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Efectos de la fibra dietética y grasas saturadas / insaturadas en ratones ácidos biliares fecales a través de TLC

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Resumen

Las dietas ricas en fibras ligan los ácidos biliares e incrementan la cantidad excretada en las heces. El objetivo de este trabajo fue evaluar si la variación de la cantidad de fibra y grasa saturada/insaturada en la dieta modifican el patrón de ácidos biliares (BA) fecales de ratones en laboratorio por TLC. Se usaron 36 ratones (*Mus musculus*). En el primer experimento se dividieron los ratones en dos grupos. El Grupo A alimentado con dieta control y el Grupo B con dietas con diferentes relaciones grasas saturadas/insaturadas. En el segundo experimento los grupos fueron alimentados con diferentes concentraciones de salvado. La fibra dietaria y la relación grasa saturada/insaturada produjeron variaciones en la concentración relativa de los BA fecales, pero no en el patrón de los mismos. La relación grasa saturada/insaturada con igual cantidad de fibra aumentó el colesterol y los ácidos glicocólico, cólico, deoxicólico y quenodeoxicólico; no varió la concentración del litocólico y el dehidrocólico. Al duplicar la cantidad de fibra dietaria se observó mayor variabilidad en la concentración de los ácidos biliares fecales. La TLC demostró ser una excelente técnica para determinar la influencia de las grasas saturadas/insaturadas y fibra dietaria en el patrón de ácidos biliares fecales en ratones.

Palabras clave: Dieta, grasa, heces, fibra, *Mus musculus*, TLC.

Effects of dietary fiber and saturated/unsaturated fat on mice fecal bile acids through TLC

Abstract

Diets rich in fiber bind bile acids and increase the amount excreted in feces. The objective of this work was to assess if the variation in dietary amount of fiber and saturated/unsaturated fat alter the fecal bile acid (BA) patterns of laboratory mice, using Thin-Layer Chromatography (TLC). There were used 36 mice (*Mus musculus*) for this study. In the first experiment, mice were divided into two groups (A and B). Group A was fed with control diet and Group B with diets containing different saturated/unsaturated fat proportion. In the second experiment

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groups were fed with different concentrations of bran. Dietary fiber and saturated/unsaturated fat proportion produced variations in the relative concentration of fecal BA, but not in the whole pattern. That proportion affected the concentration of deoxycholic and chenodeoxycholic acids; as the relation decreased, the amount of deoxycholic acid increased. Greater amount of fiber decreased the concentration of these acids. TLC proved to be an excellent technique to determine the influence of diet on the pattern of fecal bile acids in mice.

Key words: Diet, Fat, Feces, Fiber, *Mus musculus*, TLC.

Abbreviations: Bile acids, BA; CA, cholic acid; CDCA, chenodeoxycholic acid; CHOL, cholesterol; DCA, deoxycholic acid; DHCA, dehydrocholic acid; GCA, glycocholic acid; GCDCA, glycochenodeoxycholic acid; LCA, lithocholic acid; TCA, taurocholic acid; TLC, thin-layer chromatography

Introduction

Bile acids (BA) are synthesized in the liver from cholesterol (CHOL) and, after conjugation with glycine or taurine, they are secreted into the duodenum. Then, they are actively reabsorbed by the terminal ileum and undergo an enterohepatic circulation. BA carry out many important physiological functions in living organisms including CHOL homeostasis, lipid absorption and generation of a biliary flux which helps in the absorption, excretion and recirculation of drugs, vitamins and toxins (Fuchs, 2003).

Several studies have demonstrated that some food fractions, like dietary fiber, bind BA, thus increasing the amount of these compounds excreted in the feces and reducing the BA pool (Ginnett et al., 2003). By binding BA, food fractions prevent their reabsorption and stimulate plasma and liver CHOL conversion to additional BA (Kahlon et al., 2007). The hepatic conversion of CHOL into BA is the prevailing pathway for the elimination of CHOL from the body (Elliott, 1985).

Several authors reported that fecal BA excretion is affected not only by the quality and quantity of dietary lipids, but also by the quantity and type of dietary fiber (Reddy et al., 1980; Story and Furumoto, 1990). Moreover, the fecal BA composition is affected differently by different types of dietary fiber and experimental conditions (Shimizu et al., 1996). Quinn and Jackman (1994) demonstrated, in their experiments with coyotes, that the presence of fiber in their diet changes the concentration of fecal BA, but not the profile of these compounds.

Although fecal BA constitute a complex mixture and are not easy to identify, chromatographic techniques allow their identification. Particularly, TLC is a non destructive chemical technique and it offers practical advantages such as simplicity, economical equipment needed, ease of operation, short analysis time and high efficiency in simultaneously analyzing of large number of samples. Moreover, from an ecological point of view, TLC is used for the identification of species trough the determination of their fecal bile acid patterns (Cazón and Sühring, 1999; Khorozyan et al., 2007; Cazón et al., 2009).

The objective of this study was to evaluate if quantitative variations of dietary fiber and saturated/unsaturated fat, modify the fecal bile acid pattern in laboratory mice, through TLC.

Material and methods

Experimental animals

36 Swiss albine mice (18 males and 18 females) *Mus musculus* (Linnaeus), aged 2 months (50 g a 70 g) were used in this study. They belonged to the same rearing and they came from Animal Facilities of Facultad de Ciencias de la Salud, UNSa.

Diets and feeding procedures

This work was divided into two experiments. In the first one (I), mice of similar weights were divided in two groups (A and B). Group A, composed

of 6 individuals, was fed a control diet (commercial balanced food "Cargill"). Group B, composed of 30 individuals, was fed prepared diets D1, D2 and D3 (10 animals per diet). Diet D1 contained saturated fat (from animal origin) (10 g/100 g of diet) but not sunflower oil (unsaturated fat), while diets D2 and D3 did not contain fat but contained oil in two different concentrations (10 and 15 g/100 g of diet) (Table 1).

Table 1

Composition of the diets D1, D2 and D3 for the first experiment, containing fat or oil (g/100 g of diet).

Composition	D1	D2	D3
Animal fat	10	---	---
Sunflower oil	---	10	15
Dextrin	76.20	76.20	76.20

In the second experiment (II), 6 groups of 5 individuals were fed two diets, a and b, (D1a, D1b, D2a, D2b, D3a and D3b) with the same overall composition of the first experiment but with different concentrations of fiber (bran) and a polysaccharide, dextrin. Diet a, contained a low concentration of fiber and a high concentration of dextrin and diet b, a high concentration of fiber and a low concentration of dextrin (Table 2).

Table 2

Composition of the diets a and b for the second experiment containing different concentration of fiber and dextrin (g/100 g of diet).

Composition	1a	1b	2a	2b	3a	3b
Animal fat	10	10	----	----	----	----
Sunflower oil	-----	----	10	10	15	15
Fiber (bran)	12.91	25.27	12.91	25.27	12.91	25.27
Dextrin	63.29	50.93	63.29	50.93	63.29	50.93

Feces collection

After 20 days of preliminary mice rearing and administration of the diets, feces were collected on daily basis during a 45-day period. Feces were dried in oven at 30°C and processed as follows.

Feces analysis

Feces were grounded and sieved. One gram of each sample was extracted with 20 mL of benzene: methanol (1:1 v/v) for 3 hours. Extracts were filtered and concentrated to a final volume of 5 ml. Each sample extract and standards for the most common mammal BA were spotted on silicagel $60F_{254}$ plates, with aluminum base, film thickness of 0.2 mm (Merck). Lithocholic (LCA), taurocholic (TCA), glycocholic (GCA), cholic (CA), chenodeoxycholic (CDCA), deoxycholic (DCA), dehydrocholic (DHCA), glycochenodeoxycholic (GCDCA) acids and CHOL. BA standard stock solutions were prepared in methanol at a concentration of 0.1%. Plates were eluted in a glass developing tank with a solution of toluene: acetic acid: water (5:5:1.5v/v), and BA were visualized by spraying the plates with a revealing solution of anisaldehyde: glacial acetic acid: sulphuric

acid (0.5:50:1v/v). Plates were heated in oven at 150°C for 15 minutes and scanned for the analysis. Technical procedures standardized by Cazón and Sühring, were used (1999).

Statistical analysis

The BA pattern was determined by the comparison of sample R_f values (relation between distance traveled by the band and distance traveled by the eluent in the chromatographic plate), color and intensity (concentration) of the bands, with those of standard solutions. R_f mean (X) and standard deviation (SD) of each compound were calculated.

Results

We found a total of 13 compounds in this work; 6 belonged to standard BA: GCA, LCA, CA, DCA, DHCA and CDCA; 6 were unidentified compounds (R_f= 0.98±0.02, R_f= 0.76± 0.03, R_f= 0.60± 0.3, R_f= 0.49± 0.02, R_f= 0.26± 0.04, R_f= 0.22± 0.03) which do not coincide with any of the standards used, and CHOL. Each standard BA showed a characteristic color and R_f value (X ± SD) which helped in the right identification of sample bands, being the most characteristic ones, orange for DHCA, green for DCA and violet for CHOL (figure 1, table 3).

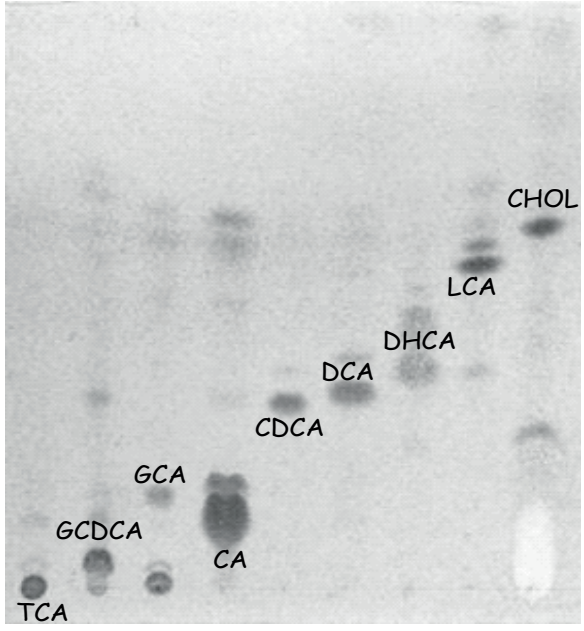

Figure 1. Standard bile acids. From left to right: TCA: taurocholic acid, GCDCA: glicochenodeoxycholic acid, GCA: glicocholic acid, CA: cholic acid, CDCA: chenodeoxycholic acid, DCA: deoxycholic acid, DHCA: dehydrocholic acid, LCA: lithocholic acid, CHOL: cholesterol.

Fecal BA pattern of mice fed a control diet was the same for both experiments, with a high concentration of all standard BA used and CHOL. TCA, TDCA and TCDCA acids did not eluted with the solvent system used, remaining at the base line as a single blue spot (figure 1, table 4).

In experiment I, feces from diets D1, D2 and D3 did not differed in the concentration of CHOL, GCA and CA; all of them were present in high concentrations, the same as in the control diet. Feces from animals fed diet D1 (animal fat) had a higher concentration of LCA than those from

Table 3
R_f (expressed as mean ± SD) of standard bile acids.

Values	CHOL	LCA	DHCA	DCA	CDCA	GCA	CA
R_f	0.55	0.53	0.45	0.34	0.34	0.13	0.10
SD	0.03	0.03	0.06	0.06	0.04	0.04	0.05
N	10	10	10	10	10	10	10
Color	Violet	Blue	Orange	Green-brownish	Grey	Grey	Grey

Table 4
Relative concentration of the compounds found in feces from the diets in experiment I and II.

	CHOL	GCA	LCA	CA	DCA	DHCA	CDCA
Control	***	***	***	***	***	***	***
D1	***	***	***	***	**	**	**
D2	***	***	*	***	***	*	***
D3	***	***	*	***	**	***	*
D1-a	**	*	***	*	**	**	*
D1-b	**	*	***	*	*	**	*
D2-a	***	***	***	***	***	**	***
D2-b	**	**	**	**	**	**	*
D3-a	**	**	***	*	**	**	*
D3-b	***	**	***	**	*	**	**

*** high concentration, ** medium concentration, * low concentration, 0 absence of the compound.

D2 and D3 which had the same low concentration of this compound (oil); it also had intermediate concentrations of DCA, DHCA and CDCA; D2 feces showed a low concentration of LCA and DHCA, and high quantities of DCA and CDCA compared to D1 and D3 diets; D3 feces had a low concentration of LCA and CDCA. Both D1 and D3 had an intermediate concentration of DCA while D2 a higher concentration of it (table 4).

Feces from all sub-diets of experiment II showed a high concentration of LCA except diet D2-b and DHCA appeared at an intermediate concentration in all the extracts from the sub-diets, compared to the control diet which had a higher concentration of this compound; DCA was more concentrated in diets *a* than in *b*, but less than in the control one except for diet D2-a which showed a high quantity of it. Mice fed with diet D1-b which contained more fiber, less

dextrin and fat but not oil, produced feces with a low concentration of GCA and CA; while feces from diets D2-b and D3-b had a higher concentration of those compounds (figure 2, table 4). Moreover, it was observed a variation in fecal bile acid concentration as a function of time assay.

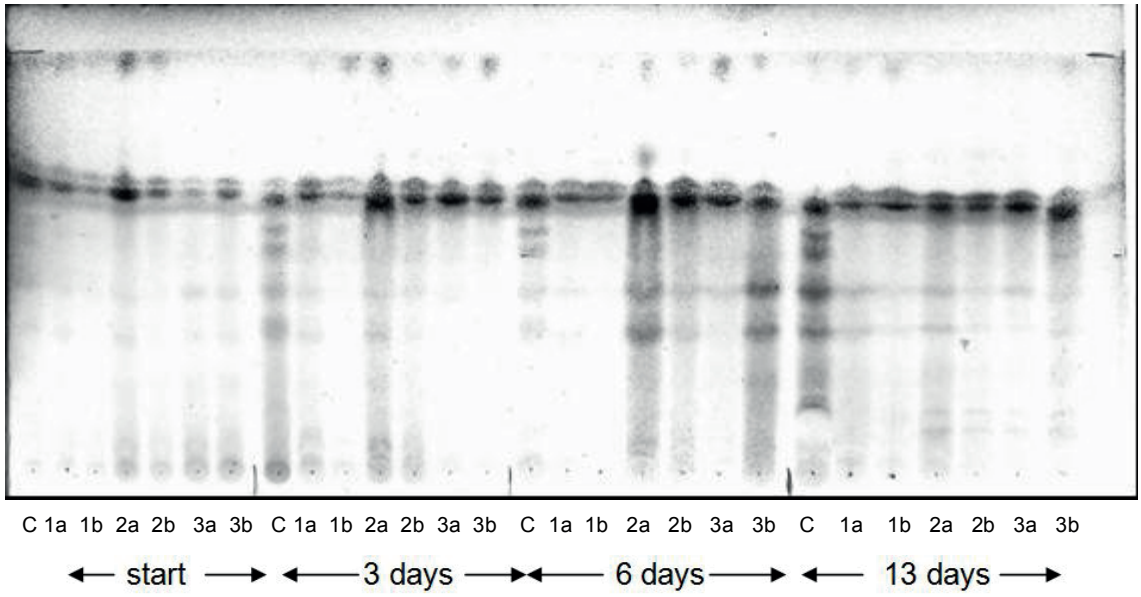

Figure 2. Chromatographic plate showing the pattern of the compounds found in extracts from mice feces, for the different diets, as a function of time assay. C: control diet.

When the effect of fiber among sub-diets is compared, we could observe that feces from D1-b had a lower concentration of DCA than those from D1-a, while for the rest of the compounds the concentrations remain the same; D2-b feces showed a lower concentration of CHOL and GCA, LCA, CA, DCA and CDCA compared to D2-a; D3-b feces had more CHOL, CA and CDCA and less DCA compared to D3-a (figure 2, table 4). D2a showed a higher concentration of all bile acids and cholesterol (oil and less fiber).

Discussion

The hepatic conversion of CHOL into BA is the prevailing way for the elimination of CHOL from the mammalian body. A major part of BA secreted into the intestine is reabsorbed, and only a small percentage is excreted in the feces (Elliott, 1985). Moreover, some food fractions such as fiber, bind BA, increasing the excretion of these compounds and thus, reducing the BA pool (Story and Furumoto, 1990).

The effects of dietary fiber and saturated/unsaturated fat on the concentration and profile of mice fecal BA were evaluated in this work. We found that these dietary components produced variations in the relative concentration of fecal BA, but they did not affect the BA profile for the studied species. This is consistent with other investigations (Quinn and Jackman, 1994).

In this study, although the addition of bran fiber to the diets produced some effects on fecal BA concentration, the component which produced the greatest variability in the results was fat; in the presence of different quantities of oil (10 and 15 g/100 g diet), some compounds increased and other decreased their fecal concentrations for the same amount of bran fiber.

Binding of BA by dietary fiber is a very complex process, in which not all bound BA are excreted. A part of the dietary fiber components are fermented by intestinal microflora; during this process bound BA are released and converted into secondary BA (Hofmann, 1994). Dietary fiber not only alter the distribution of CHOL on lipoproteins, which could affect the amount and type of BA synthesized and excreted into the gut, but also affects the absorption and enterohepatic circulation of BA (Reddy et al., 1980).

Several studies have reported the effects of different types of fiber on BA metabolism. Dietary fibers do not yield the same physiological effects and they act differently on BA metabolism (Sung et al., 2006). Trautwein et al. (1998) suggested that the most frequently mechanism responsible for the CHOL-lowering effect of some type of fiber, was the interference with intestinal CHOL and BA absorption, leading to an increase in BA excretion, which is directly associated with an increase in CHOL turnover from the body (Schneeman, 1998).

Fecal BA excretion and BA concentration were shown to be affected by different kinds of soluble fibers in laboratory animals like hamsters and rats (Sung et al., 2006). Drzikova et al. (2005) showed that the *in vitro* interactions with barley extrudates were higher with dihidroxy-BA than with trihidroxy-BA. Our results has show that an increase in dietary fiber produced a decrease in the concentration of most fecal BA, except for DHCA which stayed at a constant concentration for all the sub-diets. These results are consistent with other studies which have showed that dietary bran fiber produced a significant decrease in fecal BA concentration (Reddy et al., 1977, Alberts et al. 1996). Moreover, we report that the effect of fiber was most pronounced when it was fed together with a low concentration of sunflower oil.

On the other hand, the type of fat influences not only bile CHOL concentration, but also BA fecal composition (Jonnalagadda et al., 1995). Dietary saturated/unsaturated fat alone had almost no effect on fecal BA concentration in our study, except for DCA and CDCA which showed a little decrease in their concentrations. When oil was added to the diets, DCA and CDCA fecal concentrations were lower in the group fed 15 g than in the one fed 10 g oil. In other investigations they found similar results; Gallaher et al. (1992) reported that they did not find a great increase in fecal BA concentration when the dietary oil content was increased; Höstmark et al. (1989), reported that daily fecal BA excretion was smaller in animals fed 20% than those fed 1% sunflower oil.

Among most mammals, CA, CDCA and DCA are the most common bile acids. CA and CDCA are primary bile acids, while DCA is a secondary one. In relation to the rate of primary and secondary BA affected by the type of diet, we found that with the aggregation of bran fiber, independently of the presence of fat or oil in the diets, the concentration of DCA decreased compared to control diet except for diet D2a (low fiber, low oil). Moreover, the rate LCA/DCA was increased when animals were fed sunflower oil in any of the two given concentrations (table 2). When fiber was added to the diets, this rate increased. This coincides with some authors who found that the aggregate of fat to any diet that contained fiber preparations, promoted the metabolic system of DCA, increasing the fecal LCA/DCA rate (Han et al., 2009; Kumar et al., 2011).

Conclusions

We established that the fecal BA profile in mice was not affected by the type of dietary components; instead, BA relative concentrations were altered by the presence of fiber and/or saturated and unsaturated fat. The effect of dietary fiber was stronger when mice were fed fiber together with sunflower oil (D2-a). On the other hand, TLC has proved to be a practical and useful technique for this kind of studies, allowing the analysis of a big number of samples simultaneously, within short times and with relative low costs.

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