J. Elementol. 2010, 15(1): 141–147

INVESTIGATION ON ADSORPTION OF FATTY AND BILE ACIDS IN THE PRESENCE OF DIETARY SUPPLEMENTS CONTAINING CHROMIUM

Jan Meler, Bożena Grimling, Janusz Pluta

Department and Division of Drug Form Technology
Wrocław Medical University

Abstract

Dietary supplements contain not only macro- and microelements, but also elements which affect human metabolism. Many products available on the market contain chromium compounds together with chitosan used as a dietary supplement enhancing the digestion of lipids. The studies involved natural chitosan from krill available on the market, with the deacetylation degree of 85 to 95%, and dietary supplements containing chitosan (Vitana®, Hitec Nutrition®) as well as a product containing ionic chromium with niacin and several aminoacids – Chromdiet®). The study has determined the capability of binding fatty and bile acids by dietary supplements containing chitosan and chromium. The process of lipids and bile acids adsorption was investigated by means of a dynamic method in a biopharmaceutical model imitating *in vitro* conditions. The findings prove that extracts of fatty acids and bile acids undergo adsorption by various kinds of adjuvant substances found in dietary supplements, which confirms a significant effect of these polymers on the bioavailability of fatty and bile acids in a human organism. The addition of chromium to a supplement does not effect the capability of chitosan to bind fatty and bile acids. Mean adsorption of bile acids by 1 g of the polymer (chitosan, inulin, fibre) ranges from 0.9 g to 1.79 g depending on the pH (which decreases the bioavailability of lipids by 15-30%).

Key words: chitosans, chromium, bioavability, adsorption, model in vitro.

dr n. farm. Jan Meler, Division of Dispensing Pharmacy, Department of Applied Pharmacy, Szewska 38/39 Str., 50-139 Wrocław, Poland, email: meler@ktpl.am.wroc.pl

BADANIE SORPCJI KWASÓW TŁUSZCZOWYCH I CHOLOWYCH W OBECNOŚCI SUPLEMENTÓW DIETY ZAWIERAJĄCYCH CHROM

Abstrakt

Suplementy diety to nie tylko makro- i mikroelementy, ale też pierwiastki, które wykazują wpływ na przemiany metaboliczne organizmu. Obecnie na rynku jest sporo preparatów zawierających związki chromu wraz z chitozanem stosowanych jako dodatek żywieniowy wspomagający trawienie tłuszczowców. W pracy przebadano naturalny chitozan z kryla o stopniu deacetylacji od 85 do 95% występujący obecnie w sprzedaży rynkowej oraz preparaty zawierające chitozany, stosowane jako suplementy diety (Vitana®, Hitec Nutrition® oraz preparat zawierający chrom–Chromdiet®). Określono zdolności wiązania tłuszczowców i kwasów żółciowych przez suplementy diety zawierające chrom i chitozan. Zjawisko adsorpcji lipidów i kwasów żółciowych badano metodą dynamiczną w modelu biofarmaceutycznym imitującym warunki in vitro. Otrzymane wyniki dowodzą, że kwasy tłuszczowe i ekstrakty kwasów żółciowych ulegają adsorpcji przez różnego rodzaju substancje pomocnicze służące do wytworzenia suplementu diety, co potwierdza znaczący wpływ tych polimerów na biodostępność kwasów tłuszczowych i żółciowych w organizmie człowieka. Średnia wielkość adsorpcji kwasów żółciowych przez 1 g polimeru w zależności od pH środowiska mieściła się w granicach od 0,90 g do 1,79 g (daje to zmniejszenie biodostępności tłuszczowców o 15-30%).

Słowa kluczowe: chitozan, chrom, biodostępność, adsorpcja, model in vitro.

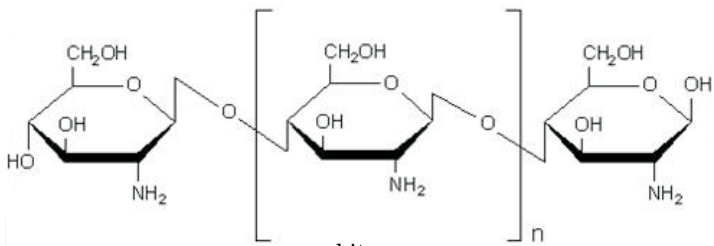
INTRODUCTION

In view of the rapid progress in pharmaceutical technology and a large variety of manufactured products, supplementation of certain microelements has gained an increasing significance. Dietary supplements not only include macro- and microelements, but also elements which affect human metabolism (Meler et al. 2002).

Many products available on the market contain chromium compounds used as a dietary supplement enhancing the digestion of lipids, and their use is based on studies indicating that chromium together with insulin increase the use of glucose (Sherman et al. 1968, Uusitupa et al. 1992, Amato et al. 2000, Trow et al. 2000, Mizerska et al. 2005). The process of digestion of lipids starts in the stomach under the effect of sublingual lipase and lipase secreted by the gastric mucosa. First of all, they decompose triacylglycerols with short fatty acids to monoacylglycerols. The main process in the breakdown of lipids takes place in the duodenum in the presence of pancreatic lipase, cholesterol esterase and phospholipase A_2. The digestion of lipids in the duodenum is facilitated by the products of lipid digestion in the stomach performed by phospholipids and bile acids, which emulsify triacylglycerols with long acid chains to form fine molecules, thus increasing the surface of the enzyme's contact with the substrate.

MATERIAL AND METHODS

The tests were performed with the use of natural chitosan from krill, available on the market, characterized by the deacetelylation degree of 85 to 95% and manufactured by Primex (85 and Chito-Clear-1015), France-Chitine (352 and 343) and Chitozan Huasu (Figure 1). Moreover, the study involved dietary supplements containing chitosans (Vitana®, Hitec Nutrition®) as well as a product containing ionic chromium with niacin and several aminoacids (Chromdiet®).



chitozan

Fig. 1 Molecular structure of chitosan as a polymer

To calculate the amount of fatty and bile acids adsorbed by various chitosans, a biopharmaceutical model of the alimentary tract was used (MELER et al. 2003). Due to the scant amount of the product, the determination method was developed which used 30 milligrams (mg) of a sample mixed with the excess of oil mass. The measurements were based on six trials, in which mean results were determined, as well as on a thorough statistical evaluation.

The investigations were performed in water bath with a shaker, under the conditions imitating those in the human alimentary tract. The amplitude of vibrations (300 rpm) as well as the temperature of the process (37ºC) were determined (MELER et al. 2003).

Next, 0.03 g of chitosan was added to 5 ml shaker vials, completed with 2 ml of 0.05 N HCl and shaken until dissolved. Afterwards, 0.05 N HCl was added in order to achieve pH 2, corresponding to the gastric pH when fasting. Next, the pH of the solution was raised to 6.4 by addition of 0.2 M Na_2CO_3. The mixture was shaken for 0.5 hours and completed with 100 mg of bile acids and 1 g of fatty acids in the form of 0.5 g of olive oil (Extra Vergine di Oliva Costa d'Oro, Spoleto, Italy) and 0.5 g of soya oil (Wielkopolskie Zakłady Przemysłu Tłuszczowego). The content of the vials was brought up to pH 7.0-7.6, which corresponds to the pH of intestinal juice in the small intestine and colon, and next incubated at 37ºC, shaking (300 rpm) for

2.5 hours. The mixture was cooled to room temperature; the vials were weighed together with their content and centrifuged (2,100·g) for 20 minutes. Next, the mixture was left for 0.5 h to stabilize, the oil layer was collected and discarded and 1.5 ml of the mixture was collected from above the sediment, transferred to clean tubes and completed with 2 ml of 1 N NaOH. The absorbance was measured in a spectrophotometer and the amount of adsorbed bile acids was calculated. The remaining solution was brought to pH 3 with 0.05 N HCl, 2 ml of ethyl ester was added, shaken for 5 minutes and left until separation of the layers. The ether layer was collected with a syringe fitted with a needle to previously weighed weighing bottle. The extraction was repeated twice. Combined ether extracts were evaporated by heating to the temperature higher than the ether boiling point. After evaporation of ether, the bottle was weighed and the amount of fat bound by the chitosan sample was calculated from the difference in weight between the empty bottle and the bottle containing lipids. Accessory substances were prepared according to the standards of *Polish Pharmacopoeia VI (Farmakopea polska VI 2002).*

RESULTS AND DISCUSSION

Table 1 presents the results of binding oil mixture containing olive oil and soya oil available on the market by various kinds of chitosan.

Table 1

Viscosity and capability of binding fatty acids by chitosans and dietary supplements in a biopharmaceutical model of the alimentary tract

Specimen	Intrinsic viscosity (η)/dm^3g^{-1}	Average mass (g) of fatty acids bound by 1 g of chitosan	Standard deviation S (±g)	Relativity coefficient Rc (%)
Primex 85	0.2852	2.91	$4.889 \cdot 10^{-4}$	1.68
Chitosan type-343	0.6402	1.21	$1.416 \cdot 10^{-4}$	1.17
Chito-Clear TM fg 95 Batch TM 1015	0.2213	7.90	$8.434 \cdot 10^{-4}$	1.07
Chitosan type-352	0.2117	1.63	$2.156 \cdot 10^{-4}$	1.32
Chitosan HUASU	0.7437	2.50	$3.260 \cdot 10^{-4}$	1.30
Chitosan Chromdiet	0.1872	4.57	$5.567 \cdot 10^{-4}$	1.22
Chitosan Nutrisearch	0.1576	2.52	$4.678 \cdot 10^{-4}$	1.86
Chitosan Witana	0.1774	6.22	$9.134 \cdot 10^{-4}$	1.47

Statistical errors and relativity coefficients determining the repeatability of the findings were calculated for all investigated samples. As shown in Table 1, the standard deviation ranged from $1.416 \cdot 10^{-4}$ g to $9.134 \cdot 10^{-4}$ g, and the relativity coefficient ranged from 1.07% to 1.86%. The investigations have demonstrated that the initial chitosans are capable of binding from 1.21 g (Chitozan type-343) to 7.9 g (Chitosan Chito-Clear) of fat.

This is probably associated with the structure of chitosan as a polymer, which exerts electrostatic effect on lipid molecules and is capable of entrapping micelles containing bile acids and fatty acids. Partly protonated chitosan binds negatively charged acid molecules and forms ionic bonds between $-NH_3^+$ groups and $-COO^-$ groups of acids (Parra-Barraza et al. 2005), which decreases the surface for subsequent enzymatic hydrolysis.

The applied method for binding lipids by chitosans in a biopharmaceutical model gives repeatable results, which was confirmed by the calculated relativity coefficients below 5% (probability higher than 95 %).

Table 2 presents the results of bile acids binding. The findings demonstrate that salts and extracts of bile acids are adsorbed by various accessory substances used to manufacture dietary supplements containing chitosan and chromium, which confirms a significant effect of polymers on the bioavailability of bile acids in a human organism. Mean bile acid adsorption by 1 g of the polymer ranged from 0.90 g to 1.79 g depending on pH (which reduces the bioavailability of lipids by about 15-30%)

The weight-reducing mechanism of the system can be explained by the effect of reduced amounts of bile acids, due to which dietary lipids undergo emulsification in the intestines (pH 7) rather than in the acid environment

Table 2

Viscosity and capability of binding bile acids by chitosans and dietary supplements in a biopharmaceutical model of the alimentary tract

Specimen	Intrinsic viscosity (η)/dm^3g^{-1}	Average mass (g) of fatty acids bound by 1 g of chitosan	Standard deviation S (±g)	Relativity coefficient Rc (%)
Primex 85	0.2852	1.30	0.0221	1.70
Chitosan type-343	0.6402	0.90	0.0046	0.51
Chito-Clear TM fg 95 Batch TM 1015	0.2213	1.79	0.0105	0.58
Chitosan type-352	0.2117	1.12	0.0136	1.21
Chitosan HUASU	0.7437	1.14	0.050	4.38
Chitosan Chromdiet	0.1872	1.31	0.0065	0.89
Chitosan Nutrisearch	0.1576	1.15	0.089	0.94
Chitosan Witana	0.1774	1.32	0.088	0.87

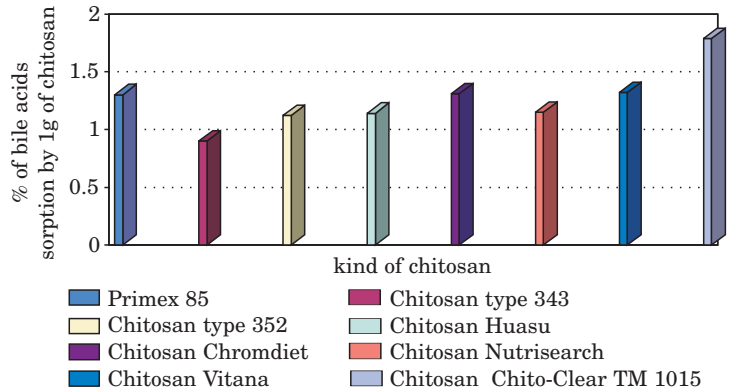


Fig. 2. Sorption of bile acids on dietary supplements including chitosans

of the stomach, but their surface is charged negatively as a consequence of dissociation of acids, which initiates electrostatic interactions between chitosan and droplets of lipids resulting in their adsorption and decreased amounts (KANAUCHI et al. 1995, PASZKO et al. 2000). Addition of chromium to a product containing chitosan, inulin and fibre (Chromdiet®) provides more effective support for weight reduction in comparison with monocomponent products. Chromium regulates the blood levels of glucose, lipids and proteins, but its effect on the binding of fatty acids and bile acids by adjuvant substances is very weak.

Summing up: Chromium present in a dietary supplement in the amount of 10 μg per dose has a minimal effect on sorption of lipids and bile acid compounds, with the highest adsorption of these substances occurring at pH above 7.

CONCLUSIONS

1. The developed method for investigations on interaction between small amounts of substances in the biopharmaceutical model of the alimentary tract enables evaluation of qualitative and quantitative changes occurring in the aqueous solution of chitosans and other accessory substances.

2. The investigation on absorption of fatty acids and bile acids confirms the previously hypothesized depressed amounts of these compounds in the intestinal passage (by 15-30% in the daily intake).

3. The binding of lipids and bile acids is accelerated at pH above 7.

4. Addition of 10 μg of chromium per dose has very little effect on the bioavailability of bile acids and lipids.

REFERENCES

AMATO P., MORALES A.J., YEN S.S. 2000. *Effects of chromium picolinate supplementation on insulin sensitivity, serum lipids, and body composition in healthy, nonobese, older men and women.* J. Gerontol. Biol. Sci. Med. Sci., 55: 260-263.

Farmakopea polska 2002. PTF, Warszawa.

KANAUCHI O., DEUCHI K., IMASATO Y., SHIZUKUISHI M., KOBAYASHI E. 1995. *Mechanism for the inhibition of FAT digestion by chitosan and for the synergistic effect of ascorbate*. Biosci. Biotechnol. Biochem., 59: 786-790.

MELER J, PLUTA J. KROTKIEWSKI M. 2002. *The influence of various kinds of chitosan on fat binding ability*. 4th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology. Florence, 617-618.

MELER J., PLUTA J., ULANSKI P. KROTKIEWSKI M. 2003. *Fat – the binding capacity of ninths the modified and modified chitosans*. In: *Progress the chemistry and application of chitin and its derivatives* (ed. H. STRUSZCZYK). Pol. Chitin Soc., Lodz, 9: 129-136.

MELER J., PLUTA J., ULAŃSKI P., KROTKIEWSKI M. 2003. *Vozdejstvie raznych form chitozana na sposobnost' svjazyvanija irov. Modern perspectives in chitin and chitosan studies*: Proc. of the VIIth Int. Conf. St. Petersburg – Repino, Moscow VNIRO Publishing, 258-260.

MIZERSKA A., PASTERNAK K. 2005. *Chrom a cukrzyca [Chromium and diabetes].* J. Elementol., 10 (3)/1:645-651 (in Polish).

PARRA-BARRAZA H., BURBOA M.G., SANCHEZ-VAZQUEZ M., JUAREZ J., GOYCOOLEA F.M., VALDEZ M.A. 2005. *Chitosan cholesterol and chitosan-stearic acid interaction at the air-water interface.* Biomacromolecules, 6: 2416-2426.

PASZKO T., GĄSZCZYK R., MUSZYŃSKI P. 2000. *Badanie efektywności desorpcji Cu^{2+}, Co^{2+} i Cr^{3+} kationami NH_4^+ w glebach mineralnych o kwaśnym odczynie [Stduies on effectiveness of desorption of Cu^{2+}, Co^{2+} and Cr^{3+} with NH_4^+ cations in mineral soils of acid pH].* Biul. Magnezol., 5 (1): 62-67 (in Polish).

SHERMAN L., GLENNON J.A., BRECH W.J., KLOMBERG G.H., GORDON E.S. 1968. *Failure of trivalent chromium to improve hyperglycemia in diabetes mellitus*. Metabolism, 17: 439-442.

TROW L.G., LEWIS J., GREENWOOD R.H., SAMPSON M.J., SELF K.A., CREWS H.M., FAIRWEATHER-TAIT S.J. 2000. *Lack of effect of dietary chromium supplementation on glucose tolerance, plasma insulin and lipoprotein levels in patients with type 2 diabetes*. Int. J. Vitam Nutr. Res., 70: 14-18.

UUSITUPA M.I., MYKKANEN L., SIITONEN O., LAAKSO M., SARLUND H., KOLEHMAINEN P., RASSANEN T., KUMPULAINEN J., PYORÄLÄ K. 1992. *Chromium supplementation in impaired glucose tolerance of elderly: effects on blood glucose, plasma insulin, C-peptide and lipid levels.* Br. J. Nutr., 68: 209-216.