CHOLECALCIFEROL (VITAMIN D3) AND RETINYL PALMITATE (VITAMIN A) DISPLAY DIFFERENT SOLUBILIZATION CAPACITIES IN MIXED MICELLE SOLUTIONS:

• **EFFECT OF INTERACTIONS WITH MIXED MICELLE COMPONENTS AND OF CHOLECALCIFEROL SELF-ASSOCIATING PROPERTIES**
Vitamins A and D in food of animal origin

- Vitamin A → retinyl palmitate (RP)
  - Molecular Formula: C_{36}H_{50}O_{2}
  - Average mass: 524.86 Da
  - Log P: 14.83 ± 0.45

- Vitamin D → cholecalciferol (D3)
  - Molecular Formula: C_{27}H_{44}O
  - Average mass: 384.638 Da
  - Log P: 9.72 ± 0.27

- Digestion process in the upper gastrointestinal tract (1):
  - Food matrix (dietary fat)
  - Emulsification
  - Micellization
  - Enzymatic hydrolysis
  - Uptake by enterocyte
  - Blood stream
  - Bioaccessibility
  - Absorption
  - Passive diffusion vs. membrane proteins (2)
  - Bioavailability

- Micellization is a key step for bioavailability

## Interactions with mixed micelles components

<table>
<thead>
<tr>
<th>components</th>
<th>ORIGIN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>diet</td>
</tr>
<tr>
<td>Bile salts</td>
<td>✓</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>✓</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>✓</td>
</tr>
<tr>
<td>Lysophospholipids</td>
<td>✓</td>
</tr>
<tr>
<td>Fatty acids</td>
<td></td>
</tr>
<tr>
<td>Monoglycerides</td>
<td></td>
</tr>
</tbody>
</table>

- **Mixed micelle lipid composition affects the solubility of fat-soluble vitamins:**
  - Substitution of lysophospholipids by phospholipids = lymphatic absorption of vitamin E $\uparrow$ in rat (Koo and Noh, 2001 J Nutr)
  - Change in FA profile (chain length and saturation degree) = absorption of D3 $\uparrow$ in perfused rat (Hollander, 1978 Gut)
  - Addition of oleic and linoleic acids = absorption of D3 $\uparrow$ (Hollander, 1978 Gut)

### Objectives:

(i) compare the relative solubility of D3 and RP in the aqueous phase rich in mixed micelles
(ii) study the interactions between these vitamins and the mixed micelle components
Methods
Incorporation efficiency of RP and D3 in mixed micelles

LDP:NaTC 1.1:5

<table>
<thead>
<tr>
<th>Concentration (mM)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Monolein</td>
<td>0.30</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>0.50</td>
</tr>
<tr>
<td>Phosphatidylcholine (PC)</td>
<td>0.04</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.10</td>
</tr>
<tr>
<td>LysoPC</td>
<td>0.16</td>
</tr>
<tr>
<td>Taurocholate (NaTC)</td>
<td>5.00</td>
</tr>
<tr>
<td>Cholecalciferol</td>
<td>0.001 - 1</td>
</tr>
<tr>
<td>Retinyl palmitate</td>
<td>0.001 - 5</td>
</tr>
</tbody>
</table>

Lipid Digestion Products (LDP) 1.1 mM

Desmarchelier et al. (2013) MNFR

1. Lipids dissolved in chloroform/MeOH (2/1) + D3 or RP/EtOH
2. Evaporation under N₂
3. Sonication: 5 min at 25W
4. pH 6 Tris buffer + sodium taurocholate 5 mM
5. Cellulose esters filter (porosity: 0.22 μm)

D3 or RP micellar solution

Vitamin analysis by HPLC

interactions between RP and D3, and the mixed micelle components

- **Surface tension measurement:**
  - Surface tension measurement
  - Critical Micellar Concentration (CMC)
  - Tensiometer K10 (Krüss)

- **Surface pressure measurement:**
  - Surface pressure measurement
  - Compression isotherms
  - Surface compressional modulus
  - Langmuir trough

- **Cryo-TEM (Transmission Electron Microscopy) analysis:**
  - Cryo-TEM analysis
  - Electron micrographs
  - JEOL 2200FS + CCD camera
Results
Solubilization of RP and D3 in mixed micelle solutions

- D3 solubilization was linear \((R^2=0.98, \text{ regression slope}=0.71)\) and higher than that of RP, which reached a plateau with a maximum concentration around 125 µM.
Interfacial behaviour of micelle components

- LDP showed better surface parameters (CMC and surface tension) than NaTC
- LDP aimed to improve NaTC surface tension and CMC by inserting well in NaTC domain
- D3 and RP solubilization depend on their capacity to penetrate the LDP and NaTC domains
Interfacial behaviour of the 2 vitamins and lipids

Based on $K_{\text{max}}$, the lipid monolayers can be classified into **poorly organized** ($K < 100 \text{ mN/m}$, for lyso-PC, monoolein, and oleic acid), **liquid condensed** ($100 < K < 250 \text{ mN/m}$, for POPC and the LDP mixture) and **highly rigid monolayers** ($K > 250 \text{ mN/m}$, for cholesterol).

D3 and LDP exhibit similar interfacial behavior, whereas RP show very different behavior compared to the micelle lipids.

<table>
<thead>
<tr>
<th>Monolayer</th>
<th>$A_c$ (Å²)</th>
<th>$\pi_c$ (mN/m)</th>
<th>$K_{\text{max}}$ (mN/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC</td>
<td>54.0</td>
<td>40.0</td>
<td>112.7</td>
</tr>
<tr>
<td>LysoPC</td>
<td>32.0</td>
<td>36.0</td>
<td>44.8</td>
</tr>
<tr>
<td>Monoolein</td>
<td>27.0</td>
<td>41.0</td>
<td>94.7</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>26.0</td>
<td>37.3</td>
<td>96.5</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>38.0</td>
<td>44.4</td>
<td>939.8</td>
</tr>
<tr>
<td>LDP mixture</td>
<td>29.0</td>
<td>41.0</td>
<td>115.4</td>
</tr>
<tr>
<td>D3</td>
<td>35.2</td>
<td>38.0</td>
<td>187.4</td>
</tr>
<tr>
<td>RP</td>
<td>56.0</td>
<td>16.2</td>
<td>42.8</td>
</tr>
</tbody>
</table>

$A_c$: collapse molecular area, $\pi_c$: collapse surface pressure. $K_{\text{max}}$: maximum compressibility modulus.
Interaction of the 2 vitamins with NaTC and LDP

<table>
<thead>
<tr>
<th>Sample</th>
<th>C_{micelle} (mM)</th>
<th>C_{vitamin} (mM)</th>
<th>Surface tension (mN/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaTC micelles</td>
<td>5</td>
<td>0</td>
<td>44.9 ± 0.1</td>
</tr>
<tr>
<td>NaTC micelles + D3</td>
<td>5</td>
<td>0.1</td>
<td>36.8 ± 0.5</td>
</tr>
<tr>
<td>NaTC micelles + RP</td>
<td>5</td>
<td>0.1</td>
<td>37.9 ± 0.5</td>
</tr>
</tbody>
</table>

- NaTC micelle barely affect by D3 or RP.
- D3 and RP insert into NaTC domain (same effect on its organization)

- For LDP/D3, surface pressure was controlled by LDP
- For LDP/RP, surface pressure was controlled by RP

D3 conferred a better rigidity of LDP monolayers than RP:

- Micelles are more stable
- Better capacity to solubilize at low concentration
Interaction of the 2 vitamins with mixed micelle

Is that also true for higher concentration?

<table>
<thead>
<tr>
<th>Sample</th>
<th>$C_{\text{micelle}}$ (mM)</th>
<th>$C_{\text{vitamin}}$ (mM)</th>
<th>Surface tension (mN/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixed micelles</td>
<td>6.1</td>
<td>0</td>
<td>$29.0 \pm 0.2$</td>
</tr>
<tr>
<td>Mixed micelles + D3</td>
<td>6.1</td>
<td>0.1</td>
<td>$31.6 \pm 0.1$</td>
</tr>
<tr>
<td>Mixed micelles + D3</td>
<td>6.1</td>
<td>1</td>
<td>$30.5 \pm 0.5$</td>
</tr>
<tr>
<td>Mixed micelles + RP</td>
<td>6.1</td>
<td>0.1</td>
<td>$29.5 \pm 0.2$</td>
</tr>
<tr>
<td>Mixed micelles + RP</td>
<td>6.1</td>
<td>1</td>
<td>$31.8 \pm 0.5$</td>
</tr>
</tbody>
</table>

Low impact of vitamin concentration in mixed micelles
cholecalciferol self-associating properties

- Since (i) D3 showed an interfacial behavior similar to that of the lipid mixture, and since (ii) it could be solubilized at very high concentrations in an aqueous phase rich in mixed micelles, then its self-assembling properties were more specifically investigated:

Cryo-TEM analysis:

- D3 can self-associate to form micelles at concentrations ≥ 0.1 mM
- D3 forms various organized systems at higher concentrations (1 mM):
  Fibers (nanotubes), aggregates
Conclusion
Summary of Findings - Conclusion

- D3 displays a higher solubility in mixed micelles than RP... WHY?

- Solubility of vitamins in mixed micelles is function of their interfacial behavior and affinity with micelle components:

- Even if [LDP] < [NaTC] in micelle, solubility of RP and D3 is dependent of their capacity to insert into both NaTC and LDP domains:
  - D3 has similar behavior than LDP, whereas RP is very different
  - Both RP and D3 can insert into NaTC

- the different abilities of the two vitamins to solubilize into mixed micelles is based on their interactions with micelle components,

- but it is also due to the fact that, conversely to RP, D3 can self-associate in structures that are readily soluble in an aqueous phase.

- Whether these D3 self-assemblies are available for absorption by the intestinal cell needs further studies.
Cholecalciferol (vitamin D3) and Retinyl Palmitate (vitamin A) display different solubilization capacities in mixed micelle solutions: effect of interactions with mixed micelle components and of cholecalciferol self-associating properties

Damián P. Prévéraud1*, Charles Desmarchelier2, David Chapron3, Ali Makky3, Véronique Rosilio3, Patrick Borel2

1Adisseo France SAS, Centre of Expertise and Research in Nutrition, Commeny, France; 2UMR 1260 INRA/1062 INSERM/Aix-Marseille University, "Nutrition, Obesity and Risk of Thrombosis", Marseille, France; 3University Paris-Sud, CNRS UMR 8612, Institut Galien Paris Sud, Châtenay-Malabry, France; *damien.preveraud@adisseo.com

Scope
- Vitamin A (as retinyl palmitate, RP) and vitamin D (as cholecalciferol, D3) : the 2 main fat-soluble vitamins found in foods of animal origin. These vitamins in the human upper gastrointestinal tract during digestion is assumed to follow that of dietary lipids[1], this includes emulsification, solubilization in mixed micelles, diffusion across the unstirred water layer and transport into enterocytes[2].
- Solubilization in mixed micelles (micellization) : a key step for their bioavailability.
- Mixed micelles are mainly made of a mixture of bile lipids (bile salts, cholesterol, phospholipids, lysophospholipids, retinyl palmitate, fatty acids and monoglycerides).
- Mixed micelle lipid composition has shown to significantly affect vitamin solubility[3]. It is possible that the properties of micelle components are linked to question the mixed micelles for its solubilization in the aqueous environment of the intestinal tract lumen.
- This study was designed to compare in vitro the relative solubility of D3 and RP in the pressure measurements the interactions between these vitamins and the mixed micelle components.

Methods

- Synthesis of mixed micelles and measurement of D3 and RE incorporation efficiency by HPLC
- Surface tension measurement and determination of the critical micelle concentration (cmc)
- Analysis of the molecular interactions between micelle components
- Synthesis of mixed micelles and measurement of D3 and RE incorporation efficiency by HPLC
- Cryo-TEM (Transmission Electron Microscopy) analysis

Conclusion
The results obtained show that D3 displays a higher solubility in mixed micelles than RP. This difference is mainly due to the different abilities of the two vitamins to solubilize into mixed micelles based on their interactions with micelle components, but it is also due to the fact that, conversely to RP, D3 can self-associate in structures that are readily soluble in an aqueous phase.

Whether these D3 self-assemblies are available for absorption by the intestinal cell needs further studies.
Thank you for your attention!

Damien P. Prévéraud

Centre of Expertise and Research in Nutrition, Commentry (France)

Charles Desmarchelier, Patrick Borel

UMR 1260 INRA/1062 INSERM/Aix-Marseille University, "Nutrition, Obesity and Risk of Thrombosis", Marseille (France)

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