Low vitamin B12 triggers lipid accumulation in human hepatocytes

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Background: There is increasing evidence that lipid metabolism in humans may be regulated by environmental factors including nutrients such as vitamin B12 (B12). B12 deficiency results in disturbance of 1-carbon metabolites [methylmalonyl coenzyme A (MMA), homocysteine and S-adenosyl homocysteine (SAH), S-adenosyl methionine (SAM) and methionine] that collectively favours lipogenesis leading to risk of cardiovascular diseases. In clinical studies, B12 deficiency is associated with higher BMI and dyslipidaemia (high triglycerides and low HDL). *In vitro* experiments in human adipocytes showed that low B12 results in hypomethylation of SREBF1, a master regulator of cholesterol biosynthesis. If similar effects happen in hepatocytes, this may explain the observation of dyslipidaemia in humans. In addition, the role of B12 in hepatic metabolism of lipids in humans is unexplored. Therefore, we investigated whether B12 deficiency affect hepatic metabolism of lipids.

Methods: Human Hep G2 cell line was cultured using custom made B12 deficient Eagle's Minimal Essential Medium (EMEM) and seeded in four different concentrations of B12 media such as 500nM (control), 1000pM, 100pM and 25pM (low) B12. Seahorse assay, oil Red O (ORO) staining, gene expression assay using RT-qPCR, total intracellular triglyceride (TG) assay with commercial kit and TG biosynthesis using radioactive flux assay were employed to examine the effect of B12 on hepatic oxidation and synthesis of lipids.

Results: Long chain fatty acid oxidation was impaired in hepatocytes under low B12 (25pM) and expression of fatty acid oxidation genes carnitine palmitoyltransferase 1A (CPT1A) and carnitine acylcarnitine translocase (CACT) were inhibited by low B12. Hep G2 cells in low B12 had more lipid droplets that were intensely stained with ORO compared with less stained few oil droplets in control B12 (500nM) condition. Total intracellular TG levels were higher in low B12 hepatocytes. The gene expressions of nuclear transcription factors sterol regulatory element binding protein (SREBF1) and low density lipoprotein receptor (LDLR) were higher in low B12 conditions compared with control. Similarly, the gene expressions of the enzymes involved in *de novo* fatty acid synthesis [ATP citrate lyase (ACLY), Acetyl CoA carboxylase (ACC), fatty acid synthase (FASN) and elongation-of very-long-chain fatty acid (ELOVL6)], cholesterol biosynthesis [3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), 3-hydroxy-3-methylglutaryl-CoA synthase 1 (HMCS1), Isopentenyl-Diphosphate delta Isomerase 1 (IDL1)] and TG biosynthesis [stearoyl CoA desaturase (SCD), glycerol-3-phosphate acyltransferase (GPAT), acylglycerol-3-phosphate acyltransferase (AGPAT), phosphatidic acid phosphatase-1 (Lipin1) and diacylglycerol acyl transferase 2 (DGAT2)] in low B12 conditions. Lastly, cellular uptake of radio-labelled fatty acid (^{14}C-oleate) for *de novo* TG biosynthesis assessed by scintillation was about 80% higher in HepG2 cells cultured in low B12 condition.

Conclusion: Our data provide novel evidence that B12 deficiency dysregulates lipid metabolism in hepatocytes. Further studies are required to quantify the effect of this on circulating levels of lipid fractions as well as its epigenetic role on hepatocyte function.