Early Programming of Obesity Throughout the Life Course: A Metabolomics Perspective

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Abstract

Background: Over the last decades, research on early life risk factors for obesity and its comorbidities in early life has gained attention within the field of developmental origins of health and diseases. Metabolomics studies that are trying to find early life biomarker and intervention targets for the early development of obesity and associated cardiovascular diseases could help break the inter-generational cycle of obesity. **Summary:** Metabolomics studies in the field of early programming are scarce and causality is lacking at this stage, as most of the studies are cross-sectional. The main metabolites in the focus of obesity are branched-chain and aromatic amino acids, long-chain polyunsaturated fatty acids, lysophosphatidylcholines, and sphingomyelins. Sex and puberty have not been considered in most of the biomarker studies, but show differences in the metabolite associations to obesity. **Key Messages:** There is still a lot unknown about the associations between early programming exposures,

metabolite concentrations, and the development of obesity. The few studies focusing on this topic find similar metabolite classes in the same age groups being associated with rapid early growth or obesity; but due to differences in the methodological and statistical approaches, the single species often differ. Therefore, more research, preferably with standardized approaches, is needed. © 2017 S. Karger AG, Basel

Introduction

Over 40 years ago, Dörner [1] proposed a “programming” effect of hormones and other metabolites in utero on the later risk of cardiovascular disease and other diseases in the offspring. Interest in this concept has risen considering the background of non-communicable diseases (NCD) like obesity that, according to the NCD Risk Factor Collaboration, has increased from 3.2% in 1975 to 10.8% in 2014 in men and from 6.4 to 14.9% in women in a large international survey [2].

During early life, the child's metabolism is especially susceptible to environmental exposures [3]. One of the more recent hypotheses amongst the developmental origins of health and diseases research is the early protein

hypothesis, proposing that a high protein intake in infancy induces rapid weight gain in early childhood, leading to later overweight and obesity [4]. Many other perinatal factors are discussed to be potential modifiers of disease risks like pre-pregnancy BMI (pBMI), smoking, birth weight, or breast-feeding [5].

Metabolomics research offers opportunities to elucidate underlying mechanisms. Metabolomic analysis characterizes molecules smaller than 1,500 Da. Analytical techniques, like mass spectrometry (MS) or nuclear MR spectroscopy (NMR), facilitate the identification and quantification of single species such as acylcarnitines (acyl-Carn), phospholipids, non-esterified fatty acids (NEFA), amino acids (AA), and organic acids (intermediates of the tricarboxylic acid cycle).

Most metabolomics research is embedded in cross-sectional studies in adult age where associations between (metabolic) diseases and the metabolome are investigated. There is a clear lack of longitudinal studies especially on the background of early programming.

Therefore, the aim of this review is to give a brief overview of the current state of knowledge regarding the effects of early life factors on the metabolome being associated to obesity or insulin resistance (IR) over the life course. We focus on the period from conception up to early adulthood (pregnancy, infancy, childhood, and adulthood). The studies mentioned were identified from a “PubMed” search for articles matching the search terms (metabolomics and programming as well as metabolomics and pregnancy/infancy/childhood/puberty/adult obesity). Afterwards, articles were screened and included if metabolites were measured and related to growth markers in the different time periods. Additionally, relevant references found in the identified papers were included.

Pregnancy

During pregnancy, the maternal body undergoes major metabolic changes in order to meet the fetal and maternal energy demand. In the context of the early programming hypothesis, some (pre-)pregnancy and perinatal factors have been repeatedly shown to correlate with offspring obesity. Macrosomia and large birth weight [6, 7] as well as small size for gestational age [6, 8, 9] are associated with increased childhood adiposity and later onset of metabolic diseases. Among the maternal factors, the most noticeable characteristics are an elevated pBMI [10], gestational diabetes mellitus (GDM) [3, 11, 12], and pre-eclampsia [13]. Furthermore, behavior-related exposures

such as diet composition and smoking during pregnancy are also known to affect the offspring outcome [3, 12].

Lindsay et al. [14] observed variations in the metabolite levels and provided evidence for an increased placental uptake of branched chain AA (BCAA) and maternal long chain polyunsaturated fatty acid (LC-PUFA) during pregnancy with enhanced maternal ketogenesis and β -oxidation rate.

Several observational studies found associations between maternal LC-PUFA levels and infant body fat and weight. Donahue et al. [15] showed a negative association between maternal omega-3 fatty acids and offspring adiposity at 3 years measured by BMI, but revealed no association between maternal omega-6 fatty acids and childhood adiposity. Other European studies repeatedly assessed a positive association between maternal omega-6 fatty acids across the gestation trimesters and childhood adiposity, defined by both BMI and body composition and measured at different time points during infancy [16–18]. Despite the differences in the single metabolites identified, which have been suggested to be explained by different diet and baseline fatty acid concentrations between the populations [16], all studies confirmed an involvement of LC-PUFA and maternal pBMI with offspring overweight or obesity.

However, intervention studies have not shown effects of LC-PUFA supplementation during pregnancy on offspring body composition [19, 20]. It was recently suggested that the maternal metabolome is predominantly influenced by pre-pregnancy obesity and less by dietary intake during pregnancy [21].

All these studies were based on maternal plasma, but the fetal exposure to fatty acids in utero could differ from what was observed in the mothers.

Over the last years, the placental fatty acid transport was identified as a key factor in fetal growth, as unexpected NEFA profiles were observed in mothers with different glucose tolerance levels or pBMI and in their children [22]. Schaefer-Graf et al. [23] detected no significant difference in the NEFA levels between healthy and GDM mothers, yet higher NEFA levels were measured in the cord blood of GDM children. An analysis of placental tissue found similar patterns in placental glycerophospholipids in both GDM and obese women [24]. These results suggest a dysregulation of placental fatty acid transport in GDM and obese mothers. In particular, the transfer of docosahexaenoic acid (DHA) from the mother to the fetus has been found to be impaired in GDM [25]. Following observations in mice [26], the major facilitator super family domain containing 2a was identified as a preferred

transporter of DHA in the human placenta [27]. This observation reinforced the hypothesis that a disturbed lipid metabolism, and not the glucose tolerance, is crucial in the fetal fat mass and subsequent adiposity onset [22–24].

An additional source of information is the cord blood metabolome. Isganaitis et al. [28] linked cord blood metabolites with rapid postnatal weight gain (0–6 months), but the analysis identified only weak associations between metabolites and growth parameters. The authors suggested various reasons for the limited strength of the associations, including the small sample size ($n = 52$). In another study by Standl et al. [29], high omega-3 LC-PUFA levels and a low omega-6/omega-3 ratio in cord blood were significantly associated with higher concentrations of the hormone adiponectin at 10 years. These authors also found cord blood lysophosphatidylcholines (LPC) were strongly positive related to birth weight, but no metabolite was associated to early weight gain and later BMI [30].

In conclusion, the metabolic profile during pregnancy is greatly influenced by the pre-pregnancy obesity status. GDM affects LC-PUFA levels in the blood, which in turn have been shown to relate to childhood obesity. However, intervention studies so far have not shown effects of enhanced LC-PUFA on later obesity or adiposity.

Infancy

Human breast milk is recommended to be the best feeding choice until 6 months of age and is suggested to promote long-term health (ESPGHAN Committee on Nutrition, JPGN, 2009, Breast-feeding: A commentary by the ESPGHAN Committee on Nutrition). It is suggested that human milk components are optimal for infant needs and thus ingredients of infant formulas (IFs) are designed according to human milk. Components that might have unfavorable effects on infant growth are a subject of current research to avoid rapid early weight gain as a well-known modifying factor in the development of obesity. A markedly higher protein (HP) intake than infant requirements through IF was hypothesized to induce an increased infant weight gain. The childhood obesity project (CHOP) was designed to investigate this “Early Protein Hypothesis” in a double-blind, randomized, multicenter intervention trial across 5 European countries. Infants were randomized to receive an isocaloric HP or lower protein (LP) content IF and follow-on formula (FOF; protein content in IF per 100 mL: HP 2.05 g; LP 1.25 g; protein content in FOF per 100 mL: HP 3.2 g; LP 1.6 g) or were breast-fed. Infants in the HP group

were shown to have a higher weight gain than the LP group during the first 2 years of life [31]. Furthermore, HP was found to induce a much higher risk for obesity at early school age with prevalence of 10% in the HP group and of only 4.4% in the LP group [32].

The underlying molecular mechanisms of the effects of early protein supply were investigated in 6-month old infants from the CHOP study. Infants in the HP group had higher plasma levels of BCAA, short-chained acyl-Carn, IGF-1, and higher urinary C-peptide-creatinine ratio which reflects an enhanced insulin secretion. The IGF-binding protein along with the long-chained acyl-Carn was lower in the HP group [33, 34]. These findings point toward an alteration in the IGF-axis and insulin release in children consuming high protein formula milk and highlight the importance of BCAA in the effect of a high protein diet on β -oxidation and fat storage. Their high level might be explained by the low liver concentration of the branched-chain keto acid dehydrogenase which catalyzes the oxidative decarboxylation of the BCAA. Hence, BCAA are less metabolized during the first pass liver metabolism [35] and the subsequently high concentrations may lead to enhanced insulin secretion. Furthermore, new evidence was provided for a possible saturation of the BCAA degradation pathway [33]. Lower BCAA levels were also found in breast-fed infants compared with formula-fed infants receiving formula milk with different protein content from age 3–12 months [36].

Looking at early weight gain, Socha et al. [34] found that IGF-1 concentrations at 6 months and early weight gain (defined as weight change in weight-for-length Z-score between 0 and 6 months) positively correlated. Thus, the relationship of early weight gain (defined as change in weight-for-age from birth to 6 months of age) and the metabolome at 6 months of age was further investigated [37]. A total of 19 metabolites showed a significant association, but only LPC 14:0 was significantly related to obesity at 5.5 years. This remained significant after adjusting for the feeding group.

Another study investigated the effect of a protein-reduced IF with alpha-lactalbumin-enriched whey, with the addition of free tryptophan, phenylalanine, DHA, and arachidonic acid on growth in 4-month-old children [38, 39]. A group receiving an isocaloric standard formula without additional DHA and arachidonic acid and breast-fed infants were also included. The authors concluded that the quality of a diet (protein source, AA composition) might have a stronger effect on the infant endocrine response than the change in absolute protein content by

about -0.2 g/100 mL formula. The supplemented DHA and arachidonic acid resulted in increased related phospholipids. The concentrations of phospholipid species with DHA in the study group were similar to those of breastfed infants, but phospholipids with arachidonic acid were lower than breast fed infants [40].

The effect of n-3 LC-PUFA concentration during pregnancy, lactation, and in formula milk on infant and child body composition was reviewed previously [40–43]. In general, the results were controversial, with positive, negative, or neutral effects of maternal n-3 LC-PUFA status on child body composition, and no conclusion could be made. Currently, standard IFs are based on cow's milk protein and vegetable oils. A recent study compared the effects of a standard cows' milk-based formula with vegetable oils to a formula based on goats' milk protein and part of formula lipids derived from goat dairy lipids on the infants' growth and metabolism. Although there were some differences in the children's glycerophospholipid profile at 4 months of age between those fed with conventional IF and goat-milk-based formula [40], there were no differences in Z-scores for weight, length, head circumference, or weight for length between the 2 groups [44].

Recently, further human milk components such as hormones like leptin or insulin became the attention of research regarding their effects on infant growth. A recent review concluded that milk levels are affected by maternal status and this might be relevant for infants, but clear data is missing to conclude clinical relevance [45].

In summary, both too low and too high early weight gain is associated with adverse later outcomes, but the underlying mechanisms are not fully elucidated. HP intake in infancy is associated with higher BCAA blood concentrations and enhanced early growth, but the underlying mechanistic pathways deserve further study.

Childhood

The metabolites most studied in the field of obesity and obesity-related IR are BCAA and metabolites involved in BCAA catabolism like glutamate, keto acids, and short-chain acyl-Carn. In contrast to several adult studies which related BCAA to IR and obesity [42, 46, 47], the results in children are rather inconsistent. Some studies found BCAA or BCAA-associated factors to be related with obesity [48–52] or even predictive for later IR [52] or triglyceride levels [53] in childhood. Other studies found no association [36, 54–56] or even a positive effect

of BCAA on β -cell function [56] and lower levels of BCAA in diabetic children [57]. Both studies showing beneficial effects of BCAA were published by the same group and conducted in the same collective. The authors state that the contrast of their beneficial findings of BCAA in adolescents and other studies showing adverse associations in adults, may be due to the adaptive (mitochondrial) mechanisms which are still present in youth but vanish with the progression of obesity [42, 46, 47].

Different hypotheses are discussed regarding the positive associations between BCAA and obesity as well as IR, for example, BCAA effects on the m-TOR pathway [58]. BCAA may activate m-TOR and cause IR in obese people. However, there are controversial results on effects of a high-protein diet on blood BCAA levels. Thus, it is conceivable that reduced insulin release or IR affects BCAA catabolism. Non-obese type 2 diabetes mellitus patients exhibit a reduced clearance rate of BCAA, when receiving an infusion containing BCAA, compared to controls [59]. Previous studies have also shown that ratios depicting BCAA degradation pathway are downregulated in IR [55]. Furthermore, key enzymes of the BCAA catabolism are less expressed or downregulated in adipose tissue and liver, but not muscle, in IR subjects [60]. Based on these results one may conclude that BCAA blood level are affected by IR and thus might be the consequence, rather than the cause of the relationship. This might explain the divergent results described in childhood. As mentioned previously, BCAA levels in childhood can predict hypertriglyceridemia in early adulthood, which can be related effects of BCAA on fatty acid metabolism [33]. Nevertheless, programming effects of exposures in the perinatal or early infancy period on the BCAA levels in childhood have not been studied yet to our knowledge.

Phospholipids, which present a mid-term marker for dietary fat intake, are another group of metabolites related to childhood obesity state. In general, phospholipids are decreased in the obese state [54, 61], but additionally the fatty acid composition is affected. Odd-chain fatty acids, like 17:0 [52, 61–63], or n-3 fatty acids [64, 65], like DHA, are decreased in obese subjects, while saturated fatty acids [65], n-6 fatty acids [62], or 24:1 [65] as well as 16:1 and 17:1 [48] are elevated. However, usually fatty acids can be related to dietary components. For instance, odd-chain fatty acids are markers for dairy fat intake, while n-3 are related to fish or fish-oil intake. Thus, fatty acid composition analysis allows drawing conclusions regarding the relation of obesity to the source of dietary fat intake. Wang et al. [61] proposed an increased dairy fat intake in the first few years of life to prevent or delay the

onset of cardiovascular disease in later life. When it comes to n-3 fatty acids, the effect is clearer, since these essential fatty acids are known to regulate the activity of genes involved in lipolytic activity [65]. Some groups also found alterations of ratios depicting desaturase activities. These desaturases are involved in the biosynthesis of MUFA and PUFA from dietary fatty acids. For instance, $\Delta 9$ -desaturase activity was found to be elevated in obese children [62, 66, 67]. The activity of this enzyme, which is also known as stearoyl-CoA desaturase-1 (SCD-1), is associated with obesity [68]. Interestingly, DHA levels, which are reduced in obese children, are inversely associated to SCD-1 activity [66]. Furthermore, the ratio of 20:3n-6 to 18:2n-6, a marker for $\Delta 6$ -desaturase activity, was also increased in obese children, while $\Delta 5$ -desaturase activity was decreased [62, 67]. In addition, several studies found 20:3n-6 increased in obese children [62, 63], while the 18:2n-6 was not affected [48] or decreased [62].

There are not only effects on the fatty acid composition, but also on certain molecular classes and species, that differ between normal weight and obese children. PC with fatty acids bound by an ether bond (PCae) are decreased in obese children [52, 54]. PCae has been linked to PUFA intake in infancy [40] and may be beneficial for membrane structure, prevention of oxidative stress, and brain development [69]. In obese German children, the LPC 18:1, 18:2, and 20:4 were found to be reduced [54]. The same LPC, in addition to LPC 17:0, 18:0, 18:3, 20:1, 20:2, 22:5, and 22:6, were also decreased in obese Mexican-American children [48], while the aforementioned LPC 14:0 in the serum of 6-month-old infants is predictive for later obesity risk [36]. The fatty acid 14:0 was also elevated in phospholipids of 15-year-old obese children [62]. Thus, certain fatty acids (e.g., 20:3, 14:0, 22:6) and lipid classes (PCae, LPC) are associated with childhood obesity. Given that blood lipids are influenced by dietary fat intake, the question remains if the findings reflect a causal relationship or are an epiphenomenon, since obese children usually have a different fat intake compared to normal weight children.

Sex Differences and Puberty Effects

The majority of the metabolomics studies analyze a general study population, with no specific focus on potential sex differences in the associations of the metabolome with obesity and IR. Therefore, it cannot be excluded that disease biomarkers are potentially biased by a sex-specific metabolism. Zheng et al. [70] state that it is the

pubertal stage, representing a critical time period regarding body development and body composition that is affected by the obesity epidemic, although this relationship is not yet clarified. In their study of pubertal boys and girls, they found differences between the sexes mainly in lipoproteins, PC, unsaturated lipids, and lower levels of choline in girls. Tanner stage was also associated with different metabolite concentrations, especially creatinine and citrate. The authors suggest that this is due to the muscle turnover, as girls enter puberty earlier than boys and therefore might have had a higher muscle mass [70].

Another study investigated the predictive ability of serum AA concentrations for triglyceride levels in adult women. AA levels were shown to be decreased during and after menarche in women, which might also point toward a lower muscle turnover [53].

The time of the menstrual cycle has also been shown to affect the plasma metabolic profile, mainly the AA levels [71]. This has not been considered in earlier studies. Thus, AA marker for diseases, such as BCAA, should be considered carefully when applied on a general population without accounting for sex and menstrual cycle phase, especially in pubescent and adult subjects.

Differences between men and women were also found to persist in adults, especially concerning polar lipids and AA [72, 73]. Mittelstrass et al. [72] even suggest in their analysis that the sex differences are not only randomly affecting single metabolites, but that specific metabolic pathways are systematically altered.

Finally, hormonal contraceptives seem to play a crucial role concerning the sex differences in the metabolome, since they affect the lipid and AA metabolism [74, 75]. Wiklund et al. [76] even excluded participants taking oral contraceptives. This might be an important point that is not considered in most of the metabolomics studies in teenagers and adults, potentially falsifying the results. Therefore, it is not yet clear if potential programming effects are biased by sex effects.

Adults

The observation that low birth weight and small size during infancy are associated with increased risk for diverse diseases in adult life, such as coronary heart disease, obesity, type 2 diabetes, and the metabolic syndrome, has been repeatedly described [77]. Other early life factors like breastfeeding have been shown to affect the BMI of 20-year-old Australians [78], although there is conflicting data on that, with some authors pointing out a publication bias [79].

There are some articles dealing with metabolomic markers for obesity in adults, but to our knowledge, there are currently no studies analyzing the potential influence of early programming factors on the metabolism in this population.

A review published in 2014 resulted in 12 articles looking for associations between metabolite concentrations and obesity [80]. Seven of those were done in adults [80]. The main results indicated changes in the β -oxidation of fatty acids and in the concentration of the BCAA valine, leucine, and isoleucine. BCAA have often been proposed as potential marker for IR, yet this association could be biased by the higher levels of BCAA in overweight and obese subjects.

In a previous study, the association between lipidomics and waist circumference/BMI as well as homeostasis model assessment (HOMA) measuring IR was analyzed in the Western Australian Pregnancy Cohort (Raine) study [81]. This study facilitated the unique opportunity to analyze metabolomics data of more than 1,000 subjects at the age of 20 years who had been followed from birth, after their mothers were enrolled during pregnancy. Data on early feeding and pre-pregnancy parameters, like parental BMI, and socioeconomic status variables, like income and education, were collected. It was found that the phospholipid profile was strongly associated with current waist circumference values and BMI. Especially increased SM levels and decreased LPC levels were associated with higher waist circumference. Associations between LPC, SM, and obesity have also previously been found [54].

A possible explanation for the association of obesity to PC and SM could be lipotoxicity, which has been previously proposed to be potentially programmed early in life [82]. This hypothesis states that with a high-fat western diet, the capacity of the adipocytes to store fat in the form of triacylglycerols (TAG) can be exceeded. Therefore, with high intake, TAGs are stored also in hepatocytes and muscle cells that are not prepared for fat storage, leading to the release of bioactive lipids to protect the cell, but at the same time desensitizing the cell for insulin [83].

Corcoran et al. [84] state that a higher intracellular amount of NEFA, due to overweight and obesity and a diet high in fat is either leading to an increased storage in lipid droplets or to a conversion to various signaling molecules, like PC, SM, and ceramide (Cer). In order to be used as energy source for muscle cells, NEFA have to be converted to long-chain acyl-CoA. Those long-chain acyl-CoA are also a source for messenger lipids like Cer and diacylglycerol. These molecules are involved in the disturbance of the glucose transporter protein and lead to a decreased insulin sensitivity.

Early life factors might indeed modify the risk, but later life factors, like nutrition, physical activity, or even genetic influences, have a more prominent effect on the actual phenotype. In summary, obesity in adults is associated with SM and PC as well as LPC.

Limitations

In general, there are 2 major challenges in investigating the early programming hypotheses on metabolomics outcomes: (1) the (cross-sectional) associations between metabolomic disturbances and NCD have not been entirely mapped yet and (2) the metabolism is also influenced by many other environmental and biological factors including diet, socioeconomic status, physical activity, smoking, sex/gender, and others. Given the lack of longitudinal studies in metabolomics, relatively little is still known about causes and consequences in these associations. Therefore, longitudinal analyses are needed in order to map the metabolic changes before, during, and after the development of the disease phenotype.

There are also methodological and analytical issues in these studies, such as the use of different analytical platforms (MS/MS or NMR), but even more variation can be found in the data analysis performed. Multivariate approaches, like random forest, PCA, or PLS, have been used to distinguish between normal weight and obese in the complete metabolomics data set or to identify underlying “factors” which are a weighted combination of different metabolites that are related to BMI, waist circumference, HOMA, or other outcomes. While univariate methods evaluating the effects of single metabolites on outcomes are rather easily comparable, results of linear or logistic regression models which have been adjusted for different confounders make comparisons and generalizations difficult. Furthermore, the correction methods to adjust for multiple testing vary between the studies and the adjusted *p* values depend on the number of metabolites. Because of the manifold sources of variability in the methodologies, statistical analyses, and time points of interest, we preferred to provide a global overview of the current metabolomics research on early programming rather than to perform a systematic review.

Conclusion

In this review, we point out associations between metabolite concentrations and obesity as well as IR that are already found in early life. LC-PUFA concentrations in

the pregnancy period and later growth have been found to be associated in observational studies, but interventional studies did not show any effect. Associations between HP intake, BCAA, LPC 14:0 concentrations, and early weight gain have been observed in infancy. Metabolomics studies conducted in childhood found BMI associated with LPC, PCae, fatty acids 20:3, 14:0, 22:6, and high BCAA, and IR with BCAA. The onset of puberty as a sensitive developmental period was associated with differences in AA concentrations depending on Tanner stage. Furthermore, metabolic differences between boys and girls of this age mainly exist in LPC and AA, including BCAA (higher in men) and SM (higher in women). In adults, a disturbed lipid metabolism was related to obesity, leading to higher NEFA, acyl-Carn, and polar lipid species in the blood due to an incomplete fatty acid oxidation. Many studies found associations between BCAA, LPC, and LC-PUFA with obesity and IR throughout the life course. The only exception in our review seems to be the intrauterine stage, but this might be due to issues in measuring the transfer of nutrients from the mother to the fetus. Although we have outlined the importance of an often neglected factor, namely gender, many other largely ignored factors, such as ethnicity or socioeconomic status, might also result in distortions.

In summary, research on early programming of the metabolome and metabolic pathways is at an early stage, but offers great future opportunities. It is neither fully understood how the metabolism underlying non-communicable metabolic disorders is disturbed, nor how it is af-

fected by early programming factors. Furthermore, depending on the platform and technique used for the quantification, the number of metabolites in the data set can differ. Thus, missing associations between metabolite and phenotype found in another study could be due to the fact that relevant metabolites were not quantified.

Despite these caveats, the strength and potential of metabolomics in the context of early programming is in leading the way to targeted early prevention strategies. Once the molecular mechanisms that ultimately lead to the onset of NCD are identified, one may be able to identify early biomarkers and to develop individualized prevention strategies.

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