Absorption and excretion of orally administered inositol hexaphosphate (IP$_6$ or phytate) in humans

Felix Grases$^{a,**}$, Bartolomé M. Simonet$^a$, Ivana Vucenik$^b$, Rafel M. Prieto$^a$, Antonia Costa-Bauzá$^a$, Joan G. March$^a$ and Abulkalam M. Shamsuddin$^c$

$^a$Laboratory of Renal Lithiasis Research, Faculty of Science, University of Illes Balears, 07071 Palma de Mallorca, Spain

$^b$Department of Medical and Research Technology, University of Maryland School of Medicine, Baltimore, MD 21201, USA

$^c$Department of Pathology, University of Maryland School of Medicine, Baltimore, MD 21201, USA

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Abstract. A study of the pharmacokinetic profile (oral absorption and renal excretion) of inositol hexaphosphate or phytate (IP$_6$) is presented. Seven healthy volunteers were following a IP$_6$ poor diet (IP$_6$PD) in a first period, and on IP$_6$ normal diet (IP$_6$ND) in a second one. When following the IP$_6$PD they become deficient in IP$_6$, the basal levels found in plasma (0.07 ± 0.01 mg/L) being clearly lower than those found when IP$_6$ND was consumed (0.26 ± 0.03 mg/L). During the restriction period the maximum concentration in plasma were obtained 4 h after the ingestion of a single dose of IP$_6$, observing almost the same renal excretion profiles for the three different commercial sources and doses. After the IP$_6$ restriction period, volunteers were on IP$_6$ND, reaching normal plasma and urinary IP$_6$ values in 16 days. Thus, the normal plasma and urinary concentrations, can be obtained either by consumption of a IP$_6$ND taking a long time or in a short period by IP$_6$ supplements.

1. Introduction

Inositol hexaphosphate (InsP$_6$ or IP$_6$ or phytate) has been an important dietary component of humans from ancient ages; high amounts of this substance are present in legumes, non-refined cereal derivatives, corn, whole grain cereals and all types of nuts. Nevertheless, for a long time IP$_6$ was considered as an antinutrient due to its capacity to form insoluble complexes with trace elements, such as zinc, iron and copper in vitro, and, as a consequence, perhaps to a decrease in the bioavailability of these elements in vivo. However, recent studies demonstrate that this “antinutrient” effect of IP$_6$ is only manifested when large quantities of IP$_6$ are consumed in combination with oligoelements-poor diet [2,10,14,23]; the daily consumption of 1–2 g IP$_6$ with balanced diets did not affect the mineral status in humans [2,23].

**Corresponding author: Prof. Dr. F. Grases, Laboratory of Renal Lithiasis Research, Faculty of Sciences, University of Illes Balears, Ctra. Valldemossa Km 7.5, 07071 Palma de Mallorca, Spain. Tel.: +31 971 17 32 57; Fax: +31 971 17 34 26; E-mail: dqufgfl@ps.uib.es.
Studies of serum and bone mineral levels in experimental animals following IP₆ administration did not show any significant change in the Ca, Mg, Fe and Zn levels [21,22]. Yet, in instances of pathological conditions, it prevents renal calcification [1,4]. It is interesting to note that the IP₆ content of healthy and recommended diets, the so-called Mediterranean diet, oscillates between 0.7–1.4 g/day [12]. On the other hand, it is noteworthy that dietary habits in developed countries tend to eliminate IP₆ from meals due to reduced consumption of whole grain foods, legumes and nuts.

Recently, important beneficial effects of IP₆ on human health have been pointed out. IP₆ is an important antioxidant [8], it protects against cancer [16,17] and prevents pathological calcifications, such as renal calculi [1,4,6]. IP₆ also has hypocholesterolemic effects [18,19] and participates in a number of important processes within the cell [3,9,15]. It has been found that the urinary and plasma levels of IP₆ are related to its oral intake through the diet [5,7].

Both to understand the mechanism(s) of the action of IP₆ and to properly design clinical trials for its use, it is crucial that the absorption, metabolism, tissue distribution and excretion of IP₆ be known. In rats, radiolabelled IP₆ was found to be quickly absorbed from the stomach and distributed throughout the body [13]. Likewise, IP₆ is also absorbed and metabolized by cancer cells [20]. However, to date no pharmacokinetic data in humans are available, one of the major reasons being the insensitivity and non-specificity of the existing methods for IP₆ determination. Using a novel and sensitive method for determination of non-radiolabelled IP₆ in biological fluids by GC/MS [11], we present here the first attempt to study the pharmacokinetic profile of IP₆ in humans to clarify its absorption and excretion. Commercially available, non-radioactive products of IP₆ were administered to healthy subjects and the kinetics of its plasma and urinary levels were evaluated. In our previous experiments with non-radiolabelled IP₆ in rats [7], we have found that orally administered IP₆ is excreted in the urine; the urinary level declines when IP₆ is withheld and increases with increasing amount of ingested IP₆, reaching a peak excretion level which is not further increased by ingestion of additional quantities of IP₆. Therefore, to answer all these questions, in particular because of existing uncertainties about the IP₆ synthesis in vivo, and to see if the same pattern as in rats will be observed in humans, we gave the human volunteers different commercial preparations and doses of IP₆.

2. Materials and methods

2.1. Study design and sample collection

Seven healthy volunteers (3 males and 4 females) were selected to study the plasma and urinary levels of IP₆, as a function of time and ingested doses. The experiment had two phases. In the first one, all participants were submitted to a IP₆-poor diet for 15 days (all types of cereals or cereal derivatives, rice, corn, legumes, all types of nuts or other vegetable seeds, coffee and potatoes, were totally excluded). On the seventh day, early in the morning (7:00) they voided the urine accumulated in the bladder overnight and drank only 150 mL of tap water. Two hours later, the first urinary sample was collected and the subjects immediately ingested 400 mg of calcium/magnesium phytate (phytin, dietary supplement, Lit-Stop®, Authex Laboratorios, Marratxí, Mallorca, Spain). Four new urinary samples were collected after 2, 4, 6, and 8 hours, and after each collection of urine only 100 mL of water were ingested (last sample was collected at 17:00). Then, the subjects continued with a IP₆-poor diet.

After three days had elapsed (as blanking period), the volunteers repeated the same procedure as described for day 7, but this time they ingested 3200 mg of IP₆ as phytin and 880 mg of inositol (dietary supplement Cell-Forte™, Enzymatic Therapy, Green Bay, WI 54311, USA) to test the effect of different
doses of IP$_6$ and that of inositol on urinary levels of IP$_6$. Then, the subjects were submitted again to the IP$_6$-poor diet for three days and on the fourth day, they repeated again the procedure described above, but this time they ingested 1400 mg of IP$_6$ as dodecasodium salt from corn (Sigma). In this last phase of the experiment, blood samples were also obtained simultaneously with each urinary sample collection (7 mL of blood). This was the end of the first phase of the experiment.

After this, the second period of the study was undertaken to see how long it will take for individuals to attain their normal urinary and plasma levels of IP$_6$. Therefore, the same 7 volunteers consuming the IP$_6$-normal diet were monitored for 16 days; every other day they collected new samples of urine to determine the IP$_6$ levels (urine accumulated over 2 h after the overnight fasting period). On the last day of this second phase, blood samples were obtained immediately after the urine collection, to evaluate the concentration of IP$_6$ in plasma. All urine and plasma samples were frozen at $-20^\circ$C until analyzed.

A global scheme of the study design appears in Fig. 1.

The study protocol was approved by the local Ethics Committe, and informed consent was obtained from all subjects before study.

2.2. Sample preparation and IP$_6$ determination

2.2.1. Treatment of plasma

Whole blood in Na$_2$EDTA was centrifuged at 3500 g for 15 min. Adequate aliquots of supernatant were treated with Na$_2$EDTA (0.1 mL of 0.1 mol/L Na$_2$EDTA per 1.0 mL of plasma for 3 h) and trichloroacetic acid (TCA) (0.2 mL of 1 mol/L TCA per each mL of plasma for 1 h). Samples were centrifuged, supernatant neutralized and used for IP$_6$ determination, as described below.
Fig. 2. The kinetic of IP₆ after ingestion of a single dose of 1400 mg sodium phytate following a IP₆-poor diet. Values are mean ± SE of 7 subjects. Student t-test was used to determine statistic significance between means. "p < 0.05 vs 0 hours-time. (1.00 mg/L = 1.52 µmol/L).

2.2.2. Treatment of urine

Urine was acidified with HCl to pH 3–4. The samples were purified using chromatographic columns with activated carbon (0.5 g per 1.0 mL of urine), and analyzed as described below.

2.2.3. IP₆ determination

IP₆ content in plasma and urine samples was determined by the same method previously described [11]. Briefly, IP₆ was retained in a chromatographic column containing a cationic resin. Then, IP₆ was hydrolyzed to myo-inositol with the enzyme phytase (Sigma). Scyllo-inositol (Sigma) was added as internal standard. The solution was frozen to −20°C and lyophilized. The residue was then derivatized by pyridine and chlorotrimethylsilane to silylated inositol, using a hexamethyldisilazane as catalyst. After reaction, the excess of reagents and organic solvents were blown off in a stream of nitrogen. The solid residue was extracted with hexane and injected in a gas chromatograph with mass spectrometry detection (Shimadzu QP-5000) using fused silica capillary column SPB-20 (Supelco) and He as a carrier. The calibration graph was obtained from peak height corresponding to the silylated compounds of scyllo- and myo-inositol.

2.3. Statistical analysis

Values in the tables and figures are expressed as mean ± SE. One-way ANOVA was used to calculate significance of differences between groups. The Student’s t-test was used to assess the differences of means. The SPSS for the Windows program was used for statistical computations. A probability of p < 0.05 was used for assessing statistical significance.
3. Results

Volunteers on a IP<sub>6</sub>-poor diet become deficient in IP<sub>6</sub>; the basal levels of IP<sub>6</sub> found in plasma on an IP<sub>6</sub>-poor diet (0.07 ± 0.01 mg/L = 0.106 ± 0.015 µmol/L) were 3 to 5-fold lower than those found in plasma when a IP<sub>6</sub>-normal diet was consumed (0.26 ± 0.03 mg/L = 0.393 ± 0.045 µmol/L). Plasma IP<sub>6</sub> levels after ingestion of a single dose of 1 g IP<sub>6</sub> are shown in Fig. 2. The maximum concentrations of IP<sub>6</sub> in plasma after the ingestion of the single bolus of IP<sub>6</sub> dose were obtained after 4 h (Fig. 2). Even this maximum peak concentrations (0.12 ± 0.02 mg/L = 0.181 ± 0.030 µmol/L) are still 2–3 fold lower than the plasma concentrations of IP<sub>6</sub> in volunteers on a normal IP<sub>6</sub> diet (Table 1), indicating that humans become deficient in IP<sub>6</sub> if they consume IP<sub>6</sub>-poor diet for as little as 2 weeks.

The increment of urinary excretions of IP<sub>6</sub> referred to the first urinary sample (when consuming a IP<sub>6</sub>-poor diet) after ingestion of three different single IP<sub>6</sub> doses (400 mg as calcium/magnesium salt, 3200 mg as calcium/magnesium salt and 1400 mg as sodium salt) as a function of time are shown in Fig. 3. The excreted amounts of IP<sub>6</sub> increased continuously during 8 h, the time at which all samples were collected. However, the collected maximum amount (0.08 mg) was still lower (33%), when compared to the levels obtained after a IP<sub>6</sub>-normal diet was consumed (0.12 mg) (see Fig. 4). It is interesting to observe that all three different sources of IP<sub>6</sub> and different doses of IP<sub>6</sub> gave almost the same excretion profiles (the observed differences were not statistically significant). This indicates that despite the differences, each of them contributed to the absorption of IP<sub>6</sub> in approximately the same amounts.

During the second period of this study, the urinary amounts of IP<sub>6</sub> were evaluated regularly during 16 days, while the subjects were consuming a IP<sub>6</sub>-normal diet (Fig. 4). The urinary levels of IP<sub>6</sub> increased continuously until the normal values were reached. IP<sub>6</sub> concentrations found in urine and in plasma on the last day of the second period after volunteers were consuming a IP<sub>6</sub>-normal diet, appear in Table 1. Levels of IP<sub>6</sub> in both urine and plasma reached the normal values, and as shown in Fig. 5, there was a clear correlation between the urinary and plasma concentrations in such a manner that increased urinary excretions corresponded to higher plasma values.

4. Discussion

This study provides an insight in the absorption and excretion of orally administered IP<sub>6</sub>. The obtained results indicate that the levels of IP<sub>6</sub> in the humans depend on its dietary intake. After 2 weeks of
Fig. 3. Increment of urinary excretion of IP₆ referred to the first urinary samples, after ingestion of a single dose of 400 mg calcium magnesium phytate (Lit-Stop), 3200 mg calcium magnesium phytate (Cell-Fort) and 1400 mg sodium phytate (Na-phytate), following a IP₆-poor diet. Values are mean ± SE of 7 subjects. Student t-test was used to determine statistic significance between means. **p < 0.05** vs 2 hours-time following the same treatment. *p < 0.05* vs 4 hours-time following the same treatment. (1.00 mg = 1.52 mol).

Fig. 4. Increasing levels of IP₆ in 2 h-urine of healthy volunteers on IP₆-normal diet following a two weeks period of IP₆-poor diet. (1.00 mg = 1.52 mol).

consuming a diet totally deprived of IP₆ (cereal derivatives and other vegetable seeds, legumes and nuts), the plasma and urinary levels of IP₆ decreased to around 75–80% of the normal values found in humans; this was in accordance to previously reported data from our laboratory [5,7]. It was also observed that after fasting IP₆ was quickly absorbed, reaching the maximum concentrations in plasma...
4 h after. However, these maximum levels still indicate an overall low percentage of absorption. The profile of the IP$_6$ urinary excretion (Fig. 3) was the same for all three different doses and formulations studied (no statistically significant differences were detected). It seems that there is an optimum ingested amount above which no increase in the excreted amount can be achieved, corresponding to the maximum absorption. Regarding the levels of IP$_6$ in the urine following removal from diet, not only its disappearance from the organism was not abrupt, but also its recuperation to normal levels was slow, needing more than 10 days (Fig. 4). A similar relationship between the dietary ingestions of IP$_6$ and its levels in the biological fluids and tissues have been previously observed in rats [7].

Furthermore, this study indicates not only that there is a maximum excretion level that can not be exceeded by ingesting higher amounts of IP$_6$ given with different formulations, but that the excreted amounts were not affected by the type of IP$_6$ salt used, either calcium/magnesium or sodium. This can be partially explained by the absorption of IP$_6$ taking place mostly through the stomach walls and mainly as its neutral protonated derivative, that might be independent of the initially present salt.

It seems that the ingested amounts that gave maximum urinary excretion can be obtained by consumption of a diet with normal IP$_6$ content. In this regard, it is important to point out now fashionable tendency worldwide to eliminate IP$_6$ from their diet (consuming more high-protein diet). Thus, to maintain the appropriate IP$_6$ levels in the organism, the consumption of IP$_6$ supplements is necessary when the diet is poor in phytate. It must be remembered that the urinary levels are directly related to the plasma values (Fig. 5) and therefore IP$_6$ in urine can constitute a marker of a deficiency in the organism. The fact that a variety of health benefits are attributed to the presence of IP$_6$ in the diet, makes it important to monitor the urinary levels of IP$_6$ to detect its deficit in the organism.

Therefore, considering the positive roles of IP$_6$, its natural occurrence in our diet, and ubiquitous presence at low levels in animal fluids and tissues, it becomes clear that this molecule might be considered as a vitamin. Furthermore, while it does not adversely affect the normal levels of minerals in the body, it most certainly prevents abnormal and pathological mineralization. At this moment we are not sure whether it is only IP$_6$ or also its metabolites, inositol or IP1-5, that are biologically active in providing...
protection for human health. Therefore, based on the knowledge obtained from the present study, additional pharmacokinetic profiles of IP₆ must be conducted, followed by the efficacy trials, that should provide more information about this molecule.

In conclusion, to maintain the adequate levels of IP₆ in the organism, it must be supplied through a healthy IP₆-rich diet. Alternatively, optimum IP₆’s status could also be achieved with supplements.

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