Project Final Report

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DORIAN

Logo: 

Project title: Developmental ORIgins of healthy and unhealthy AgeiNg: the role of maternal obesity

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1.1 Executive summary

Obesity has reached epidemic proportions and is the main health and social concern in the developed world and, increasingly, in developing countries. It affects a growing proportion of women of reproductive age. The intrauterine environment affects organ and tissue development and subsequent disease susceptibility during adult life. Our project aimed at linking studies of early developmental processes with those of ageing from a life course perspective. We hypothesized that; as a consequence of maternal obesity the offspring could be predisposed to develop cardiovascular disease, type 2 diabetes and other chronic diseases during their life-course. The project focused on the involvement of insulin resistance and glucocorticoid overexposure, oxidative stress, telomere shortening, epigenetic changes, and social stress to promote disease susceptibility during the life course. We have used human and translational preclinical research to identify pathways that lead to the development of age-related disorders, recognize predictive biomarkers and develop strategies to intervene. Our findings document that maternal gestational metabolism influences the offspring metabolic and hormonal profile at birth or during early post-natal life, and insulin resistance and glucocorticoid metabolism may modulate the duration of gestation, and subsequent fetal and infant size/growth. Maternal obesity has deleterious effects on maternal and offspring behaviour, metabolism and neuroendocrine profiles during adulthood and ageing. In adult offspring, maternal obesity is an independent predictor for common non-communicable diseases and ageing related outcomes, especially cardiovascular disease (CVD) and type 2 diabetes (T2D) in humans, showing some gender differences. Resistance to oxidative stress attenuates, and chronic social stress during post-natal life deteriorates the effects of maternal obesity on the health of the offspring. We observed gene environment interactions involving DNA methylation and DNA ageing, as many genes were found to be differentially methylated in neonates born to obese versus lean mothers, and a higher maternal BMI with excessive weight gain during pregnancy resulted in shorter telomere length in the offspring, as observed both at birth and in adult offspring. Our findings highlight that a healthy lifestyle through life can partially restore the abnormalities observed. We showed beneficial effects of maternal weight loss or antioxidant therapy over the health of the offspring. We noted that subjects born to obese mothers have lower proneness to physical activity, and we documented the beneficial effects of exercise training in the adult offspring of overweight mothers. We have identified one additional potential therapeutic target to prevent the above adverse effects, namely the glucocorticoid responsive gene, FKBP51. Overall, the results of this project have generated a better understanding of the basic mechanisms of early life development and ageing, calling for strategies that could translate into the improvement of health and quality of life during the life course. This project opens the perspective to diagnose the individual at risk by early biomarkers and prevent or treat later consequences of maternal obesity, i.e. reduce the burden of unhealthy ageing in Europe and promote the European industrial sector. The epidemics and the harmfulness of maternal obesity underscore the expected social and health-care impact of the efforts of this Consortium. Our project calls for attention to mental wellbeing in mothers and offspring, strategies to improve dietary practices in mothers before conception, careful gestation planning, and promotion of physical activity. Recognizing that research becomes successful only by sharing results and meanings with end-users, together with our dissemination partners (EASO, MMM) we recently disseminated a press releases which attracted massive media attention.

1.2 Summary description of project context and objectives

Background and Aims

Obesity has reached epidemic proportions and is the main health and social concern in the developed world and, increasingly, in developing countries. It affects a growing proportion of women of reproductive age. The concept of fetal programming, i.e. that an insult during organ and tissue development leads to permanent disease susceptibility during adult life, shares similarities with ageing, as both conditions are characterized by a limited capacity to compensate and repair, due to system exhaustion in ageing and system immaturity in fetal programming. Healthy pregnancy is a state of insulin resistance (IR), low-grade inflammation, visceral fat accumulation, dyslipidaemia and prothrombosis. The developing fetus is protected against the action of maternal insulin and glucocorticoids (GC). The limited data so far available in humans indicate that the fetus of obese mothers is exposed to higher levels of insulin, due to maternal IR, increasing neonatal fat mass, perhaps leading to IR also in the offspring. The placenta in obese mothers expresses higher levels of proinflammatory mediators, and dysfunctional adipokine and vascular patterns. Overall, gestational IR and GC overexposure would favour hyperglycaemia, relative fat excess, greater birth weight and higher blood pressure in the offspring, which are recognized risk factors for cardiovascular disease (CVD), type 2 diabetes (T2D) and cognitive impairment.

The general objectives of the DORIAN project were to:

• Ob.1: establish if maternal obesity is an independent predictor for common non-communicable diseases and ageing related outcomes, focussing on CVD, T2D, cognitive decline and frailty (risk of premature ageing) in humans by discovering and measuring biomarkers and occurrence of unhealthy ageing and disease

• Ob.2: establish if the effects of maternal obesity on unhealthy ageing of the offspring differ between genders

• Ob.3: demonstrate that human newborn/infants of obese mothers carry hallmarks of premature ageing, and to explore the potential underlying mechanisms involved

• Ob.4: exploit targeted animal models of maternal obesity (separate from post-natal overnutrition) to test mechanistic hypotheses of its effects on disease pathogenesis, senescence and lifespan

• Ob.5: use these mechanistic studies (Ob.3-Ob.4) to discover and develop novel human biomarkers of vulnerability and possible early primary prevention and therapy interventions

• Ob.6: determine the roles of gene-environment interactions and of innate genetic variability on the outcome of maternal obesity, and on the efficacy of the therapeutic strategies evaluated under Ob.5

• Ob.7: promote knowledge exchange and develop public understanding and a health promotion program

Work strategy and general description

Our project aimed at linking studies of early developmental processes with those of ageing from a life course perspective. We hypothesized that; as a consequence of maternal obesity the offspring will present early disease features, predisposing them to CVD, T2D and cognitive impairment. The project focused on the involvement of IR and GC overexposure, oxidative stress/inflammation, telomere shortening, epigenetic changes, interacting with social stress to promote disease susceptibility during the life course. Results of this project have generated a better understanding of the basic mechanisms of early life development and ageing, calling for strategies that could translate into the improvement of health and quality of life during the life course. We have used human and translational preclinical research to identify pathways that lead to the development of age-related disorders, recognize predictive biomarkers and develop strategies to intervene. This project opens the perspective to diagnose the individual at risk by early biomarkers and prevent or treat later consequences of maternal obesity, i.e. reduce the burden of unhealthy ageing in Europe. The epidemics and the harmfulness of maternal obesity underscore the expected social and health-care impact of the efforts of this Consortium. DORIAN has integrated human clinical projects with research focussing upon basic mechanisms. It comprised four research work packages (WPs). There were two additional WPs: WP5 was dedicated to dissemination and WP6 to management. The Figure below is the pert diagram of DORIAN, illustrating the interconnection between six work packages.

Management structure and procedures

The Project Coordinator ensured the smooth operation of the project and guaranteed that all efforts were focused towards the objectives. She submitted all required progress reports, deliverables, financial statements to the European Commission, and, with the assistance of GABO:mi she was responsible for the proper use of funds and their transfers to participants. The DORIAN office was established by and based at the coordinator in CNR, Pisa and at GABO:mi in Munich. The Project Office at the Coordinator was concerned with the scientific management and the co-ordination of all research activities. The Project Office at GABO:mi was responsible for administrative, financial and contractual management and the organisational co-ordination of the project activities.

The General Assembly was in charge of the political and strategic orientation of the project and acted as the arbitration body. It met once a year unless the interest of the project required intermediate meetings. The Project Steering Committee consisted of all work package leaders and the Coordinator and was in charge of monitoring all activities towards the objective of the project in order to deliver as promised, in due time and in the budget. The Steering Committee met via phone conference, approximately every six months during the funding period. Furthermore, a scientific advisory board was implemented to ensure a high standard of research and monitor the progress of the project by taking part in the annual General Assembly Meetings.

1.3 Description of the main S&T results/foregrounds of DORIAN

The main results of DORIAN are given according to each of the main objectives of the project. Objective 7 is addressed in the following section 1.4.

• Ob.1: to establish if maternal obesity is an independent predictor for common non-communicable diseases and ageing related outcomes, focussing on CVD, T2D, cognitive decline and frailty (risk of premature ageing) in humans by discovering and measuring biomarkers and occurrence of unhealthy ageing and disease

• Ob.2: to establish if the effects of maternal obesity on unhealthy ageing of the offspring differ between genders

- WP1 included population and clinical studies providing the epidemiological and clinical evidence base of the involvement of maternal obesity as relevant cause of unhealthy ageing and common non-communicable diseases (Folkhälsan). A total of 13,345 men and women born in Helsinki during 1934 – 44 belonging to the Helsinki Birth Cohort Study were included in the epidemiological part of the study. Data on maternal weight and height in late pregnancy were available from hospital records. Using validated national registers, we reported on the following outcomes in relation to maternal BMI: death, cancer, coronary heart disease, stroke, and diabetes among the offspring. Furthermore, 2003 individuals underwent measurements of body size, body composition and clinical characteristics at a mean age of 62 years. Our results document that maternal BMI was positively associated with each of the later health outcomes of the offspring. The associations were strongest for cardiovascular disease and type 2 diabetes. The association with type 2 diabetes was stronger in women. Maternal BMI was positively associated with BMI in the offspring. Higher maternal BMI was associated with less favorable body composition in the offspring. There was a significant interaction between birth weight and maternal BMI on offspring body fat. In mothers with low BMI, a higher offspring birth weight was associated with lower fat percentage, while among those with maternal BMI in the highest fourth, higher offspring birth weight predicted higher body fat percentage. Our findings suggest that a disadvantageous body composition is programmed in early life. This may in part underlie the association between maternal obesity and later cardio-metabolic health of the offspring. These findings support the importance of prevention of overweight in women of childbearing age. These results were reported in 2 publications.
- In WP4, Folkhälsan and UTU addressed the relationship between maternal obesity and frailty in the offspring. Frailty confers high risk for adverse outcomes in ageing people, such as falls, disability, hospitalization and mortality. It is characterized by physical weakness and insulin resistance. We studied whole body and tissue specific insulin resistance, bone mineral density, and grip strength in frail elderly women born to obese and lean mothers. We used positron emission tomography – computed tomography and the hyperinsulinemic euglycemic clamp. Results indicate that frail women offspring of overweight/obese mothers tended to be more insulin resistant than the offspring of lean mothers. In fact, skeletal muscle insulin sensitivity was signficantly lower in some of the studied thigh compartments, and in the thigh muscle as a whole. Instead, bone mineral density was reduced (higher osteoporosis) in offspring of lean/normal weight mothers in three regions analysed. In summary, our findings support a long-lasting effect of maternal obesity on insulin resistance in the offspring. Considering that insulin resistance is a widely recognized underlying factor in the pathogenesis of type 2 diabetes, cardiovascular disease and cancer, the current data provide potential mechanistic explanation to the findings in WP1 above.

Objectives 1-2 have been fulfilled, by documenting that maternal obesity is an independent predictor for common non-communicable diseases and ageing related outcomes, especially CVD and T2D in humans, showing some gender differences, and supporting insulin resistance as underlying mechanism.

• Ob.3: to demonstrate that human newborn/infants of obese mothers carry hallmarks of premature ageing, and to explore the potential underlying mechanisms involved

- Our findings in WP1 (CNR, UEDIN) show and/or confirm that obese mothers have higher blood pressure, and increased levels of markers of the metabolic syndrome, especially if gaining more weight during pregnancy.

Obese pregnant women are also characterized by greater social deprivation, higher parity and are more likely to be current smokers. On average, they deliver at an earlier gestation than lean women because of a higher proportion of elective caesarean deliveries due to previous caesarean sections.

- Birthweight did not differ between lean and obese in unadjusted analysis, but was significantly higher in obese after adjustment for gestation at delivery, gender, maternal age and maternal smoking status. Regardless of maternal BMI, maternal weight gain during pregnancy was a significant predictor of birth weight and subsequent body weight during the first year of life in the available subset. Newborns of obese mothers with high weigth gain were also characterized by an increased cardiac size during the early life phases in agreement with the preclinical observation in WP4. Our preliminary data on cognitive development indicate a positive relationship between maternal overweigth and offspring cognitive performance in humans and mice during the lactating period. Since the literature suggests that preschool and school age children born to obese mothers have lower cognitive function than those born to lean, we are planning to expand the sample size and carry on a follow-up in both models. This has been included in a recent application for funding (exploitation of DORIAN findings).
- As early mechanisms connecting maternal metabolism and the risk of later disease, we addressed the relationship between maternal insulin resistance (IR), glucocorticoids (GC) and GC regulatory paths, and offspring gestational age, body weight, and metabolic and hormonal markers. In a cohort of 91 (CNR), our data show that maternal short stature, weight gain during pregnancy, and the HOMA insulin resistance index, circulating triglycerides, inflammatory markers, and low placental growth factor are predictors of insulin resistance in the offspring. HOMA in the offspring relates to shorter gestational age, greater ponderal index at birth and during the first year of life .Body growth during the first year of life is a recognized potent determinant of body weight in later life. In a cohort of 286 obese pregnant women and 137 lean controls (UEDIN) studied at 16, 28, and 36 weeks of gestation and between 3-6 months postpartum, total serum cortisol concentrations increased similarly in obese and lean women throughout pregnancy but were significantly lower in obese than in lean at each time-point during pregnancy and postpartum. Cortisol binding globulin and corticotrophin showed similar patterns. Cord blood cortisol was not different between groups, whereas saliva cortisol was significantly lower in offspring of obese women at 3-6 months of age. Maternal cortisol at 28 weeks was lower in lean women giving birth to macrosomic babies compared to appropriate for gestational age babies. In obese, lower corticotrophin at 28 weeks correlated with longer gestation. In summary, levels of cortisol and hormones that regulate its bioavailability were lower in obese pregnancy suggesting decreased activity of the HPA axis. These findings may offer novel mechanism underlying shorter (IR) or prolonged (GC) pregnancy and increased fetal and infant size in obesity. They underscore the importance of achieving adequate metabolic and stress control in pregnant women, especially in proximity of, and during delivery.
- Our studies focusing upon early biomarkers at birth have also focused on examination of expression of key genes within the placenta obtained from 60 women. Genes important in placental growth and development and also glucocorticoid signalling were selected, with a particular focus on genes regulating steroidogenesis, inflammation, placental invasion and growth. The results suggest that the changes in placental mRNA transcript levels of these three key genes (*11βHSD2*, *GR* and *IGF2*) may be, at least, useful biomarkers or even contributory factors in the determination of offspring birth weight and pregnancy outcome in obese pregnancy.

In response to Objective 3, we have been able to establish that maternal metabolism influences the offspring metabolic and hormonal profile at birth or during early post-natal life, and that IR and GC may modulate the duration of gestation and fetal and infant size at birth and during the first year of life. More markers and mechanisms were explored in WP3, and are reported in conjunction with Objective 6 below.

• Ob.4: to exploit targeted animal models of maternal obesity (separate from post-natal overnutrition) to test mechanistic hypotheses of its effects on disease pathogenesis, senescence and lifespan

In WP2, ISS, MPG and UEDIN examined the mechanistic consequences of maternal obesity on offspring behaviour, metabolism and neuroendocrine profiles using three animal models.

- UEDIN generated and characterised mice lacking HSD2 solely in the fetal brain or the placenta to determine the major site of HSD2 expression that protects the fetus from glucocorticoid programming. Brain-specific HSD2 KO mice as adults exhibit increased depressive behaviours but not anxiety, suggesting that the HSD2 barrier in the brain is important to protect the developing brain from fetal glucocorticoids and consequent programming of depression. To see if the residual phenotype is dependent on placental HSD2, a placental-specific HSD2 KO

mouse was made. Contrary to expectations, placental growth and function was normal. Fetal growth was only transiently decreased. As adults there was no overt abnormality consistent with programming of anxiety/depression or metabolic parameters. However, when the female control and placental-specific HSD2 KO mice were placed on a high fat diet (HFD, compared to low fat control diet) for 3 months, the glucose handling in response to a glucose tolerance test was impaired in both genotypes but accentuated in the KO mice. HFD in the placental specific HSD2 KO mice also caused an increase in HPA activity and elevated plasma corticosterone levels. This suggests that placental-specific HSD2 KO mice exhibit adverse programming of metabolism when given a high fat diet, and indicates an interaction of maternal obesity and prenatal glucocorticoid overexposure on metabolic and neuroendocrine behaviours.

- Using models of diet-induced obesity (DIO) and diet resistance (DR), MPG has produced evidence suggesting that maternal insulin resistance (IR) has long-lasting effects in the offspring. Besides inducing hyperactivity, maternal IR did not reveal any further robust long-lasting effects under basal conditions. However when challenged as adults to a chronic social defeat stress procedure (CSS), maternal IR resulted in impaired metabolic-related phenotypes and dysregulation of the stress response system. Offspring from obese mothers have similar bodyweight to controls when young but as they age they are lighter suggesting increased frailty with age. This is accompanied by increased susceptibility to anxiety-like and depressive-like behavior with age, effects that are paralleled by adult exposure to chronic stress. Investigation of key stress associated genes has identified FKBP51, a chaperone of the glucocorticoid receptor, as a novel candidate gene regulated by maternal obesity, hence a potential therapeutic target.
- HFD increased maternal mortality and cannibalistic behaviour, suggesting an effect on maternal anxiety. Partner ISS showed that dams resistant to oxidative stress, p66Shc-/- dams, appeared overall protected. Adverse metabolic effects of HFD were observed in the offpring of control but not p66Shc-/- mice suggesting an elevated resilience to metabolic changes in the internal *milieu* in the p66Shc-/- phenotype that might lead to a metabolic protection in conditions of excessive exposure to fat. Early exposure to HFD also resulted in an increased reactivity of the HPA axis, and altered behaviours (increased emotionality), both of which are attenuated in P66Shc-/- mice. A reduced locomotor activity was observed in all mice exposed to HFD in utero. The above results were observed in young adults, and then also in aged animals, since p66Shc-/- offspring from obese mothers were protected, in a sex specific manner, from both the deleterious metabolic and affective behavioural consequences associated with maternal obesity.
- Being at the crossroad of signaling pathways involved in both central and peripheral stress responses and in the regulation of energy homeostasis, p66Shc expression was examined in peripheral blood mononuclear cells (PBMC) of aged women from WP4 (Helsinki Birth Cohort), who had been born to mothers. Aging frail offspring of obese mothers were characterized by a greater expression of the p66Shc gene in PBMC, whereas similarly frail offspring of lean mothers were not. This suggests that variations in the expression of p66Shc might reflect a condition of altered oxidative stress related to the long-term effect of a metabolically stressful condition experienced in utero rather than to a condition of frailty per se

Since data collected during the DORIAN project highlighted the detrimental effects of maternal HFD-feeding during pregnancy both on the dams, increasing aggressiveness towards pups and dam mortality rate in the perinatal period, we felt that a deep understanding of maternal physiological alterations driven by HFD before and during pregnancy could inform on predictive factors for susceptibility to HFD-related metabolic and mood disorders, with public health implications. For this reason, a study was added in WP2 during the second project period to investigate the effects of HFD feeding before and during pregnancy on the neuroendocrine, metabolic and behavioural profile of dams in the perinatal period, both before and after delivery, and on the molecular adaptations associated with parturition, delivery and the onset of maternal behaviour. The study highlighted that HFD dams had greater glucocorticoid levels, and offered lower placental protection against glucocroticoids to the fetus. They showed reduced locomotor activity, social avoidance, and altered behaviour (sniffing, grooming) towards pups. Consistently, C-Fos gene expression which is a marker of neuronal activity within the sensory cortex, olfactory bulb, caudate putamen and paraventricular nucleus (PVN) of the hypothalamus was reduced in the olfactory bulbs and in the PVN of HFD dams in the perinatal period.

Objective 4 was fulfilled in spite of some difficulties due to maternal neglect of pups due to high-fat feeding. Maternal obesity had deleterious effects on maternal and offspring behaviour, metabolism and neuroendocrine profiles during adulthood and ageing. Mice resistant to oxidativee stress exhibited attenuated effects and mice exposed to maternal insulin resistance or elevated glucocorticoids prenatally or to chornic social stress during post-natal life showed more severe effects. We have identified two crucial

therapeutic targets to prevent these adverse effects, namely oxidative stress and the glucocorticoid responsive gene, FKBP51.

• Ob.5: to use these mechanistic studies (Ob.3-Ob.4) to discover and develop novel human biomarkers of vulnerability and possible early primary prevention and therapy interventions

In WP4, we examined **a)** the effects of maternal weight gain and moderate weight loss on the cardio-metabolic health of the offspring in a minipig model, **b)** the effects of a 4-month exercise training period in adult frail women born to overweight or lean mothers, and **c)** the effects of a pharmacological intervention (antioxidant therapy in mice) to alleviate the effects of maternal obesity on glucose tolerance and behavior. In addition, **d)** work was developed around a new candidate molecule (FKBP51) for the treatment of obesity.

- CNR studied the offspring of two consecutive pregnancies, the first from lean vs high-fat-fed obese mothers, and the second after a 12% maternal weight loss in the high-fat-fed model vs control group. Notably, lean mothers were considerably grown in their weight between pregnancies. Glucose uptake in body organs was increased at birth, followed by insulin resistance in early and adult life in the myocardium, brain, liver, muscle and adipose tissue in the offspring of obese mothers. Changes in cardiac and carotid intima media sizes were observed. Perssitent inflammatory cells and lipid infiltrations were noted in the livers of offspring born to obese mother. Our data supported maternal weight loss as a strategy to reduce the risk of cardiac hypertrophy in the offspring of obese mothers, but the intervention did not seem sufficient to prevent cardiovascular vulnerability, and a longer weight stabilization period may be required to cancel the memory of prior maternal high-fat feeding from the vascular walls and myocardial metabolism of the offspring. Systemic metabolic and brain specific data were confirmed in a different preclinical model.
- UTU and Folkhälsan studied the effects of a 4-month exercise training period on the metabolic health of adult (born 1934-1944) female offspring of overweight and lean mothers, showing signs of frailty. In addition, the relationship between maternal obesity and physical activity was evaluated in a younger birth cohort of subjects born in 1985-1986 (Arvo Ylppö Study). We found that young subjects born to obese mothers had lower physical activity and fitness levels. Elderly women born to obese mothers had lower muscle strength and more muscle insulin resistance than women born to lean mothers. Results support our lifestyle strategy to improve whole body and skeletal muscle insulin sensitivity, and skeletal muscle mass, especially in the offspring of overweight mothers. Our data highlight the beneficial effect of a short exercise period on bone mineral density, reducing the degree of osteoporosis, but they document that this effect is lost few months after the end of the intervention. Finally, our results support exercise training as a strategy to improve the cerebral control of food intake and prevent obesity, especially in the offspring of overweight mothers.
- ISS**,** starting from previous work showing that the detrimental effects of prenatal exposure to a maternal HFD were counteracted by the lack of the p66Shc (reduced oxidative stress and resistance to diet-induced obesity), developed a pharmacological model of reduced oxidative stress by means of prenatal administration of N-acetyl-cysteine (NAC). This drug is an analogue and precursor of reduced glutathione with remarkable antioxidant properties. We studied female mice that were fed a HFD or control diet for 13 weeks and were exposed to NAC drug for 8 weeks until right before delivery. Offspring had higher birth weight than controls, regardless of diet and gender. The NAC drug replicated the metabolic effect of the p66Shc gene deletion, conferring glucose tolerance to young adult male offspring. More importantly, prenatal NAC overturned the effect of maternal HFD on the emotional and behavioral phenotype of the offspring.
- MPG addressed the characterization of a novel candidate in metabolic regulation. FKBP51, namely a known negative regulator of glucocorticoid receptor signaling, which appears to play a role in body weight regulation and glucose homeostasis. In particular using 51KO and WT mice, we have been able to show that the loss of FKBP51 protects against high fat diet-induced body weight gain and glucose intolerance. Indeed FKBP51 may represent a novel therapeutic target against obesity and/or insulin resistance. Ongoing work in our lab focuses on the ability to target FKBP51 and its related metabolic phenotypes using highly selective FKBP51 antagonists.

Objective 5 was fulfilled by the demonstration of organ-specific, metabolic and behavioural alterations induced by maternal obesity, confirming IR, GC/stress, and oxidative stress as markers/mediators. In addition, we identified FKBP51 as a mechanism and biomarker in diet-induced obesity (WP4) and in

maternal obesity (WP2). Our findings highlight that a "healthy" lifestyle through life can partially restore the abnormalities observed. We showed beneficial effects of maternal weight loss or antioxidant therapy over the health of the offspring. We noted that subjects born to obese mothers have lower proneness to physical activity, and we documented the beneficial effects of exercise training in the adult offspring of overweight mothers.

• Ob.6: to determine the roles of gene-environment interactions and of innate genetic variability on the outcome of maternal obesity, and on the efficacy of the therapeutic strategies evaluated under Ob.5

In WP3, Biomol in collaboration with CNR and MPG carried out genome wide methylation analysis of humans and mice in association with maternal obesity or chronic stress, using methyl binding (MBD) domain enrichment of methylated DNA, followed by second generation sequencing. Loci exhibiting differential methylation patterns were described together with the genomic partition of methylated sites. Gene ontology based tables were produced in both models. In human neonates, we have produced a table with the relationship between statistically significant methylation peaks and the associated genes, including information of more than 700 genes, putatively implicated in the relationship between obesity in mothers during pregnancy and epigenetic changes in genes related to brain and cardiovascular function, diabetes and/or cholesterol levels in adults, via differential methylation of fetal DNA.

In WP3, CNR and Folkhalsan addressed the effects of maternal obesity on offspring DNA ageing at birth and in adulthood, and whether appropriate exercise training can revert DNA ageing in a group of aged women. Telomere length was measured in a clinical birth cohort of 1082 subjects aged 67-79 years (HBCS II, from WP1), in a smaller birth cohort of 90 couples of mothers and their newborns (from WP1), and in a group of frail aged women before and after an intervention protocol consisting of four months of physical exercise from WP4. Data from the clinical cohort of adult individuals showed that shorter telomeres are associated with bad general health and with the presence of specific metabolic and cardiac disease, such as diabetes, coronary thrombosis and angina pectoris. Moreover, shorter telomere length correlated with older age, male gender, and increased weight and waist circumference. Subjects born to mothers with a high BMI at end-pregnancy tended to have shorter telomeres, and this relationship was significant in women. During pregnancy, women with higher BMI and gestational diabetes showed shorter telomere length, and offspring birth telomere length was mostly affected by the additive effect of higher maternal BMI combined with excessive weight gain during pregnancy. The latter condition was accompanied by increased serum concentration of inflammatory markers. In the same newborns, we addressed the methylation status of genes involved in replicative senescence and the mitochondrial DNA content, showing that higher maternal BMI tended to associate with the methylation of the p16 gene, which in turn was significantly associated with higher birth weight. Finally, data on telomere variation showed that reduction of body mass index and improvement of whole body insulin sensitivity and glucose metabolism achieved by controlled exercise training can revert DNA ageing and associate to telomere elongation.

Objective 6 was fulfilled by showing that gene environment interactions appear to involve DNA methylation and DNA ageing, as many genes were found to be differentially methylated in neonates born to obese mothers, and a higher maternal BMI with excessive weight gain during pregnancy may result in shorter telomere length in the offspring, as observed both at birth and in adult offspring. Additionally, our studies confirm that short telomere length is a hallmark of DNA ageing associated with risk factors of metabolic and cardiovascular disease and with the occurrence of the disease itself. Our data support gene-lifestyle interactions to combat unhealthy ageing, since we showed that when exercise is successful in improving whole body metabolism, telomere elongation may occur, as seen especially in the offspring of obese mothers.

1.4 The potential impact

Socio-economic impact and the wider societal implications of the project

Contribution to Community and social objectives

The most important aim of the study was to investigate whether maternal obesity is or is not a risk factor for the long-term health among the offspring. Our data show a strong link, lasting through the whole life course. The chronic diseases related to maternal obesity are common ones, including cardiovascular disease, stroke, type 2 diabetes and cancer. These conditions are responsible for major societal burdens, involving physical and mental health and economy, and they markedly affect quality of life in the ageing population. The observation that the risk to develop such diseases depends in part on the prenatal environment and in particular on maternal obesity and gestational weight gain has very important implications, as it indicates that primary prevention is possible. First, our data suggest that efforts directed at pregnancy planning, family education and continuing multidisciplinary support before conception and during pregnancy could protect the offspring from an important source of disease. Second, if this is not possible, we believe that introducing prenatal risk factors in the clinical history of patients (as other risk factors like smoking and blood pressure etc are normally considered) may improve the estimation of disease risk and reinforce efforts towards disease prevention in these targeted groups. Among such efforts, our findings support physical activity from younger age onwards. One important finding in this context is that although a short period of physical activity results in immediate health improvements, some benefits are lost if exercise is interrupted. Therefore, it seems important that the habit to exercise becomes part of our natural way of living. More efforts in this direction are needed.

On the economic side, in a dedicated DORIAN workshop organized to exchange with stakeholders and patients, the main concern addressed by the scientific and policy makers' representatives was that though lifestyle strategies (including exercise) are proven efficacious once implemented in research studies that are carefully followed-up in a medical setting, the promotion of a healthy lifestyle in the broad population keeps failing (lack of effectiveness), and more political efforts and investments are needed in education and infrastructures.

Some of our findings may translate in biomarkers (e.g. epigenetic, hormonal, placental, metabolic changes) or therapeutic targets (FKBP51 by FKBP51 antagonists, oxidative stress by antioxidants) to be exploited by the private industry. In addition, our results encourage industry in the fields of leisure time and fitness to develop age-targeted programs and tools. Therefore, our results provide some evidence base for the attraction of private partners in research, and to the future benefits of the European industrial sector.

One very important aspect, with strong social impact, emerging from many sub-studies in our project, is that of mental wellbeing in the context of obesity in general, and specifically in that of maternal obesity and offspring health. Our preclinical models show negative behavioural changes in obese mothers, including social avoidance. Interestingly, social avoidance was also documented in human mothers. Such behavioural changes were so strong as to compromise survival in the offspring in our preclinical models. In humans, we could expect much more subtle changes in maternal behaviour which may likewise affect the interaction between mothers and babies, deserving further and urgent investigation. In the same line, chronic stress during life in the offspring was able to potentiate health-related consequences of maternal obesity. We know very well that a high sensitivity to stress, and reduced stress coping abilities characterize human ageing, increasing disease vulnerability in older people. Consistent with this line of reasoning, in a dedicated dorian workshop organized to exchange with stakeholders and patients, one main concern addressed by the patients' representative was that obese children suffer from stigmatization by their peers, and self-emargination is an source of discomfort and pain, perpetuating a vicious cycle of sedentariness (as obese people may become ashamed to attend gyms) and overeating. Therefore, obesity translates in social discomfort, adverse hormonal changes and adverse behaviour with consequences for mothers and offspring. What we feel is that psychological support should be delivered together with medical support as part of standard practice during conception planning, pregnancy and early life phases. Mothers should absolutely seek for help in case they experience mood discomfort during their gestation and otherwise.

Another important point underlined by the patients' representative above was that people want to be more involved and informed about research progress, results, and their practical meaning. Recognizing this important point already in the planning phase of our DORIAN project, we included as full partners two prestigious organizations, namely the European Association for the Study of Obesity with their broad scientific network and European Patients Council, and the International Association of Mothers (Make Mothers Matter). We were, and are aware that our research can only

be successful and meaningful in practise if reaching the end-users in an appropriate format and language. Thanks to our collaboration with these two important partners, the social and societal impact of our research was readily shown by the massive attention given by media to our recent press release. This was prepared in conjunction with the current report, therefore underlining that findings were still preliminary and mostly to be published in the future. In the first 2 days after the release, we have been able to collect wide media coverage (**see Main dissemination / exploitation below**), but more is to come, and we will continue to inform our stakeholders and end-users and call for their feedback.

Main dissemination activities and exploitation of results

• Ob.7: promote knowledge exchange and develop public understanding and a health promotion program

We have been active in the exploitation and dissemination strategy. The number of accepted publications is still limited, but much work has been done and several publications are in progress, and we expect that several publications will be submitted in this year. We have been presenting our findings in a number of relevant International Congresses. The DORIAN Consortium disseminated their research results during the last 2 ½ years of the project. The groups have closely worked together and are going to publish their results in relevant journals and probably also in high impact factor journals. They are going to disseminate the results of their research programme as widely as possible to the scientific community.
DORIAN has been promoted at several international conferences and meetings. We have organized a dedicated DORIAN workshop at the 2013 annual EASO (European Association for the Study of Obesity) meeting, i.e. the European Congress on Obesity (ECO). This was a learning workshop in which we invited one scientist, one policy maker and one patients' representative to address the reasons for the failure of the anti-obesity campaigns. We have also presented DORIAN in a special 'Lessons from EU Projects' session at the 2014 annual EASO meeting and promoted DORIAN as part of the ECO2014 'EU Project Village'. DORIAN fliers were also distributed at relevant scientific meetings including EASO meetings, the Obesity Week meetings in the US and at the European Association for the Study of Diabetes (EASD) annual meetings. We have regularly updated our webpage and followed the number of visitors monthly. The publicly-accessible area of our website has a specific section for dissemination in which published papers are listed (with external links) and in which relevant meetings, workshops and key DORIAN actions are listed and described (with external links where necessary). A scientific review of DORIAN results will be submitted as soon as the relevant findings are published. We have made a plan on how the review will be structured.

These activities have all been coordinated by EASO in collaboration with MMM.

As mentioned in the "Socio-economic impact …" section above, the project has been so far exploited by distribution of a press release to media. The press release was written by all partners, in conjunction with the current report, therefore underlining that findings were still preliminary and mostly to be published in the future. It was disseminated within and outside of Europe thanks to the networks and efforts of EASO and MMM. The press release has attracted massive attention in the media. In the first 2 days after the release, we have been able to collect wide media coverage, but more is to come, and we will continue to inform our stakeholders and end-users and call for their feedback.

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http://www.pourquoidocteur.fr/L-obesite-pendant-la-grossesse-fait-courir-plusieurs-risques-a-l-enfant-9837.html

For exploitation, see also the next section.

Outlook and future research

The Consortium has consolidated existing and new collaborations during the period of the project. We have been engaged in planning future steps and responding to new calls to support the continuation of this collaboration. One important point will be to follow-up children born and the cohorts characterized under DORIAN, and expand recruitment. Intervention studies aimed at improving mental and physical health in mothers are warranted. In particular, it would be important to take intervention studies in mothers and offspring, including physical activity from the controlled research environment to the broad population level, i.e. from efficacy to effectiveness.

As further exploitation potential, as mentioned in the previous section, some findings may translate in biomarkers (e.g. epigenetic, hormonal, placental, metabolic changes) or therapeutic targets (FKBP51 by FKBP51 antagonists, oxidative stress by antioxidants) to be exploited in conjunction with the private industry. In addition, our results encourage collaboration with industry in the fields of leisure time and fitness to develop age-targeted programs and tools. Therefore, our results provide some evidence base for the attraction of private partners in our future research.

In addition to the measurements planned in the project, the recruitment and follow-up of study subjects offered the opportunity to collect fecal samples in mothers, fathers and offspring through growth. This has generated a quite unique collection to be exploited in future funding and research, given the growing relevance linking the gut microbiome to human health and the risk of non-communicable diseases.