

## Study of the Absorption of *Myo*-Inositol Hexakisphosphate (InsP<sub>6</sub>) through the Skin

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Recently, some properties of *myo*-inositol hexakisphosphate (InsP<sub>6</sub>) are related to its dermatological use as discolouring agent, on preventing calcinosis cutis or due to its important role on premature aging. Some studies also seem to demonstrate a capacity of InsP<sub>6</sub> to inhibit skin cancer. In this paper, a first study of the absorption of InsP<sub>6</sub> through the skin is developed. Due to the correlation between InsP<sub>6</sub> absorption and its urinary excretion, these last values were used to evaluate this process. It was found that using a moisturizing cream as vehicle, the InsP<sub>6</sub> sodium salt was absorbed at significantly higher amounts than the InsP<sub>6</sub> calcium–magnesium salt. Maximum InsP<sub>6</sub> urinary concentrations were observed approximately at 14 d of 2% InsP<sub>6</sub> topical cream application, and gave  $66.35 \pm 5.49$  mg/l urinary InsP<sub>6</sub> when the sodium salt was used and  $16.02 \pm 2.61$  mg/l urinary InsP<sub>6</sub> when the calcium–magnesium salt was applied. When the InsP<sub>6</sub> topical cream administration ceased, the InsP<sub>6</sub> urinary excretion fell dramatically approximately during a period of 10 d. From these results, it can be deduced that by topical administration InsP<sub>6</sub> can achieve important concentrations in tissues and biological fluids, this demonstrating that it is possible to propose the topic use as a new InsP<sub>6</sub> administration route.

**Key words** InsP<sub>6</sub> skin absorption; urinary excretion; cream application; dermatological use

*Myo*-inositol hexakisphosphate (InsP<sub>6</sub>) is an abundant component of plant seeds. In whole grain cereals it ranges from 1.5 to 6.4% and it is mostly associated with calcium and magnesium ions, the so-called phytin.<sup>1,2</sup> Recently it was found that InsP<sub>6</sub> is also present in all mammalian organs, tissues and fluids but at significantly low amounts. Moreover, it was demonstrated that the levels found in biological fluids (blood, urine, interstitial liquid) and mammalian tissues clearly depended on the dietary intake.<sup>3–5</sup>

Diverse studies performed by Mellanby demonstrated that a high InsP<sub>6</sub> content in some diets, as sodium salt, reduced calcium absorption and induced rickets.<sup>6</sup> Ever since up to now, several studies have attributed “anti-nutritional” properties to phytate.<sup>7–11</sup> Nevertheless, other studies<sup>12–16</sup> have shown that those findings are not quite so clear and simple as mentioned. Moreover, from the 1980s to the present, important physiological functions of InsP<sub>6</sub> have been suggested as its properties as an antioxidant<sup>17,18</sup> and its role in colon cancer prevention.<sup>19,20</sup> The InsP<sub>6</sub> present in urine and biological fluids also exhibited an important role in preventing pathological calcifications as renal calculi<sup>21–23</sup> or calcinosis cutis,<sup>24</sup> due to its powerful capacity to act as crystallization inhibitor of calcium salts.

Finally, the most recent observations about the properties of InsP<sub>6</sub> are related to its dermatological use. The majority of those applications are referred to their important action on premature aging or as discolouring agent of the skin.<sup>25</sup> Also some studies seem to demonstrate a capacity of InsP<sub>6</sub> to inhibit skin cancer.<sup>26,27</sup> In the present paper a first study of the absorption of InsP<sub>6</sub> through the skin is developed.

### MATERIALS AND METHODS

**Animals, Diets and Experimental Design** Twenty-four female Wistar rats of approximately 225 g from Harlan Iberica s.l. (Barcelona, Spain) were acclimated in the course of 7 d to our animal house. Animals were kept in Plexiglas cages (two animals per cage) at a temperature of  $21 \pm 1$  °C

and relative humidity of  $60 \pm 5\%$  with a 12-h on–off light cycle. After this period, animals were randomly assigned into four groups of six rats respectively. Rats were fed with 4068.02 Reference Diet (HopeFarms BV, Woerden, The Netherlands), a synthetic purified diet (Table 1) in which InsP<sub>6</sub> is undetectable.

After a period of 16 d consuming such diet, during which the urinary InsP<sub>6</sub> became undetectable, rats were topically treated once a day with 4 g of a standard cream with a supplement of 0.4, 1.2 and 2.0% of InsP<sub>6</sub> as sodium salt or 2.0% of InsP<sub>6</sub> as calcium magnesium salt (phytin). The surface of treatment was about 50 cm<sup>2</sup>. The application area was located on the back skin of the animal and was previously shaved using an electric shaver (each 4 d). During cream treatment animals were located individually to avoid licking cream. pH of all creams was adjusted to 4–4.5 (see Table 2). Samples of 24-h urine were collected at days 0, 7 and 14 to evaluate InsP<sub>6</sub> excretion. After 14 d of treatment, rats treated with the standard cream containing 0.4 and 1.2% of InsP<sub>6</sub> as sodium

Table 1. Composition of 4068.02 Reference Diet, a Synthetic Purified Diet in Which InsP<sub>6</sub> Is Undetectable

Ingredient	% in diet
Acid casein	20
Corn starch	10
Cellulose	5
Soybean oil	5
Glucose	52.8
CaPO <sub>4</sub> ·2H <sub>2</sub> O	1.3
CaCO <sub>3</sub>	1
KH <sub>2</sub> PO <sub>4</sub>	0.40
KCl	0.70
NaCl	0.30
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.40
MgO	0.20
DL-Methionine	0.20
Choline chloride	0.40
Standard vitamin mix	1
Standard mineral mix	1

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Table 2. Composition of Moisturizing Creams Used

Component	Percentage composition			
	0.4% InsP <sub>6</sub> (sodium salt) enriched cream	1.2% InsP <sub>6</sub> (sodium salt) enriched cream	2.0% InsP <sub>6</sub> (sodium salt) enriched cream	2% InsP <sub>6</sub> (as phytin) enriched cream
InsP <sub>6</sub> salt	0.6	1.7	2.9	2.6
Almond oil	4	4	4	4
Thomil ISF	3.8	3.8	3.8	3.8
Stearic acid	1	1	1	1
Lactic acid	1.6	1.6	1.6	1.6
Vitamin F 09929	2.5	2.5	2.5	2.5
Monestriol GAE	4	4	4	4
Propyl Paraben	0.1	0.1	0.1	0.1
Pumol 1618	4	4	4	4
Controx VP	0.03	0.03	0.03	0.03
Water	72.3	71.2	70	70.3
Triethanolamine	0.1	0.1	0.1	0.1
Laurylate S-90	0.3	0.3	0.3	0.3
Glycerine 3699 USP	4.87	4.87	4.87	4.87
Methyl Paraben	0.2	0.2	0.2	0.2
Abiol	0.3	0.3	0.3	0.3
Essence	0.3	0.3	0.3	0.3

salt were sacrificed, whereas the treatment was maintained for rats treated with the standard cream containing 2.0% of InsP<sub>6</sub> as sodium salt or calcium/magnesium salt, until InsP<sub>6</sub> urinary excretions became constant (34 d). Then, the cream application ceased but collection of 24-h urine samples continued until InsP<sub>6</sub> urinary levels decreased and became constant. When finishing the experiment, animals were sacrificed. The procedures used in this experiment were carried out according to the Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes and official permission to perform this animal experiment was obtained from the ethical committee of our University.

**InsP<sub>6</sub> Determination** The determination of InsP<sub>6</sub> levels in urine samples was performed using an analytical methodology based on the determination of total phosphorus by atomic emission spectrometry ICP.<sup>28)</sup> This methodology allows a measurement of total InsP<sub>6</sub> with a detection limit of 60 µg/l.

**Procedure:** 5 ml of urine (acidified with HCl 1:1 until pH=3–4) was transferred to a column containing 0.2 g of anion exchange resin (the inner diameter was 4 mm). The first eluate was discarded, then the column was washed with 50 ml of HCl 50 mM. The second eluate was discarded. Then, the column was washed with 3 ml of HNO<sub>3</sub> 2 M. The determination of InsP<sub>6</sub> was carried out through direct phosphorus analysis of this last eluate by ICP-AES using the corresponding calibration curve.

The ICP-AES conditions used were the following: outer argon flow 15 l/min, auxiliary argon flow 1 l/min, inner argon flow 1 l/min, nebulizer uptake rate 1 ml/min and wavelength 213.618 nm.

**Statistics** Values in the figures are expressed as mean (S.E.). The Student *t*-test was used to assess differences of means. Conventional Windows software was used for statistical computations. A value of *p* < 0.05 was considered to assess statistical significance.

## RESULTS

The obtained results about the topically treated rats with a cream containing different InsP<sub>6</sub> amounts and salts are shown in Figs. 1–3. As can be observed the InsP<sub>6</sub> sodium salt was absorbed at significantly higher amounts than the InsP<sub>6</sub> calcium–magnesium salt. Thus, after 7 d of topical cream application containing a 2% InsP<sub>6</sub>, the sodium salt corresponded to 23.35 ± 2.46 mg/l InsP<sub>6</sub> urinary excretion whereas the calcium–magnesium salt corresponded to a 11.75 ± 3.96 mg/l InsP<sub>6</sub> urinary excretion. For this period of time, the InsP<sub>6</sub> excreted urinary amount of the sodium salt clearly depended on the InsP<sub>6</sub> cream concentration. Maximum InsP<sub>6</sub> urinary concentrations were observed approximately at 14 d of 2% InsP<sub>6</sub> topical cream application and gave 66.35 ± 5.49 mg/l urinary InsP<sub>6</sub> when the sodium salt was used and 16.02 ± 2.61 mg/l urinary InsP<sub>6</sub> when the calcium–magnesium salt was applied. When the InsP<sub>6</sub> topical cream administration ceased, the InsP<sub>6</sub> urinary excretion fell dramatically approximately during a period of 10 d.

## DISCUSSION

In spite of some dermatological applications of InsP<sub>6</sub> being established at present,<sup>25)</sup> and several topical InsP<sub>6</sub> based creams can be found in the market, no studies on the InsP<sub>6</sub> absorption through the skin can be found in the literature. In a recent paper, it was demonstrated that InsP<sub>6</sub> topically administered, notably and significantly reduced the development of subepithelial dystrophic calcifications in soft tissues,<sup>29)</sup> this demonstrating that InsP<sub>6</sub> was unquestionably absorbed through the skin. The results of the present paper clearly demonstrated that InsP<sub>6</sub> was absorbed through the skin layers, crossing the epidermis and dermis, entering the blood stream and increasing the urinary excretion. It is interesting to compare the absorption through the skin with the absorption through the gastrointestinal tract. Thus, in both cases there seems to be an optimum dose, above which no

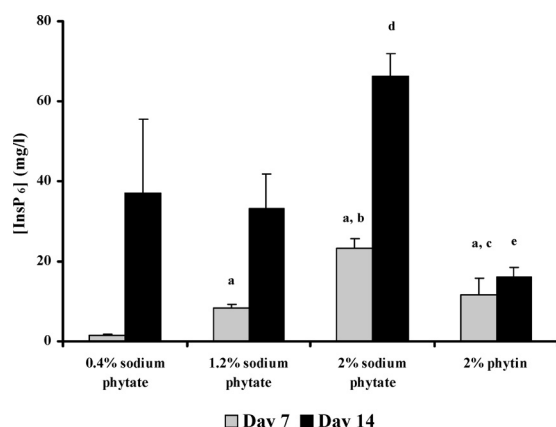


Fig. 1. Influence of InsP<sub>6</sub> Concentration in the Topically Applied Cream and InsP<sub>6</sub> the Salt Used on Urinary InsP<sub>6</sub> Concentration

<sup>a</sup>  $p < 0.05$  vs. 0.4% InsP<sub>6</sub> (day 7), <sup>b</sup>  $p < 0.05$  vs. 1.2% InsP<sub>6</sub> (day 7), <sup>c</sup>  $p < 0.05$  vs. 2.0% InsP<sub>6</sub> (day 7), <sup>d</sup>  $p < 0.05$  vs. 1.2% InsP<sub>6</sub> (day 14), <sup>e</sup>  $p < 0.05$  vs. 2.0% InsP<sub>6</sub> (day 14).

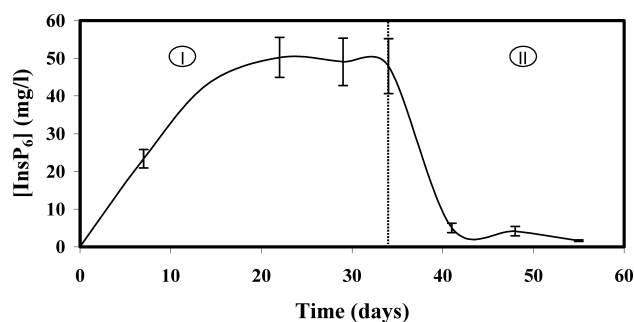


Fig. 2. InsP<sub>6</sub> Urinary Concentration in Rats

Section I: topically treated rats with a cream containing 2.0% of InsP<sub>6</sub> as sodium salt. Section II: the treated rats had stopped the topical cream application.

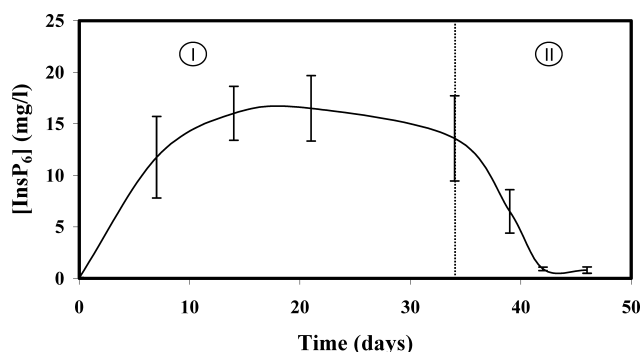


Fig. 3. InsP<sub>6</sub> Urinary Concentration in Rats

Section I: topically treated rats with a cream containing 2.0% of InsP<sub>6</sub> as phytin (calcium/magnesium salt). Section II: the treated rats had stopped the topical cream application.

further absorption occurs. Nevertheless, in the case of the gastrointestinal tract this amount was independent of the type of InsP<sub>6</sub> salt consumed<sup>4</sup>) but in the case of the skin, the InsP<sub>6</sub> sodium salt was absorbed much more favorably than the InsP<sub>6</sub> calcium-magnesium salt, thus the InsP<sub>6</sub> urinary levels were triple when the sodium salt was used. On the other hand, it is important to emphasize that the attained InsP<sub>6</sub> urinary levels found when InsP<sub>6</sub> was administered orally were almost always inferior to those observed when InsP<sub>6</sub> was top-

ically administered. Thus, maximum InsP<sub>6</sub> urinary levels found when InsP<sub>6</sub> was administered orally through the diet or using specific complements, in no case allowed to attain values superior to 6–7 mg/l InsP<sub>6</sub>,<sup>3</sup>) however through topical application urinary values of around 60 mg/l were achieved. This different behaviour of gastrointestinal and skin absorption could be in part explained considering that formation of insoluble no absorbable salts with divalent and trivalent cations and proteins in the gastrointestinal tract is more feasible due to the presence of food or food derivatives. In fact, from the results presented here it is observed that the InsP<sub>6</sub> sodium salt was clearly more favorably absorbed through the skin when compared with the calcium-magnesium salt.

From the presented results it can be deduced that by topical administration InsP<sub>6</sub> can achieve important concentrations in tissues and biological fluids, this demonstrating that it is possible to propose the topic use as a new InsP<sub>6</sub> administration route.

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