



Metabolomic study of TNAP deficiency in mice organs and its implications in lipid metabolism.

Eva Drevet Mulard, Véronique Gilard, Stéphane Balayssac, Anne Briolay, Laurence Bessueille, Gilles J. P. Rautureau, David Magne

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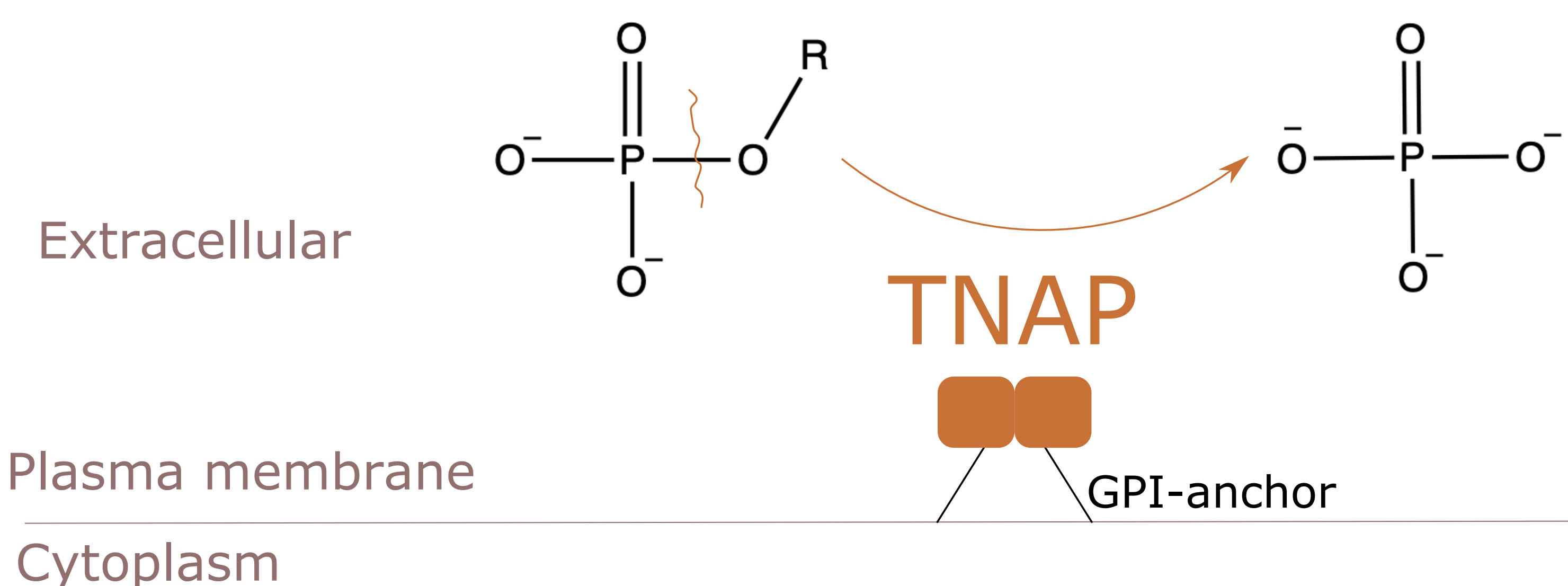
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Drevet Mulard E.¹; Gilard V.²; Balayssac S.²; Briolay A.¹ ; Bessueille L.¹ ; Rautureau GJP.¹; Magne D.¹

¹ Université de Lyon, ICBMS UMR CNRS 5246, Bâtiment Raulin, 43 Bd du 11 novembre 1918, 69622 Villeurbanne Cedex, France

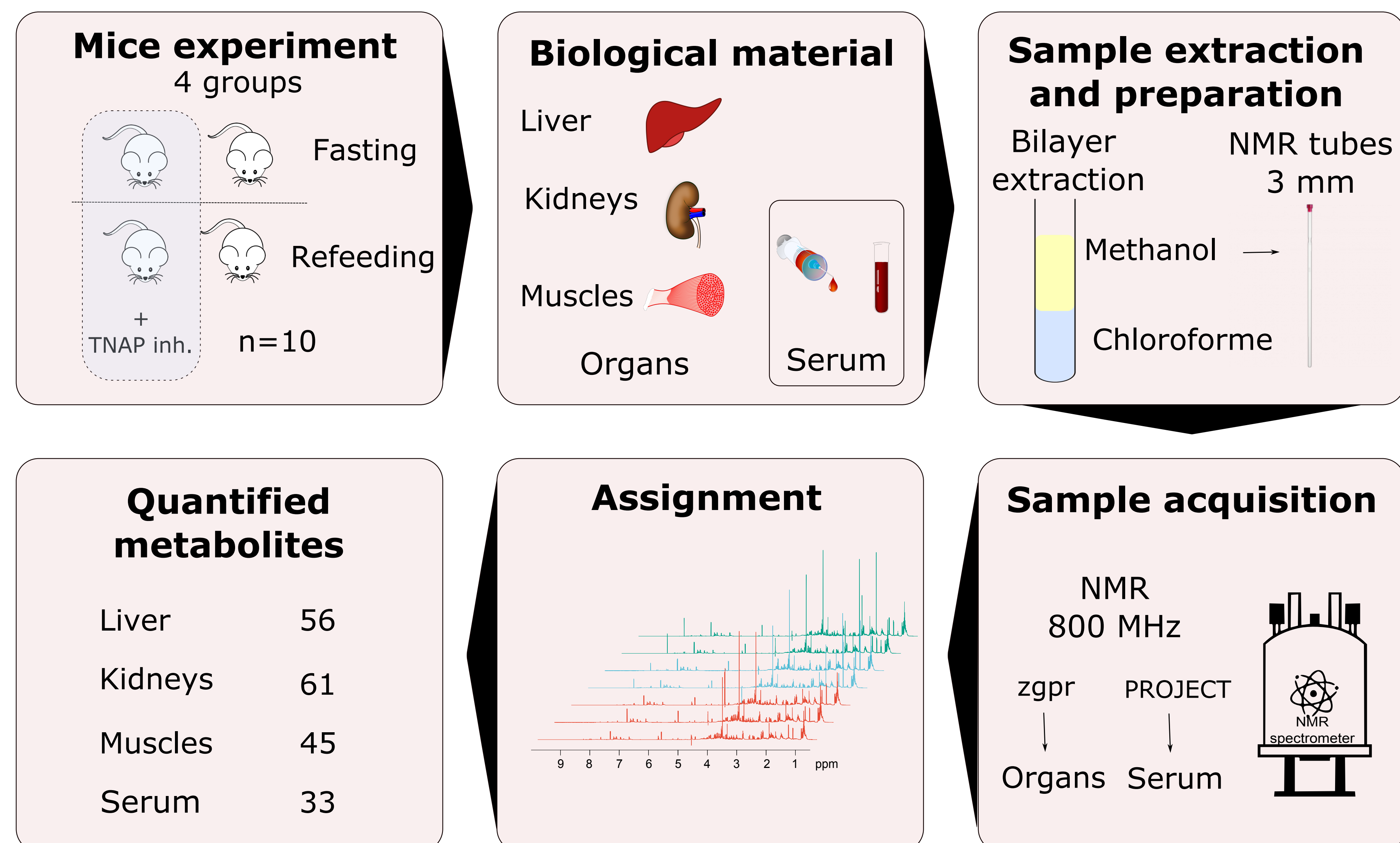
² Groupe de RMN Biomédicale, Laboratoire SPCMIB (CNRS UMR 5068), Université Paul Sabatier, Université de Toulouse, F-31062 Toulouse Cedex-9, France

Hypophosphatasia, a rare genetic disorder resulting from Tissue Nonspecific Alkaline Phosphatase (TNAP) deficiency, manifests with a spectrum of symptoms ranging from epileptic crises, bone fractures, and hypotonia in moderate forms (1 in 100,000 individuals) to life-threatening conditions in the most severe cases (1 in 300,000 individuals). While TNAP is an enzyme distributed throughout the body, the intricacies of its action in various organs remain largely unresolved, particularly its potential involvement in energy metabolism. In this study, we employed Nuclear Magnetic Resonance (NMR) spectroscopy to delineate the action of TNAP in crucial mice organs (liver, kidneys, and muscles) as well as serum. All mice (n= 40) underwent a 12-hour fasting period and half were refed while the others remained fasted. Half the mice received an intraperitoneal injection of a TNAP inhibitor. We then determined the metabolomic profiles using NMR (800 MHz) of organs after metabolite extraction, and serum. This experimental design allowed us to generate tables detailing metabolite concentrations in organs under four conditions: with or without refeeding, and with or without TNAP inhibition. Employing a non-targeted approach, our findings unveil new potential TNAP substrates, shedding light on their potential implication in lipid metabolism.



TNAP, a membrane enzyme distributed throughout the body, exhibits the capability to dephosphorylate molecular endings. While its well-established substrates include pyridoxal phosphate in the brain and pyrophosphate in the bones, the question arises regarding its activity in other organs.

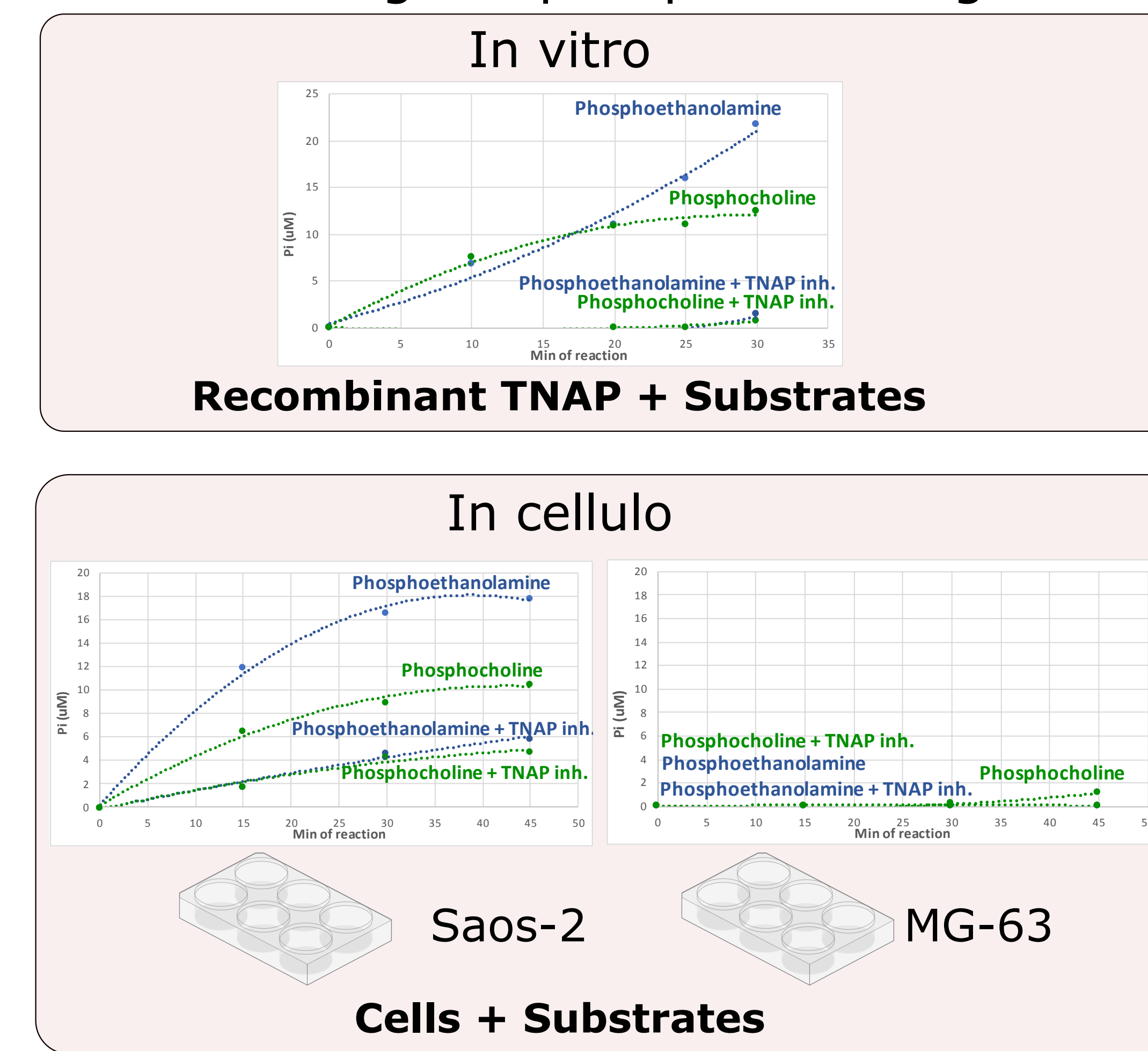
1) Metabolic NMR Workflow



Univariate statistics (ANOVA) revealed TNAP related significant metabolites

Organs	Significant metabolites	Potential TNAP substrates
Liver	Dimethylamine Choline Ascorbate Formate Succinate	Phosphocholine
Kidneys	Ethanolamine Uridine	Phosphoethanolamine
Muscles	Dimethylamine	
Serum	No metabolites	

2) Results TNAP substrats confirmation by inorganic phosphate dosage



Two potential TNAP substrates were identified: phosphocholine and phosphoethanolamine. To validate this, an in vitro assay was conducted, measuring inorganic phosphate (Pi) release in the presence of these substrates and the recombinant TNAP enzyme. The results demonstrated that TNAP effectively dephosphorylates both phosphocholine and phosphoethanolamine. Notably, the addition of a TNAP inhibitor, prevented this dephosphorylation reaction. This observation was further substantiated by a Pi dosage conducted on two osteoblast cancer cell lines: Saos-2, characterized by high TNAP expression, and MG-63, exhibiting very low TNAP expression. The specificity of phosphocholine and phosphoethanolamine dephosphorylation by TNAP was confirmed, as the presence of the TNAP inhibitor led to a significant reduction (at least by half) in the released Pi. These consistent findings provide evidence confirming phosphocholine and phosphoethanolamine as TNAP substrates.

3) Discussion

In mice treated with a TNAP inhibitor, a notable reduction in liver choline levels was observed. This phenomenon can be explained by the identification of phosphocholine as a potential TNAP substrate in the liver, suggesting that TNAP dephosphorylates phosphocholine to facilitate its cellular uptake. Thus, inactivating TNAP resulted in diminished choline and phosphocholine levels within the cells, and both are integral components of phosphatidylcholine, a major lipidic compound in the liver. Phosphatidylcholine serves two main functions in the liver: the release of triglycerides from the liver to the adipose tissue by forming a VLDL (Very Low Density Lipoprotein), and the formation of the bile. A well-established consequence of choline deficiency in the liver is the impaired ability to eliminate fatty acids, leading to hepatic steatosis. This aligns with findings by Gámez-Belmonte *et al.*, where TNAP deficiency in mice resulted in hepatic steatosis.¹ Similarly, in the kidneys, TNAP inhibition led to a significant decrease in ethanolamine concentrations. This observation suggests that TNAP facilitates ethanolamine uptake by dephosphorylating phosphoethanolamine. A plausible hypothesis is that a similar mechanism occurs in the liver, resulting in a shortage of ethanolamine, thereby disrupting its participation in phosphatidylcholine synthesis. **In conclusion, this metabolomic study has unveiled new TNAP substrates intricately involved in energetic metabolism, shedding light on a new role of TNAP related to triglyceride elimination, particularly within the liver.**

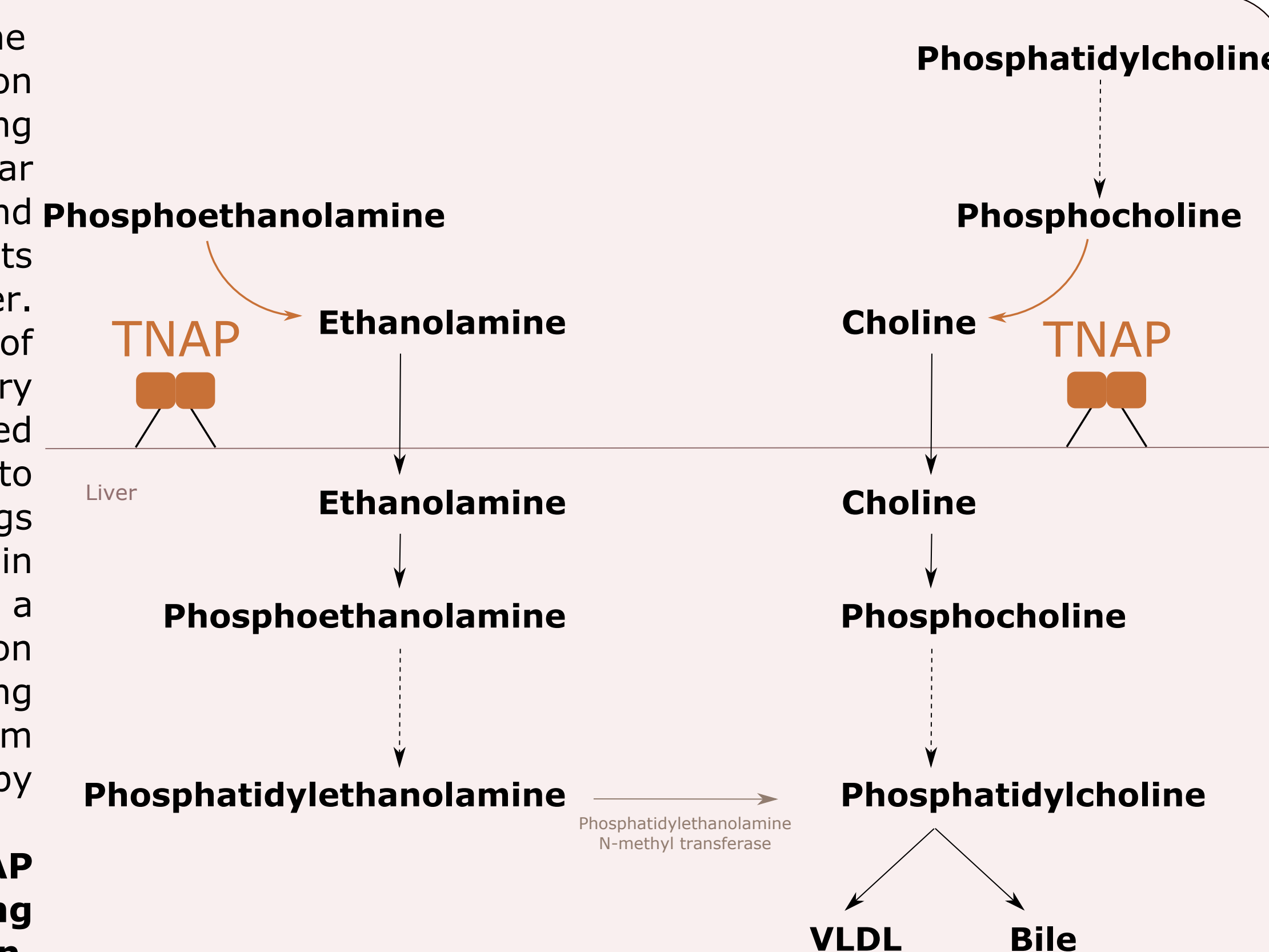


Figure: Phosphoethanolamine and phosphocholine intakes in the liver by TNAP and contribution to the formation of phosphatidylcholine in liver. Figure inspired by Bessueille *et al.*²

1. Gámez-Belmonte, R. *et al.* Deficiency in tissue non-specific alkaline phosphatase leads to steatohepatitis in mice fed a high fat diet similar to that produced by a methionine and choline deficient diet. *Int J Mol Sci* 22, 1–14 (2021).
2. Bessueille, L. *et al.* Inhibition of alkaline phosphatase impairs dyslipidemia and protects mice from atherosclerosis. *Translational Research* 251, 2–13 (2023).