Qualitative Report for the Period 1 Jan 2010 to 30 June 2012

Introduction The Centre for Fetal Programming (CFP) was the result of a combination of two phase I applications [from Drs. SF Olsen and MO Nielsen, whose disciplinary approaches were mainly EPIDEMIOLOGY and ANIMAL EXPERIMENTS, respectively] and an already existing network [where the approach was mainly CLINICAL PHYSIOLOGY], all of which dealt with fetal programming research. Based on these three structures, The Danish Council for Strategic Research (DCSR) funded CFP with a total of 34mil (reduced from 40+40+0 in the phase II application) DKK. Consequently, CFP was established with THREE PILLARS corresponding to the three disciplinary approaches mentioned; with Dr. SF Olsen as the Centre Leader and Dr. MO Nielsen as Vice-Centre Leader; and with a secretariat at Statens Serum Institut. A Steering Committee was also established–currently composed of Drs. BH Bech, I Bygbjerg, TB Henriksen, M Melbye, KF Michaelsen, JH Nielsen, MO Nielsen, SF Olsen (Chair), K Raun, B Quistorff, and A Vaag–as well as a Scientific Advisory Board, composed of Drs. F Bloomfield, MA Gillman, B Koletzko, and SE Ozanne.

DESCRIBE AND EVALUATE ACTIVITIES ADDRESSING: *RESEARCH RESULTS*

The account below is structured according to the three main disciplinary pillars of CFP

I.EPIDEMIOLOGY This pillar is based on several unique prospective databases, e.g. children born to 965 pregnant women who had their diet assessed, gave blood samples and delivered in 1988-9; children from a trial with 533 pregnant women who were randomized to fish oil and delivered in 1990; and the Danish National Birth Cohort (DNBC), with dietary data from 70,000 pregnant women who delivered in 1996-2003.

Programming of outcomes related to metabolic syndrome In the trial, with follow up over two decades, supplementation with fish oil in the third trimester of pregnancy was found to have no impact on markers of metabolic syndrome in the young adult offspring. In the observational study from 1988-89, with similarly long follow-up, fetal exposure to perflourinated compounds was found to be associated with increased obesity propensity in young adult women; studies are underway of impact of physical activity, dietary glycemic index, and other nutritional and life style factors in pregnancy on markers of metabolic syndrome in young adult offspring. Preliminary results from DNBC suggest that iron intake, particularly from read meat, is a risk factor for developing gestational diabetes (GDM).

Programming of other outcomes: the 'broader programming hypothesis' *Asthma and allergies:* Fetal exposure to PCBs was found to be associated with increased risk of asthma in a follow up over two decades, whereas a preliminary study has replicated earlier findings that supplementation with fish oil in pregnancy reduces offspring asthma risk. 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; and preliminary results indicated that higher fish consumption during pregnancy protects against child asthma development, consistent with results from earlier trial data, whereas daily (v. no) intake of artificially-sweetened beverages in pregnancy modestly increased risk of child asthma and allergic rhinitis. *Reproductive health:* Fetal exposure to perflourinated compounds was associated with poorer semen quality and reproductive hormone profile measures in young men. Fetal exposure to maternal smoking in pregnancy was associated with earlier age of menarche in young women. *Mental health:* Preliminary findings suggest that fetal exposures to perflourinated compounds, PCBs or pesticides such as pp_dde and HCB are not associated with risks of cognitive or mental health problems within the first 20y of life; whereas fish oil supplementation in pregnancy was associated with less risk of depression in the

offspring; and, in DNBC, breastfeeding and maternal intake of n-3 PUFA as well as dietary intake of iron, but not iron from supplements, were positively associated with offspring IQ measured at the age of 5. *Other offspring health outcomes:* Studies are ongoing of associations between dietary factors in pregnancy and risks of bone fracture (reflecting bone fragility), type 1 diabetes, and cancer in the offspring.

Data and materials developments More than 3000 hospital records have been reviewed to identify participants in DNBC who had gestational diabetes in the index pregnancy. A comparison was undertaken between three different methods/data sources to ascertain asthma in epidemiological studies: self-report via web-based questionnaire, diagnoses extracted from the National Patient Registry, and drug prescriptions recorded in the Medicinal Products Registry. A common definition of food groups was developed and agreed upon that can be applied to maternal dietary data from DNBC and the Norwegian Mother-Child Cohort (MoBa); this will facilitate coordinated and pooled analyses based on dietary data from the two cohorts. A common structure for a dietary questionnaire for 14 year olds has been developed and agreed upon to be used in both cohorts; the questionnaires were tested and validated for certain aspects in both countries. For the fish oil trial in China, 5000 women have been randomized; dietary data from women recruited in the Provinces of Gan-Su and Siaan-Xi have been made available for descriptive and comparative analyses; and, in specific regions, postpartum follow-up of women and children has been initiated.

II.ANIMAL EXPERIMENTS This pillar employs a number of sophisticated animal, organ and cellular models.

Sheep studies 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*. Twin-carrying sheep are exposed to either overnutrition or undernutrition in late gestation; after birth offspring are exposed to either a normal diet or an extreme high-carbohydrate-high-fat diet. The model has been thoroughly validated and results include 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. A large study is in progress but not yet completed, where sheep are reared until puberty (at 6 months) and further until young adulthood (at 2 years, i.e. in 2013), enabling assessment of age-dependent manifestations of fetal programming. Furthermore, in another model employing intravascular infusions during late gestations—conducted in collaboration with Liggins Research Centre, NZ—fetal intervention studies have been undertaken with metabolites hypothesized capable of interfering with fetal programming effectuated by diet; results are still pending due to maternity leave.

Rat studies—impact of gestational and lactation overnutrition on lipid metabolism and inflammation This rat model compared a high fat+sucrose diet to a regular diet during gestation and/or during lactation, and from postnatal weeks 21 to 26, the rats underwent high fat challenge. Overnutrition throughout gestation and lactation resulted in reduced delta6-desaturase and elongase activities at 4 and 26 postnatal weeks, as reflected in changed fatty acid compositions in plasma lipids and triacylglycerols. At 26 postnatal weeks, higher levels of free fatty acids were also observed in the liver. Part of the patterns could be explained by altered gene expression. Furthermore, overnutrition led to decreased concentrations of medium-chained fatty acids in the breast milk. It is hypothesized that the resulting reduced exposure of the newborn liver to fatty acids could alter gene expressions implicated in fatty acid metabolism. Further studies will focus on immunological markers and genes in adipose inflammatory response.

Rat studies—impact of gestational overnutrition on metabolic function During gestation and lactation rats were fed either regular rat chow or a diet with access to chocolate and sucrose-rich drinks. After weaning, offspring from each group continued on chow, or the chow plus chocolate diet, until 26 weeks of age. Results based on metabolic parameters measured in plasma indicated that a prenatal high fat diet may be protective against metabolic negative consequences of a high fat load in post natal life up to 26 weeks of age. Further studies will focus on expression of metabolic pathways in the liver and leptin pathways in the brain. A focus point will be to examine if the apparent protection against negative effects of high fat diet in postnatal life in offspring from high fat mothers will last throughout life; or if rats as they age get higher risk of metabolic dysfunction due to the high fat feeding.

Mouse studies—role of taurine In earlier studies taurine supplementation had been found to rescue up to 40% of significant gene expression changes in liver and muscle observed after fetal programming by a low gestational protein. Further studies are underway employing a taurine uptake inhibitor. Preliminary data indicate that low protein influences cell differentiation mechanisms.

Mitochondrial studies The Goto Kakisaki rat is a recognized model for type 2 diabetes (T2DM). These rats develop T2DM as they age and it is therefore possible to study the important question of how the progress of T2DM affects the metabolism of the animal and in particular the temporal involvement of the organs in the disease process. With this model CFP investigators have now shown, for the first time, that liver mitochondria is seriously involved at an early stage of the T2DM development–at a time where skeletal muscle mitochondria are still phenotypically normal. The involvement of the liver mitochondria is displayed as a significantly decrease of the TCA flux progressing with the development of the T2DM. The succinate dehydrogenase flux is strongly inhibited with fatty acid substrate but not by carbohydrate substrate, and we are suggesting an intricate redox control as the key mechanism. This finding, if present also in human beings, may explain the dyslipidaemia which has been suggested as the cause of insulin resistance in skeletal muscle by other studies. In other words, the early metabolic defect of T2DM, and the role of the liver as the prime site of action, may have been uncovered.

These studies have been followed up by experiments on normal rats showing that the same type of mitochondrial defect can be induced by a high carbohydrate diet early in life, and interestingly, that this defect is strongly influenced by fetal programming as it is induced by gestational low protein diet fed to the mothers. The CFP investigators think they may now have discovered a functional biochemical marker of fetal programming as well as of early T2DM in the specific dysfunction of liver mitochondria.

Further studies of fetal programming mechanisms—endocrine pancreas Comparison of gene expression in perinatal pancreas in offspring of pregnant rats 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. Neonatal pancreas from offspring of pregnant rats fed chocolate (mentioned above) and DIO (Diet-Induced Obese) rats have been collected and are currently being analyzed for gene expression. 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.

Mouse studies—programming of mammary function In intrauterine and juvenile overfeeding mice models, it was planned to study expression of adipocytokines, aromatase, and other markers relating to

altered milk composition previously seen in rats, as well as cell proliferation and apoptosis in mammary tissue. The studies have not yet been done due to technical issues, but the colony is now established and the work will proceed during the rest of 2012 and the first half of 2013.

III.CLINICAL PHYSIOLOGY As described in the Introduction, during the application phase, DCSR encouraged inclusion of the ongoing strategic research network, "Global Fetal and Maternal Care in Prevention of Adult Non-Communicable Disease, DSRC grant no 2101-07-0025", in the construction of CFP. Because the approach of the network was mainly clinical-physiological, it would constitute the CLINICAL PHYSIOLOGY PILLAR, i.e. CFPs 3rd pillar. Here, we report on new developments which have happened in this pillar as part of CFP activities (whereas, notably, results from the network as such do generally not form part of the present report; they have been thoroughly reported in the final DCSR report from the network, which formally ended after four years, on 31 December 2011):

Right from the inception of CFP a close interaction took place between the EPIDEMIOLOGY and CLINICAL PHYSIOLOGY PILLARS. During Dr. SF Olsen's 3m sabbatical at Harvard School of Public Health in 2010, an opportunity arose to participate in a collaborative project between the Nurses Health Study II (NHSII) and DNBC. Women participating in NHSII and DNBC, who had had GDM in a pregnancy, would be examined for development of type 2 diabetes ten years or more after the pregnancy. This project, which on the Danish side is a collaboration between Drs. A Vaag and SF Olsen, obtained funding from NIH/NICHD in late 2010 (7mil. DKK for the Danish component). In 2011 the two investigators were successful in raising further funds (11 mil. DKK from DCSR) making it possible to also undertake physical examinations of the children of the GDM mothers, as well as of a group of children born to mothers with a normal pregnancy. The physical examinations program, which includes clinical physiological assessments of early markers of metabolic syndrome in the child, was initiated in spring 2012 and will continue throughout 2013. The aim is to explore in detail the already established programming role of GDM in the development of the metabolic syndrome in the offspring, as well as the potential modifying roles of maternal and offspring diet in this relation and any possible underlying epigenetic mechanisms.

DESCRIBE AND EVALUATE ACTIVITIES ADDRESSING: *INDUSTRIAL AND SOCIETAL RESULTS*

Representatives from the Danish Food Administration and National Board of Health were invited to and participated in all our bi-annual center meetings; the authorities are thereby currently and directly, albeit informally, informed about any new results. Dr. SF Olsen is member of one of the expert groups (notably, the Pregnancy and Lactation Group), which are working on revising the Nordic Nutrition Recommendations (NNR5). Meetings, which will be open to researchers and the public, are under planning to take place during spring 2013, where results from CFPs first three years will be presented and discussed; this will ensure dissemination of pertinent results obtained in CFP to the wider scientific community and the public.

DESCRIBE AND EVALUATE ACTIVITIES ADDRESSING: *RESEARCH EDUCATION*

As shown in the quantitative report, the first years of CFP have yielded, or contributed to, several PhDs (around 10) and CFP has so far been able to retain several PhDs (5) to continue as post docs. Great efforts have been made in raising funds for the next period of CFP (as apparent from the long list of applications in the report form), which is needed to maintain this success. The course, "Developmental programming of metabolism and behaviour", organised by Dr Vaag, was held in Copenhagen 1-4 Febr 2011.

DESCRIBE AND EVALUATE ACTIVITIES ADDRESSING: *COLLABORATION, INCLUDING 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'. The first bi-annual meeting detailed overviews of research agendas of the main partners; in the second, senior scientists from each pillar gave lectures on methods and basic ways of thinking in their respective disciplines; in the third, a poster session was held where main results were discussed afterwards in plenum; in the fourth, expert talks were given 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; in the fifth meeting, overviews of the most important results from each pillar were presented and discussed. In addition, journal club meetings and specific project meetings have been held where participants from different institutions representing different disciplines participated and a post doc group, with participants from different disciplines, will plan and organize the next CFP meeting. During these first 2 ½ years of CFPs life we have indeed experienced that these barriers of understanding and communication have been reduced and that discussions at our biannual meetings have become freer with more efficient exchange of ideas and opinions across disciplines; this was also noted and pointed out by CFPs Scientific Advisory Board in their evaluation of our June 2012 meeting. The synergy that has come of this has contributed to the development of several new project ideas that we wish to pursue in CFPs next phase.

International collaboration Two meetings have been held at Harvard School of Public Health between Dr W Willett's group and researchers from CFP; and CFP has had visits by two Harvard doctoral students, of whom one is now employed as a post doc in CFP and will be part of the newly formed post doc group. Several meetings have been held between investigators from the dietary groups of DNBC and the Norwegian MoBa cohort. The above mentioned project, with coordinated follow-up of participants in the US NHSII and the Danish DNBC, represents a major new international collaborative effort of CFP. Site visits have been undertaken in China, and the PI of the Chinese trial, Dr W Zhou, has, together with a research assistant, visited CFP. A close interaction has also taken place between CFP and The Liggins Research Centre, NZ.

We are grateful that DSCF funded CFP and are committed to continue our efforts to examine and understand fetal programming phenomena in determining health and well-being, hopefully to the benefit of generations to come.

11 September 2012

On behalf of CFP

Yours sincerely

Sjurdur F Olsen, Leader of Centre for Fetal Programming