Qualitative End Report for Centre for Fetal Programming (CFP)

The Danish Council for Strategic Research – now Innovation Fund Denmark – funded the Centre for Fetal Programming (CFP), with Dr. SF Olsen as the Centre Leader and a Centre secretariat based at Statens Serum Institut in Copenhagen. CFP was initiated in January 2010 with an overall aim to study a range of fetal programming phenomena. While 'fetal programming'[1] is broadly understood as a concept that connects factors operating in fetal life to the individual's later health and development, CFP had particular focus on studying the effects of two types of factors operating in fetal life: exposure to maternal hyperglycemia (gestational diabetes) and to maternal nutritional exposures (including food born toxicants).

The present report – requested by Innovation Fund Denmark – gives a narrative account of CFP's main results and activities, whereas listings of published articles etc. are provided in CFP's quantitative report for Innovation Fund Denmark. It is structured according to the Fund's guidelines for the qualitative report.

RESEARCH RESULTS

CFP was built around three pillars, corresponding to its three disciplinary approaches to studying fetal programming: Epidemiology, Clinical Physiology, and Animal Experiments. In the account below, results from the Epidemiology and the Clinical Physiology pillars will be reported under a common heading as these studies were integrated, whereas results from the Experimental pillar will be reported separately.

EPIDEMIOLOGY / CLINICAL PHYSIOLOGY

The results from these pillars were based on several different study resources. One was an observational follow-up study of children born to 965 women, who had their diet assessed and gave blood samples during their pregnancies, and who delivered in the years 1988-1989. Another was a follow-up study of children born to 533 women, who had been randomized to receive fish oil or a control preparation during their pregnancies, and who delivered in 1990. These two birth cohorts were clinically examined two decades later when the children were 19-20 years old, and were also followed through registries. A third study resource was the Danish National Birth Cohort (DNBC), consisting of around 90,000 live-born children, whose mothers had their diet assessed and gave blood samples during their pregnancies and delivered in the years 1996-2003. While DNBC is followed through questionnaires and registries, a specific sub-cohort of DNBC – consisting of approximately 1200 children (half of the children had mothers who had developed gestational diabetes and the other half experienced a pregnancy without gestational diabetes) who underwent a clinical examination program when they were 9-16 years old. Still another study resource was a trial conducted in China among 5531 women, who were randomized to receive two different dosages of fish oil or a control preparation during pregnancy.

Fetal programming of cardio-metabolic health Results from our examinations of the 19-20 year old children led us to conclude that a high protein intake in pregnancy may lead to higher blood pressure, and that exposure to persistent organochlorine pollutants ('POPs') in fetal life may lead to overweight in young adulthood. On the other hand, our results could not support our expectations regarding a beneficial impact of long chain n-3 fatty acids in pregnancy, nor regarding an adverse impact of low vitamin D status in pregnancy, on overweight, hypertension and blood lipids assessed in the young adults. – Results from the DNBC sub-cohort showed increased adiposity, an adverse cardio-metabolic profile and, among girls, earlier

[1] A further elaboration on the definition of the term 'fetal programming' can be found in this article written by CFP's leading team and published in this theme issue on Fetal Programming of *Acta Obstetricia Gynecologica Scandinavica*.

onset of puberty in the children whose mothers had had gestational diabetes. Exposure to gestational diabetes was also associated with DNA methylation changes in the 9-16 year old children, supporting the notion that epigenetic mechanisms could possibly mediate effects of gestational diabetes, and, in girls, with shorter telomere lengths, which could possibly reflect cell damage that had already taken place in fetal life. In the sub-group with gestational diabetes, associations were observed between fasting plasma glucose concentrations during pregnancy and adiposity measures in the child assessed at ages 1.0, 1.5 and 7 years.

Programming of asthma and allergies Our follow up through registries over more than two decades of the 533 children, whose mothers had participated in the randomized *Danish trial with fish oil*, provided strong support for the hypothesis that intake of long chain n-3 fatty acids in the latter half of pregnancy, reduces the child's risk of developing asthma and allergies. However, we could detect no association between low vitamin D status in pregnancy and child's risk of developing asthma and allergies during the first two decades of life. In DNBC, peanut and nut exposure in pregnancy was found to be associated with lower risk of offspring asthma and allergies, whereas low fat yoghurt intake was identified as a risk factor. Preliminary results indicated (consistent with results from the trial data) that higher fish consumption during pregnancy protects against child asthma development, whereas intake of artificially-sweetened beverages in pregnancy was modestly associated with increased risk of child asthma and allergic rhinitis.

Programming of brain health Fetal exposures to perflourinated compounds, polychlorinated biphenyls ('PCBs'), or pesticides such as dichlorodiphenyldichloroethylene (a degradation product of DDT) and hexachlorbenzen (a fungicide formerly used as a seed treatment), were not associated with risks of cognitive or mental health problems within the first 20y of life. In the same cohort analyses based on biomarker measurement of 25(OH)D of blood samples drawn during pregnancy combined with long-term follow-up showed no support for a beneficial fetal programming effect of vitamin D status with regard to behavioral and affective disorders and scholastic achievement. In DNBC, breastfeeding and maternal intake of long chain n-3 fatty acids as well as dietary intake of iron, but not iron from supplements, were positively associated with offspring IQ measured at the age of 5 years.

Programming of reproductive health Prenatal exposure to persistent organochlorine pollutants ('POPs') in pregnancy was associated with poorer semen quality and reproductive hormone profile measures in men and fewer ovarian follicles in women, assessed at age 20. Fetal exposure to maternal smoking in pregnancy was associated with earlier age of menarche.

Programming of growth We found intake of milk in pregnancy to be associated with greater infant weight and length at the time of birth, and a propensity for greater weight and height in young adulthood, with higher maternal milk intake in pregnancy.

Programming of type 1 diabetes We found that maternal gluten intake during pregnancy was associated with the subsequent risk of their offspring developing type 1 diabetes, with a twofold risk in the offspring when comparing mothers who had the highest versus lowest gluten intakes ($\geq$20 v. <7 g/day). Moreover, our data suggested that smoking in pregnancy protects the offspring against type 1 diabetes, but we could detect no association between vitamin D status in pregnancy or at birth, and child's type 1 diabetes risk.

Programming of bone health Against the expectation, we could detect no association between low vitamin D status in pregnancy and fracture risk (a measure of bone fragility) during childhood. This was the case both when we used biomarker measurement of 25(OH)D of blood samples drawn during pregnancy combined with long-term follow-up and when we used a vitamin D prediction score in DNBC. Also in the DNBC, high mid-pregnancy consumption of Western diet and artificially sweetened soft drinks, respectively, indicated positive associations with offspring forearm fractures.

Maternal diet and pregnancy outcomes CFP participated in a Cochrane review based on published randomized controlled trials, which concluded that supplementation with long chain n-3 fatty acids is an effective strategy for reducing the incidence of preterm birth. Although our randomized *Chinese trial with fish oil* could lend no conclusive support for this notion, there were data in the trial to suggest potentially clinically important delays of 5-50 days in timing of delivery during n-3 fatty acid supplementation, but also that this effect seemed to cease rapidly after stopping supplementation. Furthermore, high concentrations of plasma ferritin in early pregnancy – which may reflect iron overload – were found to be associated with increased risk of developing gestational diabetes.

Developments and validations of research resources For the fish oil trial in China, 5531 women were recruited and randomized to three different dosages of marine n-3 fatty acids; in specific regions, where the trial had been conducted, postpartum follow-up for cognitive and immunological outcomes in women and children has now been initiated. For DNBC, based on information from interviews and registries in combination with detailed reviews of 3633 hospital records, we were able – in close collaboration with clinicians – to verify a gestational diabetes diagnosis according to standardized criteria in 783 DNBC pregnancies; this information will be important for future DNBC studies of causes and consequences of gestational diabetes. Further, for DNBC, a comparison was undertaken between three different methods/data sources to ascertain asthma: self-report via web-based questionnaire, diagnoses extracted from the National Patient Registry, and drug prescriptions recorded in the Medicinal Products Registry; the study revealed a substantial non-overlap (with kappa coefficients for agreement ranging from 0.21 to 0.38) between cases identified by the three methods. For DNBC and the Norwegian Mother-Child Cohort (MoBa), the two research groups developed and agreed upon – and published – a common definition of food groups; this data harmonization is important, as it will enable coordinated and pooled analyses based on dietary data from the two cohorts. Furthermore, a common structure for a dietary questionnaire for 14-year-olds was developed and agreed upon between the two groups, for usage in both cohorts; the questionnaires were tested and validated for certain aspects in both countries. Around 34,000 14-year-old children have completed the questionnaire in DNBC, and analyses based on these data have already yielded important results. One analysis showed that maternal diet quality during mid-pregnancy is associated with diet quality in the offspring at age 14 years, i.e. 14-15 years later, and another that low birth weight children tend to have a poorer diet quality at 14 years than children born with a normal birth weight. Both of these findings will have potential implications for future observational studies of dietary etiologies of diseases postulated to have fetal origins such as type 2 diabetes, obesity and asthma.

ANIMAL EXPERIMENTS

This pillar employs a number of sophisticated animal, organ and cellular models. Impact of malnutrition during late gestation on development of metabolic and endocrine disorders in a life-time perspective, and the interactions with dietary exposures in early postnatal life, was studied in the *Copenhagen sheep model*. I this model, twin-carrying sheep are exposed to either over-nutrition or undernutrition in late gestation; after birth, offspring are exposed to either a normal diet or an extreme high-carbohydrate-high-fat diet. Under the CFP grant the model has been thoroughly validated and results included that prenatal undernutrition has long-term effects on several endocrine systems (including the glucose-insulin and thyroid hormone axes), fat deposition patterns, subcutaneous adipose tissue and hepatic lipid profiles; and that postnatal nutrition has strong, but reversible effects. The studies supported that differential thyroid hormone signaling in adipose v. other tissues may be part of a mechanism whereby fetal malnutrition can predispose for obesity and other metabolic disorder; that a maternal high-carbohydrate-high-fat diet

interferes with offspring beta-cell function, whereas maternal undernutrition does not lead to any changes in offspring of mothers fed a low energy and protein diet, suggesting that fetal programming interferes with hypothalamic integration of important endocrine axis; and that maintenance of glucose tolerance in sheep exposed to pre-natal undernutrition relies on pancreatic hypersecretion of insulin to compensate for reduced insulin sensitivity. Studies using this model also provided support for a role of subcutaneous adipose tissue in the development of visceral adiposity, which in adult humans is known to precede the development of the metabolic syndrome. They further revealed that maintenance of glucose tolerance in sheep exposed to pre-natal undernutrition relied on pancreatic hypersecretion of insulin to compensate for reduced insulin sensitivity; and that early post-natal, but not late pre-natal diet, impacts on glucose-insulin homoeostasis could be reversed by dietary correction later in life. Rodent studies supported the hypothesis that the reduced beta cell mass in low protein offspring is caused by a change in the intra-uterine environment that favors premature maturation of the beta-cells, and suggested that long-term taurine supplementation improves glucose tolerance and normalizes hepatic triglyceride content following long-term fructose feeding. Comparison of gene expression in perinatal pancreas in offspring of pregnant rodents fed low protein diet compared with normal chow, identified that reduced expression of prolactin receptor, growth hormone receptor, neurogenin-3, and Gas-6 that may be involved in beta-cell differentiation and growth. Serum from pregnant women was shown to stimulate human beta-cell proliferation and increase the expression of markers of beta-cell differentiation in late fetal rat pancreas, suggesting the presence of factors that promote beta-cell neogenesis. Protein fractionation of serum from pregnant women identified a number of peptides derived from known proteins, including alpha-1 antitrypsin, apolipoprotein A1, angiotensinogen, and HM kininogen implicated in beta-cell growth, survival and function. Employing a rodent model of obesity prior to and during pregnancy, no evidence was found of altered myometrial mitochondrial function or morphology following obesity.

INDUSTRIAL AND SOCIETAL RESULTS

Representatives from the Danish Food Administration and National Board of Health were invited to and participated in most of our bi-annual center meetings; the authorities were thereby currently and directly, albeit informally, informed about all new results. Dr. SF Olsen was member of one of the expert groups – notably, the Pregnancy and Lactation Group – who revised the Nordic Nutrition Recommendations (NNR5). CFP team organized, in 2013 and 2014, two international symposia on 'Fetal Programming', both of which took place in Novo Nordic Foundation's Auditorium in Copenhagen, and with >100 participants each time; see program and abstracts from the 1st symposium; and program of the 2nd symposium. Results from CFP were presented and discussed, for both symposia, researchers were broadly invited to ensure dissemination of important results obtained in CFP to the wider scientific community. As regards the potential translational value for society of results based on the Centre's research, the following is of particular note: Centre research contributed significantly to substantiate the notion that increasing the intake of long chain n-3 fatty acids in pregnancy – in women with a beforehand low intake of long chain n-3 fatty acids – may help prevent preterm birth; and, also, that it may help prevent asthma in the offspring.

RESEARCH EDUCATION

As shown in the quantitative report, the CFP grant has helped finance 18 PhD and 5 Post Doc stipends, in which fetal programming research formed part of the research training. The Centre meetings and symposia, as well as international conferences, served as excellent fora where CFP PhD students and Post Docs got a chance to have their ideas and results presented and challenged by audiences consisting of

researchers – junior as well as senior – with highly diverse skills, which usually included epidemiologists, physiologists, clinicians, nutritional experts, as well as experts in animal and other types of lab experiments. SF Olsen taught topics related to fetal programming at courses at University of Copenhagen and Harvard School of Public Health in Boston, and he wrote chapters on fetal programming for teaching books (see pp. 64-99 in ISBN 978-87-628-1458-5, and pp. 362-370 in ISBN 978-87-628-0884-3). CFP enjoyed long term visits by three Harvard doctoral students, one of whom became employed as a post doc in CFP. Continued teaching and research in this field, at termination of CFP, has been secured by University of Copenhagen's recent appointment of SF Olsen as professor of epidemiology with special focus on fetal programming research.

CROSS-INSTITUTIONAL, INTERDISCIPLINARY, AND INTERNATIONAL COOPERATION

Cross-Institutional and interdisciplinary collaboration A fundamental challenge in such cooperation is to overcome barriers in understanding each other's discipline-specific language and methodological approaches. The Centre meetings have been the primary instruments for interaction between institutions and disciplines in CFP. Five biannual Centre meetings have been held, which all, in different ways, strongly emphasized interaction and breaking down barriers in mutual understanding of methodological approaches, with the aim to facilitate 'cross-fertilization'. Examples of such main topics for different bi-annual meetings included: *1. Detailed overviews of research agendas presented by the main partners. 2. Lectures by senior scientists from each pillar on methods and basic ways of thinking in their respective disciplines. 3. Poster session, where results were discussed afterwards in plenum. 4. Expert talks on dietary recommendations relevant to the fetal programming field, and on principles underlying generating and developing official dietary and nutritional recommendations by health and food authorities. 5. Overviews results from each pillar.* In addition, journal club meetings and specific project meetings were held, where participants from different institutions representing different disciplines participated. The experience was that barriers of understanding and communication were reduced, and that discussions at our biannual meetings became freer with more efficient exchange of ideas and opinions across disciplines. The synergy that came of this contributed to the development of several new project ideas. Examples of interdisciplinary 'cross-fertilization', include work on 'gluten and offspring T1D' [30232082] (undertaken by epidemiologists with researchers doing animal experiments) and 'protein and offspring metabolic health' [28679553] (undertaken by epidemiologists with researchers doing clinical physiology).

International collaboration Several meetings were held at Harvard School of Public Health between Dr. W Willett's group and researchers from CFP, and between investigators from the dietary groups of DNBC and the Norwegian MoBa cohort. Meetings were also held with another large cohort, the Japan Environment Children's Study (JECS). In connection with the large trial in China, several site visits were undertaken, and Prof. W Zhou and Dr. L Min, visited CFP. These interactions, facilitated by the Centre grant, were of paramount importance for the success of several of the main projects in the Centre's portfolio, as well as for identifying new collaborative projects and grant opportunities.

Concluding remark Research in the Centre opened novel avenues for fetal programming research. Several of these will be continued, and further developed, in the new professorship in epidemiology with special focus on fetal programming established at University of Copenhagen at the termination of the Centre.

Statens Serum Institut, 22 January 2020
Sjurdur Frodi Olsen, professor of epidemiology with special focus on fetal programming