

Summary

The Staub-Traugott effect refers to the apparently improved glucose tolerance following a sequence of two glucose loadings. Similarly, whey protein (WP) intake as a pre-meal followed by a main meal improves the apparent glucose tolerance. WP intake as a pre-meal influences postprandial metabolic regulation through different mechanisms and can add to the dietary strategies improving postprandial metabolism in subjects with metabolic syndrome (MetS).

Rapid and excessive weight gain during infancy increases the risk of obesity in later life. Although exclusive breastfeeding is the ideal feeding regime for the first six months of life, some exclusively breastfed infants experience excessive and rapid weight gain. The differences in breastmilk composition may contribute to excessive and rapid weight gain in infants. One plausible mechanism explaining the variation in growth rate in these infants can be related to the end product of bacterial fermentation: the short chain fatty acids (SCFAs). Human milk oligosaccharides (HMOs) and other breastmilk components potentially alter SCFA dynamics in the infant gut and thereby interact with energy homeostasis and appetite regulation.

The central nervous system regulates energy homeostasis through the neural, hormonal, and nutrient mediated feedback after food intake. The regulation of energy homeostasis is a complex process influenced by many dietary factors and by the cross-talk between the host and microbiota. This thesis aims to investigate the role of certain dietary factors on metabolic regulation using untargeted and targeted metabolomics. The first aim is to investigate the metabolic fingerprint of protein pre-meals consumed before a high-fat main meal in subjects with MetS. A second aim is to review alterations of gut microbiota and the role of SCFAs in early life malnutrition. Further aims are to explore the differences in breastmilk components and their association with fecal SCFA concentration in relation to infants' growth.

In Paper-I, postprandial plasma metabolites were profiled from two cross-over meal studies to investigate the effect of pre-meal protein intakes followed by fat-rich breakfast meals in subjects with MetS. Early peaks of branched chain amino acids (BCAAs), aromatic amino acids, and PC (32:1) contributed to the postprandial insulin and glucose response to the fat-rich main meal. Numerous other metabolites were also altered postprandially. A higher

level of short- and medium chained acyl carnitines indicates an up-regulated BCAA metabolism while lower postprandial levels of linoleic acid, β -hydroxypalmitic acid, and L-palmitoyl-carnitine indicate an enhanced lipid clearance from the plasma after the pre-meal treatment. The pre-meal WP treatment affected postprandial lipemia by altering the particular PC, LPC and LPE species, rather than the total triglyceride or FFA response. The pre-meal WP treatment caused a postprandial metabolic pattern that is likely to facilitate glucose and lipid disposal from plasma in response to a fat-rich main meal in participants with MetS. WP and casein pre-meals exerted almost similar effects, whereas a pre-meal with gluten was less efficient in stimulating the early BCAA and aromatic amino acid responses. The timing of the pre-meal (-15 min vs. -30 min) did not alter the postprandial metabolome.

Paper-II reviews the gut microbiota changes in relation to childhood malnutrition and the possible role of SCFAs in energy homeostasis. There is some evidence that gut microbiota perturbation is a causal factor in childhood malnutrition. However, it is more likely that there is a complex bidirectional relationship between microbiota perturbation and childhood malnutrition. Undernourished microbiota is characterized by gut microbiota immaturity, altered diversity, enrichment in potentially pathogenic and inflammogenic species, depletion in obligate anaerobes, and by less efficient nutrient utilization. *Bifidobacterium* colonization in early life is negatively associated with later childhood weight gain, whereas *Bacteroides fragilis* colonization is positively linked with childhood weight development. SCFA production via gut microbiota is an energy harvest mechanism from the undigested fibers and proteins reaching the colon. Moreover, SCFAs interact with energy metabolism through the gut-brain axis, by modulation of adipose tissue metabolism and possibly by influencing the IGF-1 production in the liver.

Paper-III integrates the breastmilk metabolome, HMO concentrations, and infants' fecal SCFA concentrations in the SKOT III cohort of mother-infant dyads to investigate the characteristics of excessive and rapid weight gain in early life. Linolenic acid, oleic acid, LPE (16:0), LPC (16:0), LPC (18:0), PC (36:2) levels as well as HMO-diversity were lower in the breastmilk for the infants with excessive growth and these breastmilk components were positively associated with fecal branched short chain fatty acid (BSCFA) concentrations, potentially contributing to the rapid weight gain. Fecal SCFA concentrations were not different

between infants with excessive and normal weight gain at 5 months of age. A lower fecal butyrate concentration was observed at 9 months of age during the complementary feeding period; the lower butyrate may contribute to – or be a consequence of - the excessive weight gain, possibly as a reflection of the differences in substrate availability for the gut microbiota of infants with normal and excessive growth.

List of publications

PAPER-I

Pekmez CT, Bjørnshave A, Pratico G, Hermansen K, Dragsted LO: Pre-meal protein intake alters postprandial plasma metabolome in subjects with metabolic syndrome. *Eur J Nutr* 2019 Jul 6. doi: 10.1007/s00394-019-02039-9.

PAPER-II

Pekmez CT, Dragsted LO, Brahe LK: Gut microbiota alterations and dietary modulation in childhood malnutrition - The role of short chain fatty acids. *Clin Nutr* 2019, 38:615-630. 10.1016/j.clnu.2018.02.014.

PAPER-III

Pekmez CT, Larsson MW, Lind MV, Vázquez-Manjarrez N, Yonemitsu C, Larnkjær A, Bode L, Mølgaard C, Michaelsen KF, Dragsted LO. Breastmilk oligosaccharides and lipids are associated with fecal branched short chain fatty acid concentrations in infants with excessive weight gain. (Under review in *Molecular Nutrition & Food Research*)

Other Publications not included in the thesis

Vázquez-Manjarrez N, **Pekmez CT**, Micheau P, Pétéra M, Durand D, Pujos-Guillot E, Dragsted LO, Manach C. Untargeted metabolomics to identify urinary biomarkers of tomato intake in a randomized controlled intervention study. (Manuscript)

Laursen MF, Sakanaka M, Von Burg NC, Andersen D, Mörbe U, Rivollier AMC, **Pekmez CT**, Moll JM, Michaelsen KF, Mølgaard C, Lind MV, Dragsted LO, Katayama T, Frandsen HL, Vinggaard AM, Bahl MI, Pedersen SB, Agace W, Licht TR, Roager HM. Breastmilk-promoted bifidobacteria produce aromatic lactic acids via a novel aromatic lactate dehydrogenase. (Manuscript)

Hartstra AV, De Groot P, Bastos D, De Clercq NC, Koopen AM, Serlie M, Soeters M, **Pekmez CT**, Dragsted LO, Ackermans M, Groen AK, Nieuwdorp M. Correlation of plasma metabolites with glucose and lipid fluxes in human insulin resistance. (Submitted to *Diabetes*)

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1. Introduction

Metabolic syndrome is a cluster of risk factors for type 2 diabetes (T2DM) and cardiovascular diseases (CVD). Abdominal obesity is the core criterion for MetS. The other components of MetS are elevated triglycerides (TG), reduced high-density lipoprotein (HDL) cholesterol, elevated blood pressure, and high fasting glucose [1]. Having abdominal obesity, along with ≥ 2 of the other MetS components is the metabolic syndrome criterion proposed by the International Diabetes Federation Task Force on Epidemiology and Prevention [1]. The MetS components can be prevented or reversed by lifestyle changes such as diet and exercise. One of the dietary factors inversely associated with MetS risk is the dairy proteins [2].

Improved glucose tolerance following a sequence of two glucose loadings is known as the Staub-Traugott effect [3]. Similarly, improved glucose tolerance has been shown after the consumption of a pre-meal with (WP) followed by a main meal with a mixed macronutrient composition [4]. WP consumption as a pre-meal influences postprandial metabolic regulation through different mechanisms such as lowering gastric emptying rate, suppressing food intake, lowering postprandial glucose, and increasing postprandial insulin, glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) [4, 5]. Pre-meal WP consumption can be one of the dietary factors that modulate postprandial metabolic regulation in subjects with MetS.

Rapid and excessive weight gain during infancy is one of the predictors of later life obesity [6] and thereby increases the risk of MetS in adulthood. Exclusive breastfeeding is the ideal feeding regime for the first six months of life. However, some exclusively breastfed infants experience rapid and excessive weight gain, opening the question of whether the differences in breastmilk composition contribute to excessive weight gain in early life.

Short chain fatty acids can interact with energy homeostasis and appetite regulation through multiple mechanisms and thereby possibly affect the growth outcomes in early life [7]. A plausible mechanism explaining the variation in growth rate in exclusively breastfed infants can be related to the SCFAs. During the breastfeeding period, SCFAs are primarily produced via fermentation of HMOs and proteins by the infants' gut microbiota. Little is known about the possible relationship between the other breastmilk components and fecal SCFAs dynamics.

The overall focus of the thesis is to explore the role of certain dietary factors on metabolic regulation in excessive weight gain/obesity in early life and adulthood using a metabolomics approach. The thesis builds on two different themes due to unexpected changes in funding sources during the Ph.D. The first theme of the thesis focuses on the effect of pre-meal protein intervention on postprandial metabolic regulation in subjects with MetS. The second theme is based on the differences in breastmilk metabolome in early life and its relationship with the fecal SCFAs' dynamics in infants experiencing rapid and excessive weight gain.

The overall aims of the thesis are:

- 1) To test the effect of pre-meal WP dose, protein quality, and timing on the postprandial metabolome in subjects with MetS.
- 2) To review the gut microbiota changes in childhood malnutrition, the role of SCFAs on energy metabolism and the possible dietary strategies to modulate gut microbiota in childhood as a treatment and prevention strategy towards childhood malnutrition.
- 3) To investigate whether the human milk metabolome of the mothers and the infants' fecal SCFA concentrations differ between infants with normal and excessive growth. Furthermore, to investigate the relationship between breastmilk components of mothers and fecal SCFA content in infants with normal and excessive growth.