined using the Lowry technique (23). Saliva samples and S-IgA levels were normalized to the total salivary protein content (S-IgA/l00 mg total protein).

BIOCHEMICAL AND INFLAMMATORY MARKERS

Whole peripheral blood samples were centrifuged at 3000 rpm for 10 min, serum was obtained and stored at –70 °C until analysis. Glucose, total cholesterol (TC), triglyceride (TG), HDL cholesterol (HDL-C), LDL cholesterol (LDLC), albumin, and C-reactive protein (CPR) levels were determined using an enzymatic colorimetric method with a Cobas Integra analyzer (Roche Diagnostics. Indianapolis IN).

Plasma cytokines were quantified using multiplexed immunoassays on a MAGPIX luminometer (Luminex, Austin, Texas, USA) and 4.2 xPONENT software. The evaluated cytokines included TNFα (sensitivity ≥ 1.47 pg/mL) and IL-10 (sensitivity ≥ 2.93 pg/mL).

Serum LPS levels were determined using an ELISA kit (Catalog IEB526Ge, Cloud-Clone Corp, sensitivity ≥ 0.057 ng/mL), following the manufacturer's instructions.

LYMPHOTOXIN (LT) mRNA EXPRESSION

Peripheral blood mononuclear cells (PBMNC) were isolated by centrifugation over a Lymphoprep® density gradient. Total RNA was obtained using TRIzol reagent, and its purity was assessed by 1 % agarose gel electrophoresis and quantified by UV absorption. RNA was kept at -70 °C until use. cDNA was synthesized using 1 µg of total RNA, a mix of random hexamers, and anchored oligo-dT primers, according to the Transcriptor First-Strand cDNA Synthesis Kit (Roche Diagnostics GmbH, Mannheim, Germany). The resulting cDNA was amplified by quantitative polymerase chain reaction (qPCR) using Forget-Me-Not SybrGreen qPCR Master Mix (Biotium Inc., Fremont, CA). The primers used were: Homo sapiens lymphotoxin alpha (LTA, NM_001159740.2), forward 5´- CAGCCCCGACCTAGAACC -3´ and reverse: 5´- GTCATGGGGAGAACCTGCT -3´; and Homo sapiens beta-actin, (ACTB, NM_001101.3), forward 5´- AGAGCTACGAGCTGCCTGAC -3´ and reverse 5´- CGTGGATGCCACAGGACT -3,' as a housekeeping gene. The reaction was performed in the LightCycler-480 II apparatus (Roche Diagnostics LTD, Rotkreuz Switzerland) with the following conditions: 1 cycle of 95 °C for 5 min, followed by 45 cycles of 95 °C, 10 sec; 60 °C, 10 sec and 72 °C, 10 sec, each; a final melting curve analysis consisting on 1 cycle of 95 °C for 5 sec; 65 °C for 1 min and 97 °C with continuous acquisition. qPCR raw data were analyzed through the delta-delta (ΔΔCT) method (24).

STATISTICAL ANALYSES

Data are presented as medians and interquartile ranges. We used the Wilcoxon test to compare continuous variables (T0 vs T3) and Spearman's test for associations between variables. Data were analyzed using SPSS software, version 21, and $p < 0.05$ was considered statistically significant.

RESULTS

We studied 11 participants with a median age of 43 years (40-56), seven of whom were male. The median BMI was 27.3 kg/m^2 (24.9-29.1). The handgrip strength was normal in 8 participants (72.7 %), according to reference values for Mexicans(25). The median phase angle was 6.7º (6.4-7.1), and the median extracellular total water/body total water ratio (ECW/BTW) was 0.371 (0.366-0.377). The lean body mass was 33.7 kg (30.9-36.5) in males and 28 kg (26.1-28) in females. The fat mass was 20.5 % (15-25.6) in males and 33.9 % (33.4-33.9) in females.

Ten participants had at least one of the following conditions: hypercholesterolemia, hypertriglyceridemia, overweight, or obesity. There was no effect on metabolic biomarkers after 3 days of fasting (glucose, TC, HDL-C, TG, CPR and LPS), except for LDL-C concentrations, which showed a significant increase (109.4 mg/dl vs 131 mg/dl, $p = 0.010$) (Table I).

There were no statistically significant differences in S-IgA concentrations, LT expression, or IL-10 levels between days 0 and 3. We did not observe any correlation between S-IgA and LT expression levels. However, we noted a significative reduction in TNFα concentration after the 3-day fasting (13.5 pg/mL vs 11.3 pg/mL, $p = 0.005$) (Fig. 2). We found no association among S-IgA, LT, or other cytokines.

Table I. Biochemical and inflammatory markers ($n = 11$)

	T0 (IR)	T3 (IR)	*p*
Glucose (mg/dl)	85.1 (73.4-122.2)	83.1 (75.3-103.5)	0.477
Cholesterol (mg/dl)	170.6 (147.8-208.1)	179.3 (155.1-222.6)	0.139
Triglycerides (mg/dl)	191.1 (94.9-237.5)	116.9 (81-125)	0.062
HDL-C (mg/dl)	24.1 (17.3-38.2)	20.6 (16.6-29.9)	0.182
LDL-C (mg/dl)	109.2 (95.1-126.8)	131 (111-160.8)	*0.010*
CRP	2.1 (0.65-5.2)	3.7 (1.3-9.7)	0.053
LPS (ng/ml)	234.2 (202-287)	349.2 (189-457)	0.182
S-IgA (mg/100 mg prot)	47.7 (15.5-107.4)	41.4 (26.1-188.6)	0.657
LT (relative units)	1 (0.6-1.2)	0.85 (0.60-0.99)	0.124
IL-10 (pg/ml)	4.5 (2.9-6.1)	6.4 (4.9-7.6)	0.110
TNFα (pg/ml)	13.5 (11.7-19.5)	11.3 (8.9-15.3)	*0.005*

*T0: basal time; T3: 72 hours of fasting; IR: interquartile range; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; CRP: C-reactive protein; LPS: lipopolysaccharide; S-IgA: secretory immunoglobulin A; LT: lymphotoxin; IL-10: interleukin 10; TNFα: tumor necrosis factor alfa. Median comparison by Wilcoxon's test. *p < 0.05.*

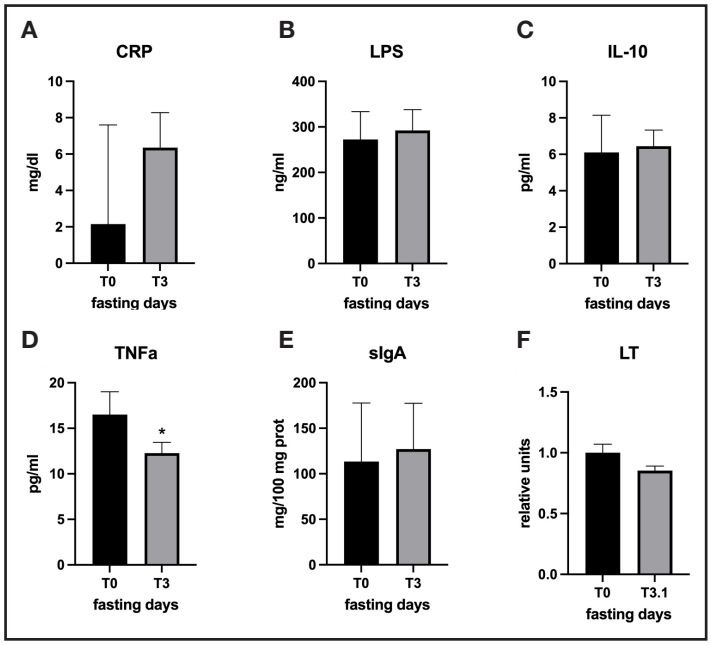

Figure 2.

Comparison between basal time (T0) and 72 hours (T3) concentrations: A. C-reactive protein (CRP). B. Lipopolysaccharide (LPS). C. Interleukin 10 (IL-10). D. Tumor necrosis factor alpha (TNFα). E. S-IgA. F. Lymphotoxin (LT) relative expression. Median comparison by Wilcoxon test. *$p < 0.05$.

DISCUSSION

This is the first study performed in humans to assess the effects of fasting on S-IgA concentrations in saliva and LT α1ß2 mRNA transcripts as markers of mucosal immune response in healthy volunteers.

Animal studies, mostly in mice and rats, have shown that fasting (understood as the lack of enteral stimulation) has deleterious effects on GALT development and S-IgA production; however, when enteral stimulation is reintroduced, these changes are reversed (3,6,11,17,26-28). All murine studies confirmed that S-IgA concentrations drastically decreased after the first day without enteral stimulation. At the same time, there was a decrease in the mucosal addressin cell adhesion molecule 1 (MadCAM-1) expression, needed for producing S-IgA and developing GALT. However, when enteral feeding was reestablished, MAdCAM-1 increased again. This suggests that damage to GALT is reversible (3,6,11,26-28) (3,6,11,27-29).

LT α1ß2 is lymphotoxin beta receptor's (LTßR) ligand, which plays a vital role in GALT differentiation and S-IgA production (30). The receptor is located inside the Peyer patches and lamina propria, making it difficult to study in humans, mainly for ethical reasons. Therefore, our study investigated the ligand expression. Other studies have reported that blocking LTßR produces similar alterations in the mucosa to when TPN is administered to mice.

Our results showed a decrease in the gene expression, and there was no difference in the expression after 72 hours of fasting; maybe due to the small sample size, we couldn't observe changes. Different types of cytokines, such as IL-10, IL-4, and IL-6, are required to produce S-IgA (31,32). We measured IL-10 production but did not observe any change over time.

In contrast, germ-free mice have low levels of S-IgA that normalize through bacterial colonization, and there is an activation of the Peyer's patches that increases the number of plasmatic B cells in the lamina propria (33,34). Furthermore, S-IgA interacts with commensal microbiota, inducing competition for the niche against pathogenic bacteria. Thus, IgA protects the host from pathogenic bacteria (34). We measured LPS concentrations in serum as an indirect marker of bacterial type and also TNFα as an inflammatory marker, but we did not observe differences between days 0 and 3 in LPS and S-IgA.

There was a statistical difference in TNFα, consistent with other studies. In an animal study (35), it has been observed how 30 days of intermittent fasting attenuates the inflammatory response of LPS by decreasing TNFα, IL-6, and IFN-γ, among other cytokines in the serum (36,37). Additionally, the study also demonstrated how rats that had 24 h of fasting could reduce the production of inflammatory cytokines, including TNFα, proposing fasting as a dietary measure to reduce inflammation in subjects with central adiposity.

We did not observe any changes in C-reactive protein (CRP) levels. It is an acute-phase protein associated with inflammation. It is associated with insulin resistance and the development of chronic diseases (38-40). Although the glucose levels in our study on fasting day 3 showed no changes, we hypothesized that the more days of fasting, the more inflammation, which may lead to a change in the intestinal mucosa barrier. In addition, we observed an increase in LPS concentration; however, this increase was not significant.

Our study had some limitations. First, we had a small sample size; however, performing studies with healthy subjects under fasting conditions was difficult. The subjects were camping in a mountain as part of the prehispanic ritual so that made difficult to obtain different kind of samples. Therefore, we could not obtain fecal samples to examine microbiota changes. We did not measure more cytokines needed for S-IgA production, such as IL-13 and IL-4. Our study's strengths are that this is one of the few studies performed in humans with complete fasting (no water and food for three days). We measured LT expression as a possible indicator of S-IgA production in the absence of LTßR expression when it is impossible to obtain an intestinal sample.

It is interesting to note that the results of previous studies may be due to the synergy between stress (injury, trauma, and diseases), malnutrition (undernutrition), and a lack of enteral stimulation. In our study, stress and malnutrition were not observed, which may explain why we could not detect changes in the mucosal immune response after fasting. Therefore, fasting (72 h) alone is not responsible for the decrease in the mucosal immunological response.

In this study, in people fasting for three days, we concluded that there were no differences in S-IgA concentrations, LT expression, LPS, and IL-10 concentrations. Further studies are needed to elucidate the role of ligand LT in the production of S-IgA and its relationship with nutritional status and inflammatory factors.

REFERENCES

1. Gesualdo L, Di Leo V, Coppo R. The mucosal immune system and IgA nephropathy. Semin Immunopathol 2021;43(5):657. DOI: 10.1007/s00281-021-00871-y
2. Sun H, Bi J, Lei Q, Wan X, Jiang T, Wu C, et al. Partial enteral nutrition increases intestinal sIgA levels in mice undergoing parenteral nutrition in a dose-dependent manner. Int J Surg 2018;49(December 2017):74-9. DOI: 10.1016/j.ijsu.2017.12.011
3. King BK, Li J, Kudsk K. A Temporal Study of TPN-Induced Changes in Gut-Associated Lymphoid Tissue and Mucosal Immunity. Arch Surg 1997;132(12):1303. DOI: 10.1001/archsurg.1997.01430360049009
4. Jonker MA, Heneghan AF, Fechner JH, Pierre JF, Sano Y, Lan J, et al. Gut lymphocyte phenotype changes after parenteral nutrition and neuropeptide administration. Ann Surg 2015;262(1):194–201. DOI: 10.1097/SLA.0000000000000878
5. Nakamura K, Ogawa S, Dairiki K, Fukatsu K, Sasaki H, Kaneko T, et al. A new immune-modulating diet enriched with whey-hydrolyzed peptide, fermented milk, and isomaltulose attenuates gut ischemia-reperfusion injury in mice. Clin Nutr 2011;30(4):513-6. DOI: 10.1016/j.clnu.2011.01.002
6. Covello C, Becherucci G, Di Vincenzo F, Del Gaudio A, Pizzoferrato M, Cammarota G, et al. Parenteral Nutrition, Inflammatory Bowel Disease, and Gut Barrier: An Intricate Plot. Nutrients 2024;16(14):2288. DOI: 10.3390/nu16142288
7. Koroleva EP, Fu YX, Tumanov AV. Lymphotoxin in physiology of lymphoid tissues - Implication for antiviral defense. Cytokine 2018;101:39-47. DOI: 10.1016/j.cyto.2016.08.018
8. Btaiche IF, Khalidi N. Metabolic complications of parenteral nutrition in adults, part 1. Am J Health Syst Pharm 2004;61(18):1938-49. DOI: 10.1093/ajhp/61.18.1938

9. Wildhaber BE, Yang H, Spencer AU, Drongowski RA, Teitelbaum DH. Lack of enteral nutrition - Effects on the intestinal immune system. J Surg Res 2005;123(1):8-16. DOI: 10.1016/j.jss.2004.06.015
10. Gramlich L, Kichian K, Pinilla J, Rodych NJ, Dhaliwal R, Heyland DK. Does enteral nutrition compared to parenteral nutrition result in better outcomes in critically ill adult patients? A systematic review of the literature. Nutrition 2004;20(10):843-8. DOI: 10.1016/j.nut.2004.06.003
11. Li J, Kudsk KA, Gocinski B, Dent D, Glezer J, Langkamp-Henken B, et al. Effects of parenteral and enteral nutrition on gut-associated lymphoid tissue. J Trauma - Inj Infect Crit Care 1995;39(1):44-51. DOI: 10.1097/00005373-199507000-00006
12. Jeejeebhoy KN. Total parenteral nutrition: Potion or poison? Am J Clin Nutr 2001;74(2):160-3. DOI: 10.1093/ajcn/74.2.160
13. Sano Y, Gomez FE, Kang W, Lan J, Maeshima Y, Hermsen JL, et al. Intestinal polymeric immunoglobulin receptor is affected by type and route of nutrition. JPEN J Parenter Enteral Nutr 2007;31(5):351-6. DOI: 10.1177/0148607107031005351
14. Lopatin U, Blutt SE, Conner ME, Kelsall BL. Lymphotoxin Alpha-Deficient Mice Clear Persistent Rotavirus Infection after Local Generation of Mucosal IgA. J Virol 2013;87(1):524-30. DOI: 10.1128/JVI.01801-12
15. Machado CSM, Rodrigues MA, Maffei HV. Assessment of gut intraepithelial lymphocytes during late gestation and the neonatal period. Neonatology 1994;66(6):324-9. DOI: 10.1159/000244125
16. Wu Y, Kudsk KA, DeWitt RC, Tolley EA, Li J. Route and type of nutrition influence IgA-mediating intestinal cytokines. Ann Surg 1999;229(5):662-7. DOI: 10.1097/00000658-199905000-00008
17. Fukatsu K, Kudsk KA. Nutrition and gut immunity. Surg Clin North Am 2011;91(4):755-70. DOI: 10.1016/j.suc.2011.04.007
18. Farokh R Demehri, Meredith Barret DHT. Changes to the Intestinal Microbiome With Parenteral Nutrition. Nutr Clin Pract 2015;30(6):798-806. DOI: 10.1177/0884533615609904
19. Buchman AL, Mestecky J, Moukarzel A, Anient ME. Intestinal Immune Function Is Unaffected By Parenteral Nutrition In Man. J Am Coll Nutr 1995;14(6):656-61. DOI: 10.1080/07315724.1995.10718556
20. Reyes-Torres CA, Flores-López A, Osuna-Padilla IA, Hernández-Cárdenas CM, Serralde-Zúñiga AE. Phase angle and overhydration are associated with post-extubating dysphagia in patients with COVID-19 discharged from the ICU. Nutr Clin Pract 2022;37(1):110-6. DOI: 10.1002/ncp.10781
21. Shannon IL, Prigmore JR, Chauncey HH. Modified Carlson-Crittenden Device for the Collection of Parotid Fluid. J Dent Res 1962;41(4):778-83. DOI: 10.1177/00220345620410040801
22. Gómez E, Ortiz V, Saint-Martin B, Boeck L, Díaz-Sánchez V, Bourges H. Hormonal Regulation of the Secretory IgA (sIgA) System: Estradiol- and Progesterone-induced Changes in sIgA in Parotid Saliva Along the Menstrual Cycle. Am J Reprod Immunol 1993;29:219-23. DOI: 10.1111/j.1600-0897.1993.tb00590.x
23. Peterson GL. A simplification of the protein assay method of Lowry et al. which is more generally applicable. Anal Biochem 1977;83(2):346-56. DOI: 10.1016/0003-2697(77)90043-4
24. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 2001;29(9):2004-7. DOI: 10.1093/nar/29.9.e45
25. Rodríguez-García WD, García-Castañeda L, Orea-Tejeda A, Mendoza-Núñez V, González-Islas DG, Santillán-Díaz C, et al. Handgrip strength: Reference values and its relationship with bioimpedance and anthropometric variables. Clinical Nutrition ESPEN 2017;19:54-8. DOI: 10.1016/j.clnesp.2017.01.010
26. Anastasilakis CD, Ioannidis O, Gkiomisi AI, Botsios D. Artificial nutrition and intestinal mucosal barrier functionality. Digestion 2013;88(3):193-208. DOI: 10.1159/000353603
27. Hermsen JL, Sano Y, Kudsk KA. Food fight! Parenteral nutrition, enteral stimulation and gut-derived mucosal immunity. Langenbecks Arch Surg 2009;394(1):17-30. DOI: 10.1007/s00423-008-0339-x
28. Janu P, Li J, Renegar KB, Kudsk KA. Recovery of gut-associated lymphoid tissue and upper respiratory tract immunity after parenteral nutrition. Ann Surg 1997;225(6):707-17. DOI: 10.1097/00000658-199706000-00008
29. Johnson CD, Kudsk KA, Fukatsu K, Renegar KB, Zarzaur BL. Route of Nutrition Influences Generation of Antibody-Forming Cells and Initial Defense to an Active Viral Infection in the Upper Respiratory Tract. Ann Surg 2003;4:565-73. DOI: 10.1097/01.SLA.0000059991.89316.B8
30. Crowe PD, VanArsdale TL, Walter BN, Ware CF, Hession C, Ehrenfels B, et al. A lymphotoxin-beta-specific receptor. Science 1994;264(5159):707-10. DOI: 10.1126/science.8171323
31. Sutherland DB, Fagarasan S. IgA synthesis: A form of functional immune adaptation extending beyond gut. Curr Opin Immunol 2012;24(3):261-8. DOI: 10.1016/j.coi.2012.03.005
32. Pietrzak B, Tomela K, Olejnik-Schmidt A, Mackiewicz A, Schmidt M. Secretory IgA in Intestinal Mucosal Secretions as an Adaptive Barrier against Microbial Cells. Int J Mol Sci 2020;21(23):1-15. DOI: 10.3390/ijms21239254
33. Macpherson AJ, Slack E. The functional interactions of commensal bacteria with intestinal secretory IgA. Curr Opin Gastroenterol 2007;23:673-8. DOI: 10.1097/MOG.0b013e3282f0d012
34. Hoces D, Arnoldini M, Diard M, Loverdo C, Slack E. Growing, evolving and sticking in a flowing environment: understanding IgA interactions with bacteria in the gut. Immunology 2020;159:52-62. DOI: 10.1111/imm.13156
35. Vasconcelos AR, Yshii LM, Viel TA, Buck HS, Mattson MP, Scavone C, et al. Intermittent fasting attenuates lipopolysaccharide-induced neuroinflammation and memory impairment. J Neuroinflammation 2014;11(1):1-14. DOI: 10.1186/1742-2094-11-85
36. Speaker KJ, Paton MM, Cox SS, Fleshner M. A Single Bout of Fasting (24 h) Reduces Basal Cytokine Expression and Minimally Impacts the Sterile Inflammatory Response in the White Adipose Tissue of Normal Weight F344 Rats. Mediators Inflamm 2016;2016:1698071. DOI: 10.1155/2016/1698071
37. Rius-Bonet J, Macip S, Massip-Salcedo M, Closa D. Effects of Fasting on THP1 Macrophage Metabolism and Inflammatory Profile. Int J Mol Sci 2024;25(16):9029. DOI: 10.3390/ijms25169029
38. Guerrero-Romero F, Simental-Mendía LE, Rodríguez-Morán M. Association of C-reactive protein Levels with Fasting and Postload Glucose Levels According to Glucose Tolerance Status. Arch Med Res 2014;45(1):70-5. DOI: 10.1016/j.arcmed.2013.11.004
39. Pickup JC. Inflammation and Activated Innate Immunity in the Pathogenesis of Type 2 Diabletes. Diabetes Care 2004;27(3):813-23. DOI: 10.2337/diacare.27.3.813
40. Tarfeen N, Ul Nisa K, Masoodi SR, Bhat H, Wani S, Ganai BA. Correlation of Diabetes Related Factors with Vitamin D and Immunological Parameters in T2DM Kashmiri Population. Indian J Clin Biochem 2024;39(4):586-92. DOI: 10.1007/s12291-023-01144-1