Abstract. The presence of myo-inositol hexakisphosphate (InsP₆) in biological fluids (blood, urine, saliva, interstitial fluid) of mammalians has been clearly demonstrated. The existence of intracellular InsP₆ in mammalian cells has also been established. Further, significant extracellular and intracellular functions of this molecule have been found. The relationship between InsP₆ ingestion and the InsP₆ distribution in various tissues of mammalians is discussed. It was found that the majority of the extracellular InsP₆ found in organs, tissues and biological fluids of mammalians has a dietary origin and is not a consequence of endogenous synthesis, whereas the intracellular InsP₆ probably originates in the cell. Little absorption of dietary InsP₆ takes place during intestinal transit and depletion of extracellular InsP₆ occurs at high rates when InsP₆-poor diets are consumed. From these results, it can be deduced that health benefits linked to extracellular InsP₆ must be related to dietary InsP₆.

The presence of myo-inositol hexakisphosphate (InsP₆) in tissues and biological fluids (blood, urine, saliva, interstitial fluid) of mammalians is well known (1-3). The existence of intracellular InsP₆ in mammalian cells has also been established (4-6), despite the problem of determining its exact subcellular localization. The role of InsP₆ in mammalian cells is a complex matter that, in spite of recent research (7), leaves a lot of key questions unanswered, such as the exact pathways of InsP₆ synthesis and metabolism and their mechanisms of control, the detailed biological function inside the cell and the relationship with the other inositol polyphosphates. Nevertheless, it seems that the presence of InsP₆ inside the cell is important.

Parallel to the above-mentioned studies, significant extracellular functions have been found as a crystallization inhibitor of pathological calcification (8-11) and as antioxidant (12). From these properties, important health benefits can be derived as, for example, prevention of renal calcium stones development (13-14) or some anticancer activity (15-18).

InsP₆ is an abundant component of plant seeds, where it acts as a phosphate store (19). InsP₆ is associated with calcium and magnesium ions (the so-called phytin) but is not homogenously distributed. For example, the endosperm of wheat and rice kernels are almost devoid of InsP₆ as it is concentrated in the germ and aleuronic layers of cells of the kernel and in the bran or hull (19, 20). For this reason, large amounts of InsP₆ are found in unrefined cereals and in other edible vegetable seeds such as legumes and nuts, constituting an important part of the mammalian diet. Nevertheless, due to cereal refinement processes, elimination of the rice shell, low fibre consumption etc., human diets are gradually becoming poorer and poorer in InsP₆. The amount of InsP₆ ingested from a healthy and balanced diet, as for example the so-called Mediterranean diet, varies between 1-2 g of InsP₆ per day. The relationship between InsP₆ ingestion and InsP₆ distribution in various tissues of mammalians is discussed below.

Animal studies

It was found that InsP₆ is present in the blood, urine, interstitial and intracellular fluids of mammals, and the levels found in urine, blood and rat tissues clearly depended on the dietary intake (0, 61, 182 and 425 mg InsP₆/L in a liquid diet) (1, 2, 21). Tissue and fluid (plasma and urine) levels of InsP₆ are limited by its rate of absorption from the gastrointestinal tract. There appears to be a maximum absorbable amount, above which no further increase in absorption occurs (see Figure 1). This amount was found to be 20.9 mg/kg/day for Wistar rats (21). When InsP₆ was eliminated from the diet, its levels in fluids (blood and urine) and organs notably decreased, as can be seen in...
Table I, while in some tissues such as bone it became undetectable (1, 2). These studies also demonstrated that, in spite of InsP6 intake (1%) directly affecting the physiological levels in biological fluids and tissues of rats, it did not affect the total InsP3 levels (Figure 2) (22). Nevertheless, InsP6 treatment of malignant cells did not induce a significant difference in the intracellular concentrations of InsP6 (Figure 3), but there was a significant increase in InsP3 concentration (22).

All these data seem to demonstrate that the majority of the extracellular InsP6 found in organs, tissues and biological fluids, such as blood and urine, has a dietary origin and is not a consequence of endogenous synthesis. The intracellular InsP6 probably originates in the cell.

Finally, a study of InsP6 topical administration through a moisturizing cream demonstrated significant InsP6 absorption through the skin, that allowed it to reach high urinary levels which in no case were attained by oral administration (around 10-fold the maximum amounts attained by oral administration) (23). This demonstrated that it is possible to propose topical application as a new InsP6 administration route.

Table I. Determined amounts of InsP6 in brain, kidney, bone (femur), urine and plasma of rats fed with AIN 76A diet and AIN 76A + 1% InsP6 diet for 12 weeks (from reference 2).

<table>
<thead>
<tr>
<th>Group</th>
<th>AIN 76A diet (without phytate)</th>
<th>AIN 76A diet plus phytate (1%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>InsP6 Brain (µg/g)</td>
<td>2.55±0.37a</td>
<td>24.89±1.14</td>
</tr>
<tr>
<td>Kidney (µg/g)</td>
<td>0.048±0.005a</td>
<td>1.71±0.06</td>
</tr>
<tr>
<td>Bone (µg/g)</td>
<td>nd</td>
<td>1.79±0.06</td>
</tr>
<tr>
<td>Urine (mg/L)</td>
<td>0.06±0.04a</td>
<td>3.20±0.06</td>
</tr>
<tr>
<td>Plasma (mg/L)</td>
<td>0.021±0.004a</td>
<td>0.22±0.01</td>
</tr>
</tbody>
</table>

nd: non detectable. a*<0.05 vs. AIN 76A + 1% InsP6 group

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Human studies

The consumption of a InsP6-free diet, in humans, as occurred for rats, significantly decreased the urinary excretion of InsP6 (about 50% after 36 hours) (24). In a study in which volunteers were submitted to an InsP6-poor diet for 15 days, the InsP6 levels found in the plasma were 3- to 5-fold lower (0.106±0.015 µmol·L⁻¹) than those found when an InsP6-normal diet was consumed (0.393±0.045 µmol·L⁻¹) (25). The maximum plasma InsP6 levels after ingestion of a single dose of 1400 mg InsP6 (as dodecasodium salt) was obtained after 4 hours (see Figure 4). The InsP6 urinary excretion was similar for the three different doses and formulations administered to volunteers on the InsP6-poor diet (25), as demonstrated in Figure 5. As in rats, there appears to be a maximum absorbable amount above which no further absorption occurs. This amount was found to be 20.9 mg/kg/day for animals (21), thus, extrapolating for a 70 kg human, the minimum intake to obtain maximum absorption was calculated to be 1463 mg InsP6 per day, and was independent of the type of InsP6 salt consumed. Volunteers, submitted for 15 days to an InsP6-poor diet, on return to the InsP6-normal diet, showed continuously increasing InsP6 urinary levels until normal values were again reached after 16 days (Figure 6).
was a clear correlation between urinary and plasma concentrations, in that increased urinary excretions corresponded to higher plasma values, as can be seen in Figure 7 (25). A study of different stomach conditions before InsP₆ administration (empty stomach, empty with an alkalinizing agent and full stomach), demonstrated that no differences in the excretion profiles between the three different conditions were produced at 8 hours, suggesting that the overall InsP₆ absorption took place independently of the stomach state, full or fasted stomach, thus indicating that some InsP₆ absorption also takes place during intestinal transit (not published). Thus, if InsP₆ supplements are consumed to maintain optimum InsP₆ levels, these supplements can be taken either during or between meals with the same efficacy.

Figure 3. Influence of InsP₆ treatment on total intracellular InsP₃ (A) and InsP₆ (B) contents. MDA-MB 231 and K562 cells were seeded onto tissue culture plates and treated with InsP₆ for 1 hour (from reference 22). The InsP₆ treatment did not induce a significant increase in the intracellular concentration of InsP₆ in either cell line. (*p<0.05).

Figure 4. Plasma levels of InsP₆ after ingestion of a single dose of 1400 mg sodium phytate following an InsP₆-poor diet. Values are mean ± SE of 7 subjects. Student's t-test was used to determine statistical significance between means. *p<0.05 vs 0 hours-time (from reference 25).

Figure 5. Increment of urinary excretion of InsP₆ 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-Forte) and 1400 mg sodium phytate (Na-phytate), following an InsP₆-poor diet. Values are mean ± SE of 7 subjects. Student's t-test was used to determine statistical 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 (from reference 25).
Conclusion

The majority of the extracellular InsP$_6$ found in organs, tissues and biological fluids of mammalians has a dietary origin and is not a consequence of endogenous synthesis, whereas the intracellular InsP$_6$ probably originates in the cell. The absorption of dietary InsP$_6$ takes place during intestinal transit at low percentages and depletion of extracellular InsP$_6$ occurs at high rates when InsP$_6$-poor diets are consumed. From these results, it can be deduced that health benefits linked to extracellular InsP$_6$ must be related to dietary InsP$_6$.

Acknowledgements

This work was supported by the Conselleria d’Innovació i Energia del Govern de les Illes Balears (Grant PROIB-2002GC1-04) and by the project BQU 2003-01659 of the Ministerio de Ciencia y Tecnologia from Spain.

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Received December 28, 2004
Revised January 24, 2004
Accepted April 13, 2005