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PRENATAL EXPOSURE TO MATERNAL OBESITY AND SFLT-1 OVEREXPRESSION AND CARDIOVASCULAR FUNCTION IN THE ADULT OFFSPRING

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PRENATAL EXPOSURE TO MATERNAL OBESITY AND SFLT-1 OVEREXPRESSION AND CARDIOVASCULAR FUNCTION IN THE ADULT OFFSPRING

by

Egle Bytautiene

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Dedication

To my Son, my Mother, my Grandmother, my Best Friend, and to Lithuania –
sources of inspiration and strength

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PRENATAL EXPOSURE TO MATERNAL OBESITY AND SFLT-1 OVEREXPRESSION AND CARDIOVASCULAR FUNCTION IN THE ADULT OFFSPRING

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Cardiovascular disease is the leading cause of death in the United States. Adult smoking, type II diabetes, and other environments and conditions, have been identified as risk factors for cardiovascular diseases; however, the effects of prenatal development cannot be overlooked. In the 1980s Barker proposed a hypothesis that poor nutrition in the fetal or early neonatal period increases susceptibility to the negative effects of an affluent diet during adulthood. Overall, there is little understanding of the exact mechanisms of the developmental origin of adult disease, but it is recognized that adult disease occur when postnatal environment is considerable different from what fetus experienced while in utero. The obesity epidemic has led to the evaluation of the effect of a high fat nutritional environment on fetal programming. Conversely, recent studies have shown that the maternal prepregnancy weight is a strong independent risk factor for preeclampsia. Preeclampsia by itself has a negative effect on offspring's future

cardiovascular function. It is possible that maternal obesity in conjunction with preeclampsia may emerge as one of the risk for impairment of cardiovascular function in adult life.

These studies were performed to determine the effect of prepregnancy obesity and sFlt1-induced preeclampsia on cardiovascular function in the offspring in a small animal model. A link between increased blood pressure and amplified vascular reactivity was revealed in offspring born to mothers fed high fat diet and with or without sFlt1 overexpression during pregnancy. In addition, investigation of metabolic, inflammatory and atherosclerotic profiles and determination protein expressions of angiotensin II, as well as its receptors, lead to a conclusion that maternal obesity is a much stronger negative factor influencing offspring's cardiovascular function than pregnancy complications. Though, if combined together, obesity and preeclampsia have even more detrimental effect. Study also identified possible mechanisms and indicated several directions for therapeutical approaches.

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Chapter 1: Introduction

Cardiovascular diseases remain the number one killer in the United States. Mortality data show, that CVD as the underlying cause of death (including congenital cardiovascular defects) accounted for 36.3% of all deaths in 2004, or 1 of every 2.8 deaths in the United States (Rosamond et al., 2008). Moreover, an estimated 80,700,000 American adults (1 in 3) have 1 or more types of CVD (Rosamond et al., 2008). The estimated direct and indirect cost of CVD for 2008 is $448.5 billion.

Adult smoking, type II diabetes, and other environments and conditions, have been identified as risk factors for cardiovascular diseases; however, the effects of prenatal development cannot be overlooked. In the 1980s Barker proposed a hypothesis that poor nutrition in the fetal or early neonatal period increases susceptibility to the negative effects of an affluent diet during adulthood (Barker & Osmond, 1986). Additional epidemiological data and animal experiments produced evidence that there is a significant correlation between malnutrition during the fetal period and coronary heart disease, stroke, hypertension, and type II diabetes later in life (Barker, 1998). Overall, there is little understanding of the exact mechanisms of the developmental origin of adult disease, but it is recognized that adult disease occur when postnatal environment is considerable different from what fetus experienced while in utero.

MATERNAL OBESITY AND FETAL PROGRAMMING OF CARDIOVASCULAR DISEASES

Though the original discussion regarding the role of the prenatal environment was based on the influence of maternal under nutrition, the obesity epidemic has led to the evaluation of the effect of a high fat nutritional environment on fetal programming. After controlling for the birth weight, birth year, and gender of the children plus the mothers'

age, race/ethnicity, education level, marital status, parity, weight gain, and smoking during pregnancy, the relative risk of childhood obesity associated with maternal obesity in the first trimester of pregnancy was found to be 2.0 times higher at age 2, 2.3 at 3 years of age, and 2.3 at 4 years of age (Whitaker, 2004). This and other increasing evidence imply that children born of pregnancies complicated by obesity are at increased risk of obesity and other features of the metabolic syndrome, and consequently are at higher risk to develop cardiovascular diseases (Catalano & Ehrenberg, 2006). The metabolic syndrome (the presence of two or more of the following components: obesity, hypertension, glucose intolerance, and dyslipidemia) is more common in those adolescents who were large-for-gestational age at birth or who were born to obese mothers (Boney et al., 2005). Men whose mothers had a higher body mass index (BMI) in pregnancy had high death rates, and hazard ratio for coronary heart disease was 1.24 (95% confidence interval 1.1-1.39) for every standard deviation increase in mother's BMI (Forsen et al., 1997). Studies in rats and mice have further explored cardiovascular function in adult offspring of dams fed high-fat diets (Khan et al., 2003; Khan et al., 2005; Samuelsson et al., 2008). The most prominent characteristics of prenatal exposure to high fat diet were increased adiposity, blood pressure and endothelial dysfunction. In offspring of lard-fed pregnant rats, female but not male offspring had elevated blood pressure, while endothelium-dependent relaxation to acetylcholine in mesenteric arteries was blunted in both genders (Khan et al., 2003). Similar results were reported in mice fed palatable obesogenic diet, which is rich in sugars and animal fat, except that hypertension was observed in both, male and female offspring (Samuelsson et al., 2008).

MATERNAL OBESITY AND PREECLAMPSIA

Obesity is becoming an alarming public health problem. The proportion of women who are obese has doubled since the 1960s (2006). In 2003, the prevalence of overweight or obesity among women (BMI >25 kg/m^2 and >30kg/m^2, respectively) was 61.6% (Thom et al., 2006). Obesity and overweight affect multiple organ systems and are associated with an increased risk of cardiovascular diseases, diabetes, gallstones, etc (Kushner & Foster, 2000; Kushner, 2002). During gestation obese women are at risk for a wide array of potential medical and obstetrics problems, which may have adverse effects on the fetus. One of those problems is preeclampsia. Recent studies have shown that the maternal prepregnancy weight is a strong independent risk factor for preeclampsia. After adjusting for maternal race and prepregnancy smoking status, the risk of preeclampsia doubled at a BMI of 26 and almost tripled at a BMI of 30 when compared with women with a BMI of 21 (Bodnar et al., 2005). A dose-dependent relation between prepregnancy BMI and severity of preeclampsia has also been demonstrated. The risk of severe preeclampsia was 2-fold for BMI of 25, 3-fold for BMI of 30, and 5-fold for BMI of 35 when compared with women with BMI of 20. Similar effects of BMI was observed with regards to mild preeclampsia and transient hypertension of pregnancy (Bodnar et al., 2007).

PREECLAMPSIA AND FETAL PROGRAMMING OF CARDIOVASCULAR DISEASES

Preeclampsia and pregnancy-associated hypertension can affect the fetus indirectly through the altered intrauterine environment, since the reduction in uterine artery and placental perfusion is a hallmark of preeclampsia. It has been demonstrated that 6-, 12-, and 17-year-old children born with maternal preeclampsia had elevated blood

pressure (Palti & Rothschild, 1989; Seidman et al., 1991; Tenhola et al., 2003; Tenhola et al., 2006).

A two-stage model has been suggested in the pathogenesis of preeclampsia: reduced placental perfusion followed by the maternal systemic manifestations (Roberts & Lain, 2002). The placenta plays a central role in the development of the preeclampsia. Several research groups have explored how placental insufficiency affects the cardiovascular function in the rat offspring using various methods, including placing sliver clips around the aorta above the iliac bifurcation and on the main uterine branches of both the right and left ovarian arteries (Alexander, 2003; Payne et al., 2003). Pregnant dams with clips had a significantly higher blood pressure than normal pregnant rats. Offspring born to pregnant rats with reduced uterine perfusion demonstrated growth restriction, hypertension, decreased endothelial vascular relaxation, and increased active stress in aortic strips (Alexander, 2003; Payne et al., 2003).

There is growing evidence that an imbalance between active circulating proangiogenic and antiangiogenic factors (such as soluble fms-like tyrosine kinase-1 or sFlt1), plays an important role in the pathogenesis of preeclampsia (Levine et al., 2004). Our laboratory has established a mouse model of sFlt1-induced preeclampsia-like-syndrome. In this model, pregnant mice on day 8 of gestation are injected with adenovirus carrying sFlt1. The group of pregnancy mice injected with adenovirus carrying mFc serves as controls for virus injection. The model exhibits significant hypertension throughout pregnancy, which rises towards the end of gestation. Pregnant animals had lower platelet counts, higher white blood cell count, and maternal hemoglobin concentrations and histological changes in the kidneys. Fetal outcomes were characteristic of preeclampsia as well — placental insufficiency and fetal growth

restriction were observed (Lu et al., 2007a). Further investigations revealed that sFlt-1 overexpression in pregnant dams results into vascular dysfunction of the offspring, which manifested as hypertension and abnormal vascular reactivity (Lu et al., 2006; Lu et al., 2007b; Lu et al., 2007c).

MATERNAL OBESITY, PREECLAMPSIA, AND FETAL PROGRAMMING OF CARDIOVASCULAR DISEASES

Several factors warrant a look into how prepregnancy obesity and preeclampsia affect offspring. Increasing rates in obesity and obesity being one of the major risk factor for preeclampsia are the most obvious arguments. One more rationale to look at the combined model of obesity and preeclampsia is based on a common physiological pathway for both conditions – inflammation.

Obesity is associated with systemic inflammation, because adipose tissue is a source of pro-inflammatory cytokines and metabolic mediators, such as TNF-alpha, interleukin-6 (IL-6), leptin and plasminogen activator-1. The levels of leptin, TNF-alpha, IL-6, and IL-8 have also been shown to be significantly increased in preeclamptic subjects when compared with healthy control pregnant and nonpregnant women (Sharma et al., 2007). BMI is significantly correlated with circulating leptin, TNF-alpha and IL-6, each of which has pro-inflammatory action. IL-6 induces acute phase responses, so elevated acute phase reactants, such as C-reactive protein, are also present. The C-reactive protein has been shown to be increased in obesity (Visser et al., 1999). The C-reactive protein also was noted to be significantly higher during the first trimester in women who subsequently developed preeclampsia, compared to those who remained normotensive (Wolf et al., 2001). Ramsay and colleagues in 2002 published an extensive study in which various metabolic and inflammatory parameters were measured, and an

in-vivo assessment of endothelial-dependent and endothelial-independent microvascular function was performed using laser Doppler imaging in lean and obese women in the third trimester. One of the findings was that obesity in pregnancy is associated with endothelial dysfunction and activation of the inflammatory system (increased C-reactive protein and IL-6 levels) (Ramsay et al., 2002). In summary, obesity triggers systemic inflammation that has been implicated in the development of preeclampsia. In turn, these events could lead to the cardiovascular dysfunction in the offspring exposed to prepregnancy obesity and preeclampsia.

HYPOTHESES

In light of increasing rates in obesity, the fact that maternal prepregnancy weight is a strong independent risk factor for preeclampsia, and inflammation is a common physiological pathway for obesity and preeclampsia, we propose to examine the effects of pregravida obesity and sFlt1-induced preeclampsia-like syndrome on the cardiovascular system in offspring later in life.

General hypothesis: **Prepregnancy obesity and sFlt-1-induced preeclampsia-like syndrome lead to cardiovascular changes in the offspring later in life.**

This general hypothesis will be tested in a mouse model by examining the following three specific hypotheses in offspring born to mothers with/without prepregnancy obesity and with/without AdsFlt1-induced preeclampsia.

Specific Hypothesis 1: **Prepregnancy obesity superimposed over sFlt1-induced preeclampsia leads to abnormal vascular function in offspring later in life.**

Specific Hypothesis 2: **Prepregnancy obesity superimposed over sFlt1-induced preeclampsia causes metabolic, inflammatory, and atherosclerotic syndromes in offspring later in life.**

Specific Hypothesis 3: **Prepregnancy obesity superimposed over sFlt1-preeclampsia leads to the changes in rennin-angiotensin system that in turn affects cardiovascular system in offspring.**

The following aims are proposed to test the corresponding hypotheses:

Specific Aim 1: To examine offspring's cardiovascular vascular function using in vivo blood pressure measurements by telemetry and in vitro vascular responses to vasoconstrictor and vasodilator agents in carotid artery.

Specific Aim 2: To investigate offspring's blood concentrations of fasting glucose, total cholesterol, triglycerides, insulin, leptin, adiponectin (for metabolic syndrome), C-reactive protein and IL-6 for inflammatory changes, and intercellular adhesion molecular-1 for atherosclerotic abnormalities using commercially available ELISAs and immunoassays.

Specific Aim 3: To determine the protein levels of angiotensin II, angiotensin receptor 1 and 2 in offspring's adipose tissue and kidneys.

The proposed animal model represents a novel tool to study and selectively evaluate contributions of prepregnancy obesity and sFlt1-induced preeclampsia to the development of CVDs in offspring later in life, since the combined effects of preexisting obesity and preeclampsia on the development of CVDs have not been addressed in basic science research.

The suitability of the animal model is based on the following: **1)** the similarities to human pregnancy in trophoblast invasion and placental development, as well as in the cardiovascular adaptations to pregnancy; **2)** mice on a high-fat diet develop obesity, which manifests as overweight, hyperinsulinemia, hyperlipidemia, and hyperglycemia

similar to humans; **3)** overexpression of sFlt-1 during pregnancy in mice leads to preeclampsia-like syndrome.

Given that pregnancy may be viewed as a screening test for future health, and can unmask underlying subtle deficits which may take years to appear, identification of the role of prepregnancy obesity and preeclampsia may lead to preventive strategies and novel new treatments for cardiovascular diseases. The long period of time between pregnancy and disease later in life presents a unique opportunity for intervention. Our proposed studies can form the basis for future investigations into mechanisms of disease and preventive strategies.

Chapter 2: Materials and Methods

ANIMALS

The mouse strain (CD-1) that we were using is a good model in which to study human pregnancy because of the similarities in trophoblast invasion and placental development (Rossant & Cross, 2001). Moreover, the cardiovascular adaptations to pregnancy in mice also seem to parallel those in humans. In mice, mean arterial pressure decreases, cardiac output increases, and the pressure response to angiotensin is blunted during pregnancy (Wong et al., 2002). These data are in accordance with the cardiovascular adaptations occurring during normal human pregnancy (Sibai & Frangieh, 1995). Uterine and mesenteric arteries from pregnant mice show enhanced vasodilatation, through endothelium-dependent, as well as endothelium-independent mechanisms (Russell & Watts, 2000; Cooke & Davidge, 2003). The CD-1 strain of mice has also been used in diet-induced obesity studies - female mice on a high-fat diet have been observed to develop diet-induced obesity (Pelleymounter et al., 1998; Banks & Farrell, 2003). Unlike other strains used in obesity research, such as in the inbred DBA/2J strain, female CD-1 mice subjected to a high-fat diet do not exhibit obesity-induced infertility (Raygada et al., 1998; Tortoriello et al., 2004).

The study protocol and all related procedures were approved by the Animal Care and Use Committee at The University of Texas Medical Branch, Galveston, Texas. The mice were maintained in the animal care facility at The University of Texas Medical Branch. They were housed separately in temperature and humidity-controlled quarters with constant light:dark cycles of 12 h:12 h. They were provided with food and water ad libitum.

Mice were fed either a standardized diet (4.3 gm% or 10 kcal% fat) or a high fat diet (34.9 gm% or 60 kcal% fat). The high fat diet (D12492) was purchased from Research Diets, Inc. (New Brunswick, NJ), while the standard fat diet (Teklad 7012: Harlan Teklad LM-485 Mouse/Rat Sterilizable Diet) was supplied by Harlan Teklad, Madison, WI. The source of fat in the high fat diet is lard, while the standard diet does not contain animal product, and the source of fat is soybean oil.

STUDY DESIGN

The overall study design is presented in Figure 1. Female CD-1 mice were obtained from Charles River Laboratories at approximately 4–5 weeks of age. Mice were randomly assigned either to the standard diet group (control mice, **SF** group) or to the high fat diet group (diet-induced obesity mice, **HF** group). After 3 months on the assigned diet, mice were mated with a CD-1 male, and maintained on standardized diet. We expected female mice after 12-14 weeks on high fat diet to weight significantly more than those in SF group, and, therefore, we categorized them as obese mice.

The day when a vaginal plug was noted was considered day 1 of pregnancy. On day 7 of gestation (E7), blood was collected from the tail vein to establish a baseline for sFlt1 levels, and then on E8, mice in each diet group were injected via tail vein with adenovirus vector (10^9 PFU in 100μl) carrying sFlt1 or mFc. Blood from pregnant mice were collected on days 14 (mid term) and 19 (term) of pregnancy.

Pregnant mice delivered four groups of offspring: 1) **HF sFlt1** – exposed to maternal obesity and sFlt-1 overexpression, 2) **HF mFc** – exposed to maternal obesity only, 3) **SF sFlt1** – exposed to sFlt1 overexpression only, 4) **SF mFc** – exposed to normal intrauterine environment.

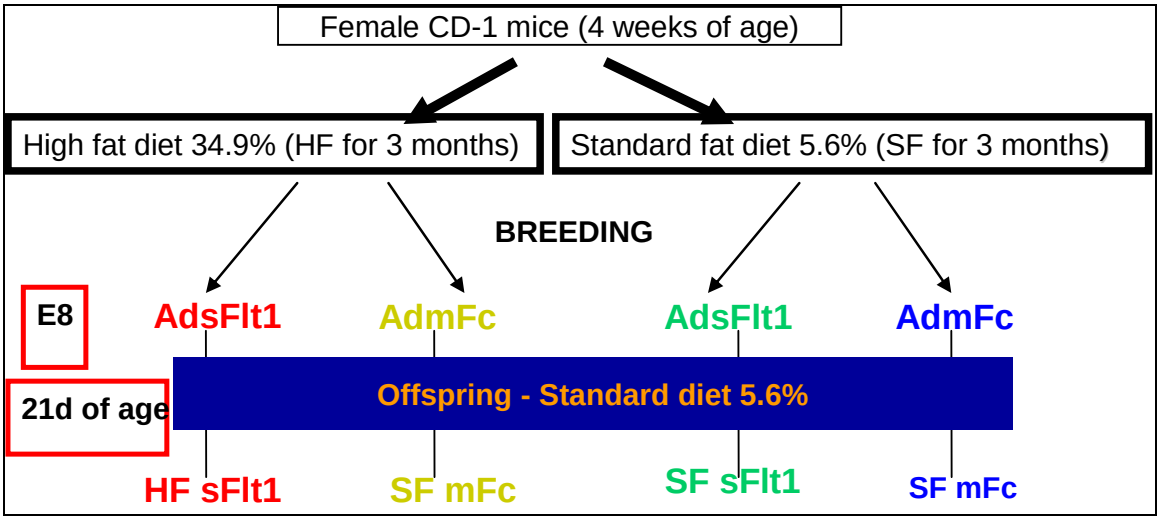

Figure 1: Study design. Explanations are presented in the text.

After delivery, pups were weighed at 1 day of age. During lactation, mothers were fed the originally assigned diet. At the age of 21 days, offspring in all four groups were weaned from the mothers onto a stand fat diet, containing 4.3 gm% or 10 kcal% fat. At 6 months of age, both female and male offspring were studied for the manifestations of cardiovascular diseases. In order to establish an age-related analog to the human model, we used the following metric: at 6 weeks of age, mice are already sexually mature, and on average their lifespan is 2 years when in captivity. Therefore, at 6 months of age, mice could be considered mature adults. If human lifespan is about 80 years, and mice live about 2 years, then 6 months in mouse would be an equivalent of about 20 years in humans.

PREPARATION, AMPLIFICATION, AND PURIFICATION OF SFLT-1 AND MFC VIRUS VECTOR

A standardized procedure to prepare adenovirus carrying sFlt1 and mFc vector was followed as described previously by Dr. Saade's lab (Lu et al., 2007a). The adenovirus vectors' stock, AdFlt1 (1-3) as the active vector and Ad-mFc as the virus control (first generation, E1 and E3 deleted, and carrying the murine IgG2α Fc fragment as the adenovirus control) were prepared and titered by the Research Vector Core, Harvard Medical School. The 293 cell line was used to grow and transfect the virus. The cells were cultured, and when they reached a 70%-80% confluence, a cell transfection procedure was performed. The transfection medium containing either sFlt-1 or mFc adenovirus was added the cells. About 20 hours later, roughly 50% of the cells were detached from their plates exhibiting the cytopathic effect (CPE). At this point, the cells were collected, then spun, and then the supernatant was removed. The cells were re-suspended and centrifuged. The supernatant was removed, and the cells were stored at -80ºC for purification. For the purification process, sterile 10mM Tris (pH=8.o) was first added to the cell pellet, thawed, then vortexed briefly, and lastly were put on the dry slurry to be frozen. This cycle was repeated 6 times. The cells were lysed by spinning them at 2000rpm/15min/4ºC, and the supernatant containing adenovirus was collected. The adenovirus then was collected following two-step centrifugation using a CsCl step gradient (d=1.43 and d=1.34). After the last spin, the adenovirus band appeared and was collected for dialysis to finally isolate the virus.

The concentration of adenovirus particles was determined using spectrophotometric analysis of an appropriate dilution of the test sample (typically 1/10) in a solution of 10mM Tris/1mM EDTA/0.1% SDS. The extinction coefficient used is 1 OD= 1.1× 10E12 particles of adenovirus and the extinction coefficient was applied to the

A260. The results were reported as particles per ml. Such particle counts are about 20-40 fold of the plaque forming unit (PFU) based on experience (Maizel, Jr. et al., 1968).

IN VIVO TELEMETRIC BLOOD PRESSURE MEASRUMENT EXPERIMENTS

There are several options for measuring blood pressure in mice, and some of these have clear disadvantages. The insertion of blood pressure catheters is a challenging and invasive procedure, considering the small size of the mice and the minute size of the blood vessels. The tail cuff method is an alternative approach; however it is less accurate than direct intravascular blood pressure measurement in mice, and does not provide continuous blood pressure and heart rate recording. Telemetric monitoring of the blood pressure, using an internal pressure transducer, is superior to either of these other two methods. It is very accurate, and moreover, the telemetric measurements do not require anesthesia or restraining of the animals during data recording, both of which are known to produce erroneous or stressed blood pressure measurements which are not truly basal.

For implanting the internal blood pressure transducers, the mice were anesthetized with a mixture of ketamine (Ketalar, Parke-Davis, Morris Plains, NJ) and xylazine (Gemini, Rugby, Rockville Center, NY). A vertical midline skin incision along the neck was made and the submaxillary glands were gently separated. The left common carotid artery located next to the trachea was carefully isolated. The catheter (diameter 0.4 mm) then was introduced into the carotid artery through a small incision in the vessel wall, and the body of the transducer (PA-C10 model, Data Systems International [DSI], Overland Park, KS) was secured in a subcutaneous pouch along the animal's right flank through the same ventral neck incision (Figure 2). The neck incision was closed using 6-0 silk. Mice

were kept warm on a heating pad and monitored closely until full recovery from anesthesia.

Recording of blood pressure began 48-72 hours after surgical implantation of the pressure transducer and was continuously monitored for 7 consecutive days using RLA 1020 telemetry receivers (DSI), BCM consolidation matrix (DSI), and an adapter, where the signal was de-multiplexed. This output subsequently was band-pass filtered and amplified. The information was fed to data acquisition and recording system, Dataquest software (A.R.T.3.1; Gartner Dataquest, Stamford, CT). Then, the mice were sacrificed, and blood and tissues were collected for later analysis, while the carotid arteries were isolated for in vitro vascular reactivity studies.

Data analysis

Blood pressure data obtained from the telemetry system were plotted as mean values over each 24-hour period and were expressed as mean ± standard error of mean (SEM) using GraphPad Prism 4 software version 4.00 for Windows (GraphPad Software, La Jolla, CA). For statistical analysis repeated-measures ANOVA with Bonferroni post hoc test was applied. A probability value (P-value) of <0.05 was considered statistically significant.

VASCULAR REACTIVITY STUDIES

Two-millimeter segments of the right carotid arteries were mounted in a wire myograph (Model 610M, DMT, Aarhus N, Denmark) using 25 μm tungsten wires (Figure 3). The preparations were bathed in Krebs solution maintained at 37°C, pH ~7.4. A mixture of 95% O2 and 5% CO2 was bubbled continuously through the solution. The force of contractions of the vascular rings was continuously recorded by an isometric

force transducer and analyzed using PowerLab system and Chart 5 data acquisition and playback software (AD Instruments, Castle Hill, Australia).

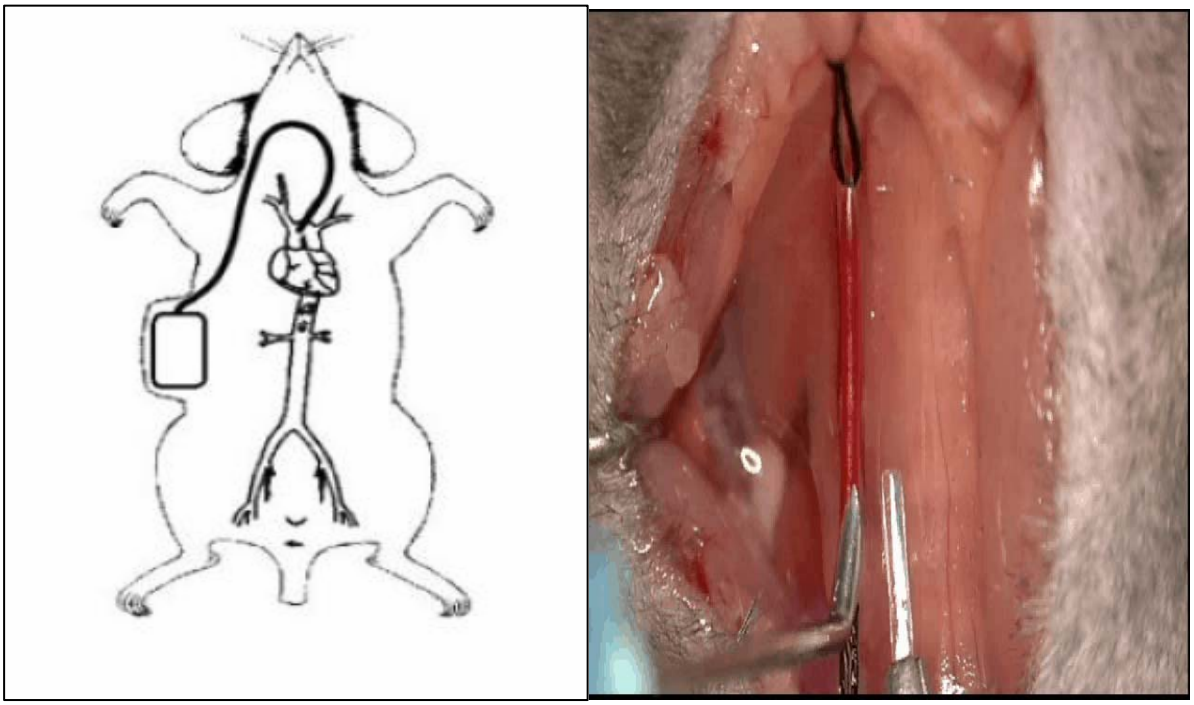

Figure 2: Transducer positioning (on the left) and catheter placement in the left carotid artery (on the right) for in vivo telemetric blood pressure measurements.

The optimal diameter and the passive tension applied to the vessels (3.5 mN), as well as the protocol and concentrations used in the vascular reactivity studies were determined from previous experiments in our laboratory. After stabilization of the tone, the vessels were contracted twice with 60 mmol/L of potassium chloride (KCl) for 10 min in order to enhance reproducibility of responses. The second response to KCl was used as a reference contraction in the final calculations. To evaluate endothelial function, the response to a single concentration of acetylcholine (10-6 mmol/L) in vessels pre-contracted with phenylephrine (10^{-6} or $3x10^{-6}$ mmol/L) was determined. Only arteries

demonstrating a substantial response to ACh (> 70-80 of relaxation) and a high constriction to KCl were used for the experimental studies.

After 1 hour of equilibration, relaxant responses to the endothelium-dependent vasorelaxant acetylcholine (10^{-10}–10^{-5} mmol/L), the endothelium-independent vasorelaxant sodium-nitroprusside (10^{-10}–10^{-5} mmol/L) and the β-adrenoreceptor agonist isoproterenol (10^{-10}–10^{-5} mmol/L) were obtained after precontraction of the vessels with phenylephrine (10^{-7}–10^{-6} mmol/L). In addition, contractile responses to the α1-adrenergic agonist phenylephrine (10^{-10}–10^{-5} mmol/L), thromboxane A2 mimetic U46619 (10^{-10}–10^{-5} mmol/L), and 5-hydroxytryptamine or serotonin (10^{-10}–10^{-5} mmol/L) were assessed.

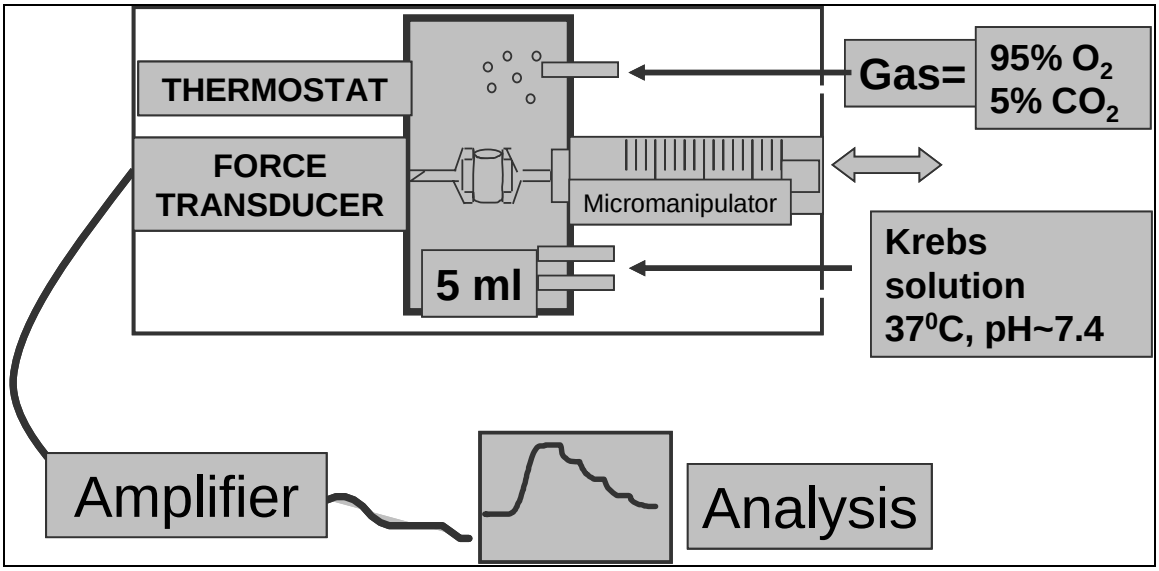

Figure 3: Schematics of small vessel myograph used in vascular reactivity studies.

Drugs and solutions

Krebs solution was composed of 119 mmol/L NaCl, 4.7 mmol/L KCl, 1.2 mmol/L KH_2PO4, 25 mmol/L $NaHCO_3$, 1.2 mmol/L $MgCl_2$, 2.5 mmol/L $CaCl_2$, 0.026 mmol/L EDTA, and 11.5 mmol/L glucose. Potassium chloride, acetylcholine

hydrochloride, phenylephrine hydrochloride, isoproterenol, serotonin hydrochloride, and sodium nitroprusside were obtained from Sigma-Aldrich (St. Louis, MO), and U46619 from Cayman Chemical (Ann Arbor, MI). All drug dilutions were done using Krebs solution.

Data analysis

Vascular reactivity results were expressed as the mean ± SEM. A second response to KCl was used as a reference to calculate the percent of contraction achieved by the contractile agents studied, while phenylephrine precontraction was utilized so as to obtain the percentage of relaxation induced by the vasorelaxant agents. In these studies, the area under the concentration response curve (AUC), the logarithm of the concentration producing 50% of the maximal effect (log EC50, a measure of sensitivity to the agent), and the maximal effect (Emax, expressed as the percentage of the reference contraction to 60 mM KCl) were calculated analyzed using GraphPad Prism 4 software version 4.00 for Windows. Data were compared between various groups by 1-way ANOVA followed by Tukey's Multiple Comparison Test. A P-value <0.05 was considered statistically significant.

SEROLOGICAL STUDIES

Blood was collected on day 7, 14, and 19 of gestation from pregnant mice and at 6 months of age from offspring.

In pregnant mice, blood was collected via tail sectioning. Mice were restrained in a 50 mL Falcon tube, the tail was cleaned with 75% alcohol, and a transverse section through the long axis of the tail 2 mm from the tip was made with a sterile scalpel blade. Blood dripping from the sectioned tail was collected using a capillary tube. In the

offspring, blood was collected via heart puncture at 6 months of age after sacrifice at the end of blood pressure measurements.

Two hundred micro litters were collected at one time via tail sectioning and about 1mL of blood was obtained via cardiac puncture. After about 20 min of clotting time, the sample was spun and the serum was collected and stored at -80ºC until further experiments were conducted.

Glucose blood levels were measured using OneTouch Ultra a blood glucose monitoring system (LifeScan, CA). Mice were fasted overnight (16 -18 hours). After clipping off about 2mm of tail, the first drop of blood was wiped off, and the next drop was guided into a narrow channel in the top edge of the test strip.

Commercially available kits, listed in Table 1, were used according to the manufacturer's instructions in order to determine serum levels of sFlt1, cholesterol, triglycerides, insulin, leptin, adiponectin, CRP, IL-6, and sICAM-1.

Data analysis

Data were expressed as the mean ± SEM and compared between various groups by 1-way ANOVA followed by Tukey's, Newman-Keuls, or Dunnett's Multiple Comparison Tests as appropriate (GraphPad Prism 4 software version 4.00 for Windows). A P-value <0.05 was considered statistically significant.

PROTEIN EXPRESION STUDIES

Proteins from adipose tissue and kidney were extracted after homogenization with a protease inhibitor cocktail and were quantified using a BCA Protein Assay kit (#23225, Pierce, Rockford, IL). Then, protein extracts were electropheretically separated by SDS/PAGE on 8-10% running gel and were transferred to nitrocellulose membranes, as

Table 1: Assays used to determine serum levels of different cytokine/chemokine levels

Cytokine/Chemokine	Technology	Catalog #, Manufacturer
sFlt1	Quantitative sandwich ELISA	Cat # MVR100, R&D systems, Minneapolis, MN
Cholesterol	Fluorometric assay	Cat # 10007640, Cayman, Ann Arbor, MC
Triglycerides	Calorimetric Assay	Cat # ETGA-200, BioAssay Systems. Hayward, Ca
Insulin	Luminex bead technology	Cat # MADPK-71K, Millipore, Billerica, MA
Leptin	Luminex bead technology	Cat # MADPK-71K, Millipore, Billerica, MA
Adiponectin	Luminex bead technology	Cat # MCVD277BK, Millipore, Billerica, MA
CRP	Two-site ELISA	Cat # E-90CRP, ICL, Inc. Newberg, OR
IL-6	Luminex bead technology	Cat # MPXMCYTO70KPMX32, Millipore, Billerica, MA
sICAM-1	ELISA	Cat # EMICAM1, Endogen Pierce, Woburn, MA

previously described (Kim et al., 2006). They were blocked overnight and incubated with primary polyclonal antibodies detecting Ang (52 kDa), type-I and II receptors (AT1, 47 kDa, AT2, 46 kDa) from Santa Cruz Biotechnology (Santa Cruz, CA) at 1:100, 1:200, 1:200 dilution, respectively. Beta-actin (β-actin, 42kDa) served as internal standard. The bands obtained in Western blots were scanned and then analyzed using ImageJ 1.42q software (NIH).

Data analysis

Signal intensity for the bands representing protein was measured by densitometry. Intensity values for the test proteins were normalized against those for β-actin. Data were expressed as the mean ± SEM and compared between various groups by 1-way ANOVA followed Dunnett's and Tukey's Multiple Comparison Tests as appropriate (GraphPad Prism 4 software version 4.00 for Windows). Statistical significance was defined as $P<0.05$.

Chapter 3: Results

SPECIFIC HYPOTHESIS 1

Prepregnancy obesity superimposed over sFlt-1-induced preeclampsia leads to abnormal vascular function in offspring later in life.

SPECIFIC AIM 1

To examine the cardiovascular vascular function of offspring using in vivo blood pressure measurements by telemetry and in vitro vascular responses to vasoconstrictor and vasodilator agents in the carotid artery.

Introduction

The unfavorable intrauterine environment, produced by the reduced placenta perfusion due to by the excess sFlt-1 levels in early pregnancy, is associated with fetal vascular programming that will lead to an altered vascular function in later life evidenced by hypertension and altered vascular reactivity in adult animals, and even more accentuated by exposure to maternal obesity.

Experimental design

As outlined in the Study Design section (page 23), females were first fed either a standard or a high fat diet. At 3 months of age, they were bred with males on a standard fat diet. Dams fed a high fat diet were significantly heavier, (i.e. they exhibited diet-induced obesity on day 1 of pregnancy, Figure 4).

On day 7 of pregnancy, blood was collected via tail vein. On day 8, dams were injected with adenovirus carrying either sFlt1 or mFc. Blood was collected again on days 14 and 18-19 of pregnancy. At all three time points of pregnancy, sFlt1 levels were measured. The results are presented in Figure 5. The sFlt1 levels were highest in the high

fat group mothers injected with AdsFlt1, followed by (in descending order) SFsflt1, SFmFc, and HFmFc dams. Mothers were allowed to deliver. There were no differences in average pups weight and pups numbers between four experimental groups (Figures 6, 7).

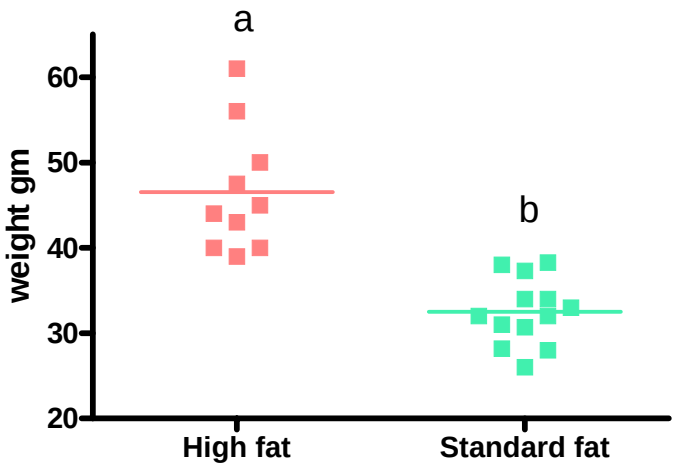

Figure 4: Maternal weight on day 1 of gestation of mice fed high fat (red, n=10) or standard fat (green, n=13) diet. Each dot represents a single animal. Line is mean value. [a,b] indicates statistically significant difference.

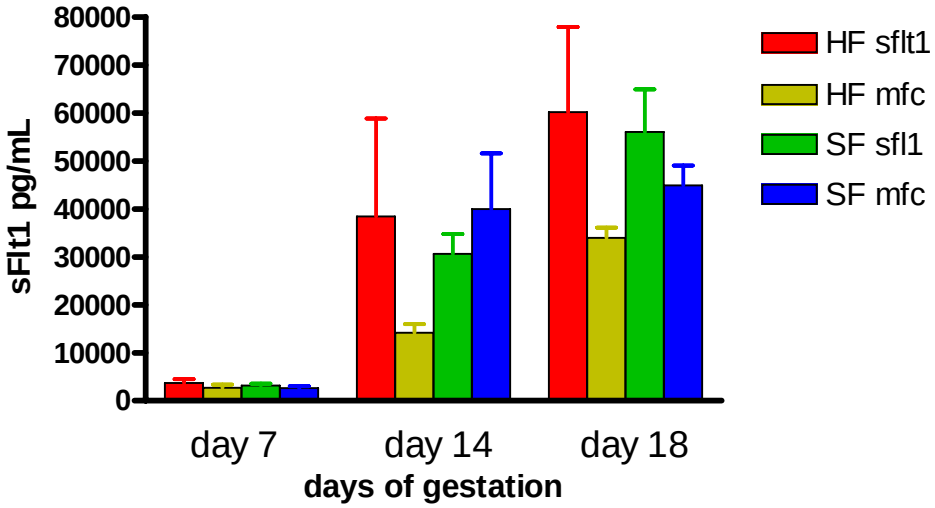

Figure 5: The sFlt1 levels in maternal blood on days 7, 14 and 18 of pregnancy accordingly to study groups: HF sFlt1 n=4, HF mFc n=4, SF sFlt1 n=5, SF mFc n=5. Bars represent mean + SEM.

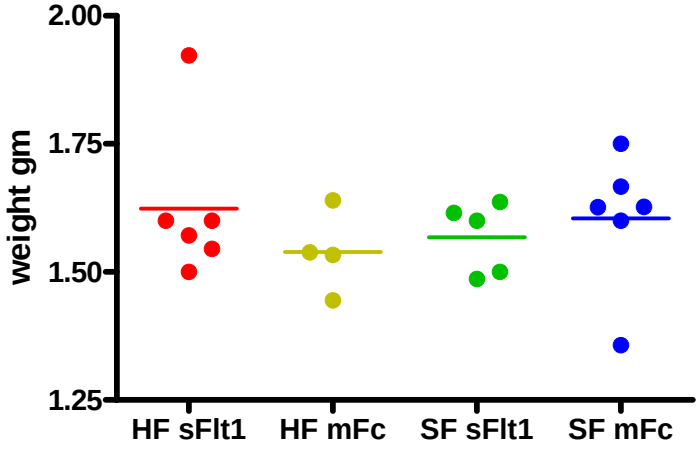

Figure 6: Average pup weight per mother at birth accordingly to study groups: HF sFlt1=6, HF mFc=4, SF sFlt1n=6, SF mFc n=6. Each dot represents pups born to the same mother. Line is a mean value. N is number of dams in each group.

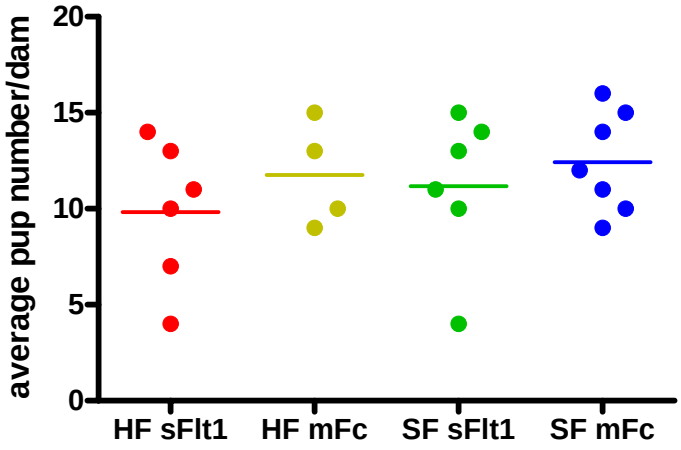

Figure 7: Average pup number per mother at birth accordingly to study groups: HF sFlt1=6, HF mFc=4, SF sFlt1n=6, SF mFc n=7. Each dot represents pups born to the same mother. Line is a mean value. N is number of dams in each group.

At 6 months of age, male and female offspring born to HF sflt1, HF mFc, SF sFlt1, and SF mFc mothers according to experimental plan outlined in Figure 1 (section "Study Design", page 24) were used for experiments. To accomplish Specific Aim 1, animals were instrumented with telemetric blood pressure transmitters. Telemetric blood pressure measurements, which allow continuous measurement of blood pressure in conscious unrestrained mice in their usual environment, have a marked advantage over other methods that require restrain and/or anesthesia (Carlson & Wyss, 2000; Mills et al., 2000; Butz & Davisson, 2001; Van Vliet et al., 2003). On day 8 after the surgery, the mice were sacrificed, blood was collected via cardiac puncture, other tissues were collected for further tests, and the right carotid artery was dissected to use for in vitro vascular reactivity experiments. Adipose tissue and the heart were dissected and weighed in order to determine adiposity and any cardiac effects, respectively.

SPECIFIC AIM 1: RESULTS

Six-Month Old Offspring Characteristics

Average body, visceral adipose tissue, and heart weights, as well as percent of adiposity, are shown in Table 2 for males and Table 3 for females.

Table 2: Characteristics of the male offspring at 6 months of age accordingly to study groups: HF sFlt1 n=8, HF mFc n=13, SF sFlt1 n=8, SF mFc n=6. Values are mean ± SEM. [a,b] indicates statistically significant differences.

Characteristic	HF sFlt-1	HF mFc	SF sFlt1	SF mFc
Weight (gm)	49.6 ± 1.6 [a]	50.8 ± 1.5 [a]	42.8 ± 1.4 [b]	41.4 ± 2.2 [b]
Weight of visceral adipose tissue (gm)	2.6 ± 0.15 [a]	2.6 ± 0.16 [a]	1.7 ± 0.28 [b]	1.1 ± 0.25 [b]
Heart weight (gm)	0.20 ± 0.006 [a]	0.20 ± 0.005 [a]	0.18 ± 0.004 [b]	0.18 ± 0.004 [b]
Percent of adipose tissue per body weight (%)	5.2 ± 0.19 [a]	5.1 ± 0.22 [a]	3.7 ± 0.57 [b]	2.8 ± 0.52 [b]

Table 3: Characteristics of the female offspring at 6 months of age accordingly to study groups: HF sFlt1 n=7, HF sFlt1 n=5, SF sFlt1 n=7, SF mFc n=7. Values are mean ± SEM. [a,b] indicates statistically significant differences.

Characteristic	HF sFlt-1	HF mFc	SF sFlt1	SF mFc
Weight (gm)	40.4 ± 1.5 [a]	42.6 ± 2.0 [a]	32.0 ± 1.9[b]	32.6 ± 1.3 [b]
Weight of visceral adipose tissue (gm)	3.1 ± 0.44 [a]	3.3 ± 0.88 [a]	1.2 ± 0.24 [b]	1.0 ± 0.19 [b]
Heart weight (gm)	0.16 ± 0.004	0.15 ± 0.006	0.15 ± 0.006	0.15 ± 0.007
Percent of adipose tissue per body weight (%)	7.6 ± 0.84 [a]	7.4 ± 1.74 [a]	2.9 ± 0.48 [b]	3.2 ± 0.61 [b]

Male offspring born to both groups of obese mothers were significantly heavier, had considerably more adipose tissue, their heart was notably larger, and their body mass had a significantly higher percentage of adipose tissue. In females, while body weight, adipose tissue and percentage of adipose tissue were significantly higher in mice born to mothers fed high fat diet, their heart weight was not significantly different between the groups.

Blood Pressure Measurements

Mean 24-hour blood pressure was measured in 6 month old offspring after exposure to prenatal maternal obesity and sFlt1-induced preeclampsia during pregnancy. Mean blood pressure (Figure 8) was significantly higher in males born to HF sFlt1 mothers when compared to other three study groups. There were no differences observed in mean blood recorded in female offspring born to HF sFlt1, HF mFc, SF sFlt1, and SF mFc mothers (Figure 9).

Vascular Reactivity Studies

Vascular reactivity studies were performed on the carotid arteries from all four groups of offspring at 6 months of age. The mice utilized for these studies were the same animals that underwent telemetric blood pressure studies as described above. Preliminary examinations in our lab have established that placement of the catheter in the left carotid artery do not affect vascular reactivity patterns in the right carotid artery.

Contractile Responses

Smooth muscle contractile response to KCl show a trend of higher sensitivity in HF sFlt1 males, followed by HF mFc and SF sFlt1 groups, with lowest response in SF mFc group (Figure 10). In females, significantly higher responses in both HF groups and

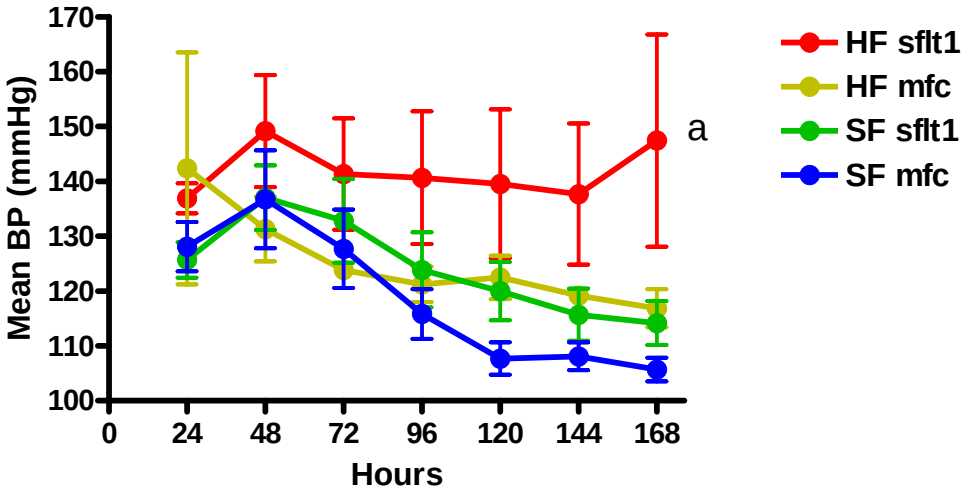

Figure 8: Comparison of mean 24-hour blood pressure in male offspring at 6 months of age according to study group: HF sFlt1 n=4, HF sFlt1 n=4, SF sFlt1 n=6, SF mFc n=6. [a] indicates statistically significant difference between HF sFlt1 group when compared to HF mfc, SF sflt1, SF mfc.

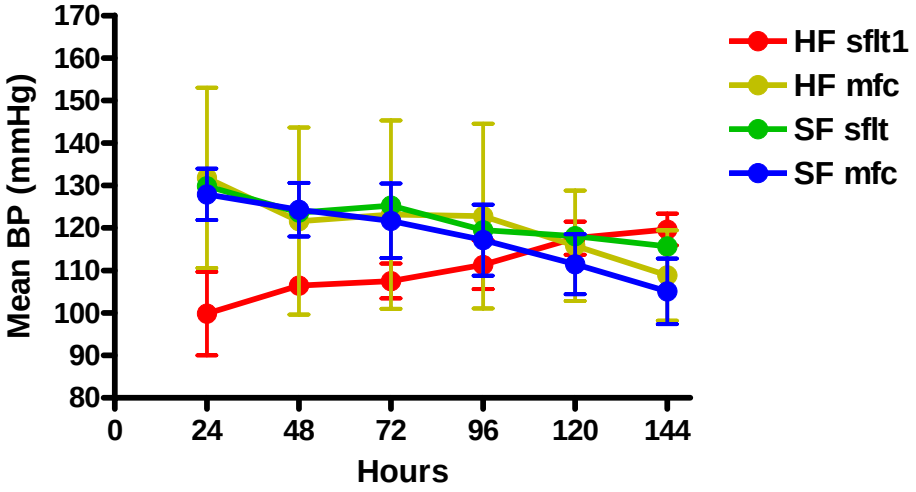

Figure 9: Comparison of mean 24-hour blood pressure in female offspring at 6 months of age according to study group: HF sFlt1 n=4, HF mFc n=4, SF sFlt1 n=4, SF mFc n=4.

SF sFlt1 group as compared to SF mFc mice indicate a high fat diet and sFlt1 related impairment in smooth muscle membrane (Figure 11). The concentration response curves to phenylephrine, thromboxane (U46619) and serotonin were obtained in the carotid artery over a dose range of 10^{-10} to 10^{-5} M. The contractile reply of the smooth muscle in the carotid artery to phenylephrine was similar in all four groups of offspring, males (Figure 12, P= 0.763) and females (Figure 13, P= 0.498). There were also no differences in AUC, logEC50 and Emax values in response to phenylephrine (data not shown).

Concentration-response curves to thromboxane are shown in Figure 14 for males and in Figure 15 for females. While overall there were no differences between male groups (P=0.9115), AUCs and log EC50 were significantly differently between males born to HF sFlt1 and SF mFc dams (Table 4). In females the only statistical difference was noted between animals born to HF mFc and sFlt1 mothers (Table 5).

In general, contractile responses to serotonin were significantly different between the four groups in males (P=0.027) and females (P=0.040). Figure 16 demonstrates that in male offspring at certain concentrations statistically significant differences were observed either between HF sFlt1 and SF sFlt1 groups or between HF mFc vs SF sFlt1 mice. The AUC was significantly higher in SF sFlt1 group when compared with others, while Emax was notably higher when comparing SF sFlt1 and HF mFc groups (table 6). In females, responses to serotonin at certain concentration and Emax were significantly different between SF sFlt1 and HF mFc groups (Figure 17 and Table 7).

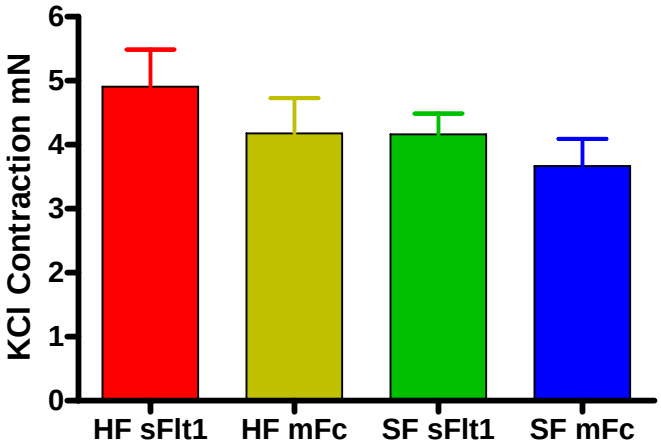

Figure 10: Contractile responses to KCl in male offspring at 6month of age according to study groups: HF sFlt1 n=8 , HF mFc n=10 , SF sFlt1 n=9 , SF mFc n=10. Values are presented as mean ± SEM.

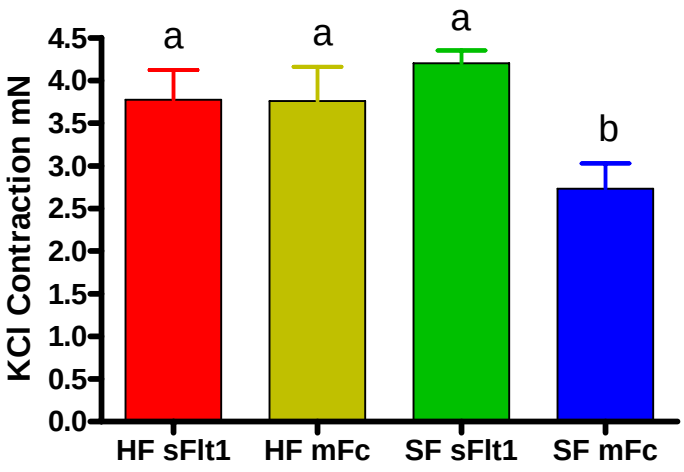

Figure 11: Contractile responses to KCl in female offspring at 6month of age according to study goups: HF sFlt1 n=8 , HF mFc n=7 , SF sFlt1 n=9 , SF mFc n=9. Values are presenet as mean ± SEM. [a,b] indicate statistically significant differences.

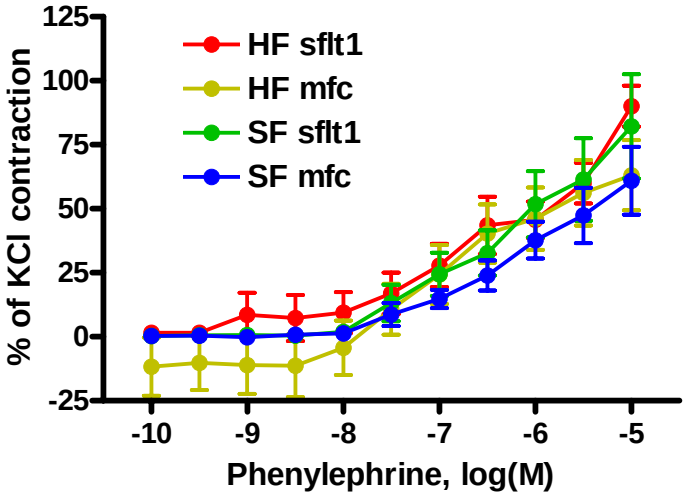

Figure 12: Contractile responses to phenylephrine in the carotid artery from male offspring according to the study group.

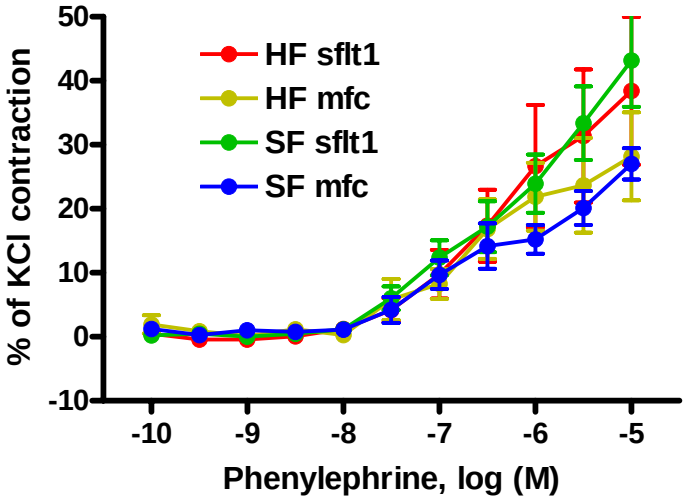

Figure 13: Contractile responses to phenylephrine in the carotid artery from female offspring according to the study group.

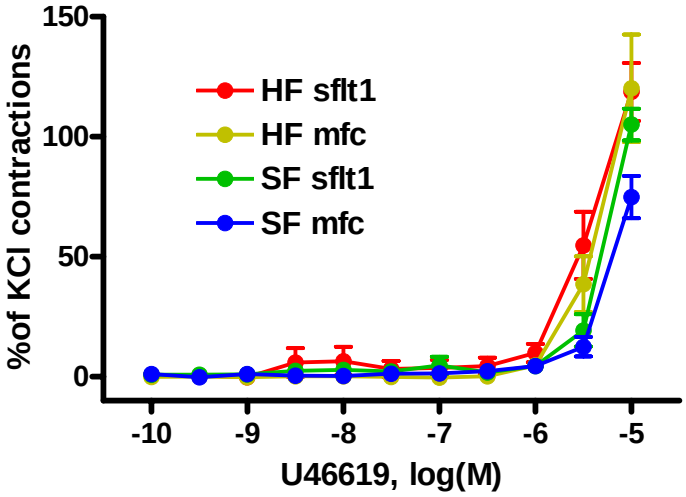

Figure 14: Contractile responses to thromboxane (U46619) in the carotid artery from male offspring according to the study group.

Table 4: Area under the thromboxane concentration-response curve (AUC, arbitrary units), maximal effect (Emax) and logarithm of molar concentration that produces log EC50 in the carotid artery of male offspring of HF sFlt1, HF mFc, SF sFlt1, and SF mFc mothers. [a,b] denotes P<0.05 for HF sFlt1 group vs SF mFc group.

Study groups	AUC	Emax	Log EC50
HF sFlt1	77.47 ± 16.55 [a]	118.7 ± 12.04	-5.05 ± 0.22 [a]
HF mFc	55.96 ± 11.56	120.2 ± 22.41	-4.93 ± 0.18
SF sFlt1	47.75 ± 7.05	105.1 ± 6.57	-4.73 ± 0.20
SF mFc	32.25 ±5.07 [b]	74.88 ± 8.77	-4.20 ± 0.25 [b]

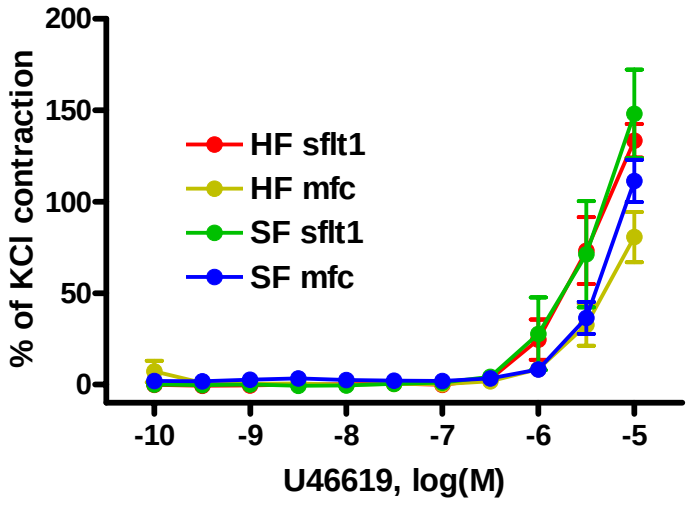

Figure 15: Contractile responses to thromboxane (U46619) in the carotid artery from female offspring according to the study group.

Table 5: Area under the thromboxane concentration-response curve (AUC, arbitrary units), maximal effect (Emax) and logarithm of molar concentration that produces log EC50 in the carotid artery of female offspring of HF sFlt1, HF mFc, SF sFlt1, and SF mFc mothers. [a,b]denotes P<0.05 for HF mFc group vs SF sFlt1 group.

Study groups	AUC	Emax	Log EC50
HF sFlt1	94.26 ± 20.51	133.3 ± 9.26	-5.12 ± 0.255
HF mFc	47.47 ± 10.03	80.68 ± 13.70 [a]	-5.97 ± 0.68
SF sFlt1	90.94 ± 31.11	148.0 ± 24.25 [b]	-5.13 ± 0.28
SF mFc	36.67 ± 6.17	111.4 ± 11.53	-4.85 ± 0.25

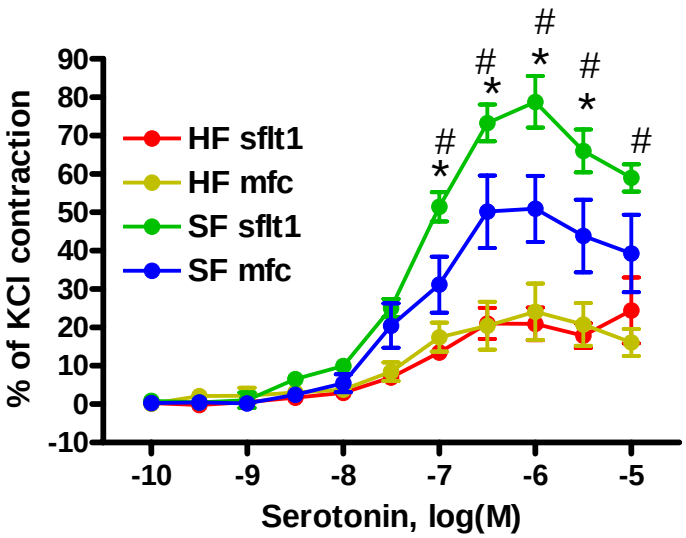

Figure 16: Contractile responses to serotonin in the carotid artery from male offspring according to the study group. * indicates statistically significant differences between HF sFlt1 and SF sFlt1 groups; #denotes statistically significant differences HF mFc vs SF sFlt1 groups.

Table 6: Area under the serotonin concentration-response curve (AUC, arbitrary units), maximal effect (Emax) and logarithm of molar concentration that produces log EC50 in the carotid artery of male offspring of HF sFlt1, HF mFc, SF sFlt1, and SF mFc mothers. [a,b] denotes P<0.05 for SF sFlt1 group vs all four other groups. [c,d] indicates statistically significant differences between SF sFlt1 and HF mFc groups.

Study groups	AUC	Emax	Log EC50
HF sFlt1	49.55 ± 6.45 [b]	24.45 ± 8.57	-6.58 ± 0.66
HF mFc	58.41 ± 13.78 [b]	16.11 ± 3.53 [d]	-8.69 ± 1.35
SF sFlt1	173.6 ± 29.05[a]	59.03 ± 12.48 [c]	-7.32 ± 0.23
SF mFc	113.0 ± 20.47 [b]	39.29 ± 10.05	-7.23 ± 0.11

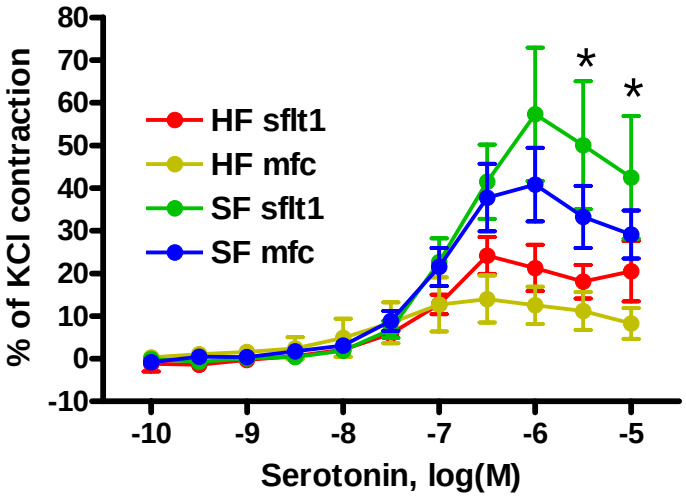

Figure 17: Contractile responses to serotonin in the carotid artery from female offspring according to the study group. * indicates statistically significant differences between SF sFlt1 and HF mFc groups.

Table 7: Area under the serotonin concentration-response curve (AUC, arbitrary units), maximal effect (Emax) and logarithm of molar concentration that produces log EC50 in the carotid artery of female offspring of HF sFlt1, HF mFc, SF sFlt1, and SF mFc mothers. [a,b] denotes P<0.05 for HF sFlt1, HF mFc and SF sFlt1 group vs SF mFc group.

Study groups	AUC	Emax	Log EC50
HF sFlt1	94.26 ± 20.51	133.3 ± 9.262	-5.12 ± 0.25 [a]
HF mFc	47.47 ± 10.03	80.68 ± 13.70	-5.97 ± 0.68 [a]
SF sFlt1	90.94 ± 31.11	148.0 ± 24.25	-5.13 ± 0.28 [a]
SF mFc	36.67 ± 6.17	111.4 ± 11.53	-4.85 ±0.25 [b]

Relaxation Responses

The concentration response curves to acetylcholine, sodium nitroprusside, and isoproterenol were obtained in the carotid artery over a dose range of 10^{-10} to 10^{-5} M after precontraction with phenylephrine (Figures 18-23). No significant differences in the response patterns to the above listed relaxants were noted between either male or female offspring born to all four groups of dams.

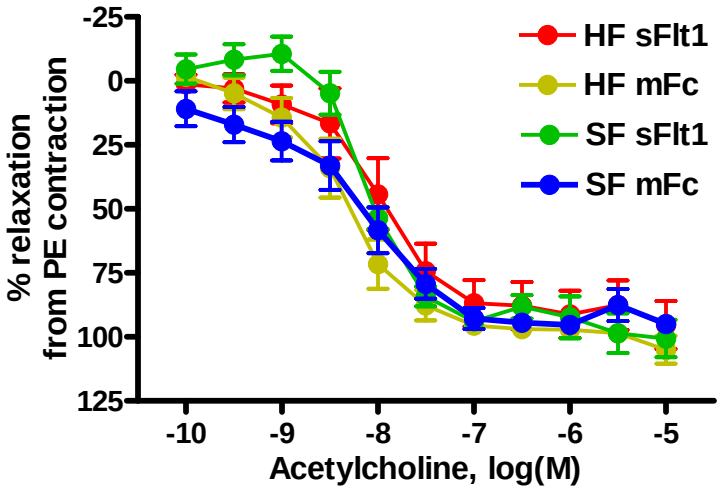

Figure 18: Relaxatory responses to acetylcholine in the carotid artery from male offspring according to the study group.

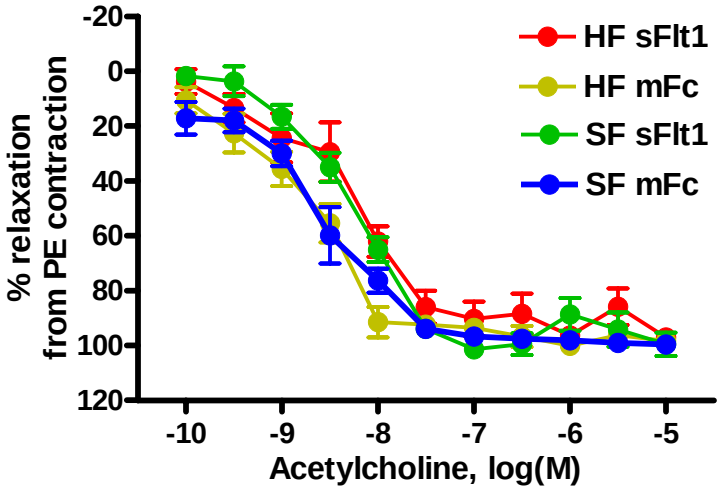

Figure 19: Relaxatory responses to acetylcholine in the carotid artery from female offspring according to the study group.

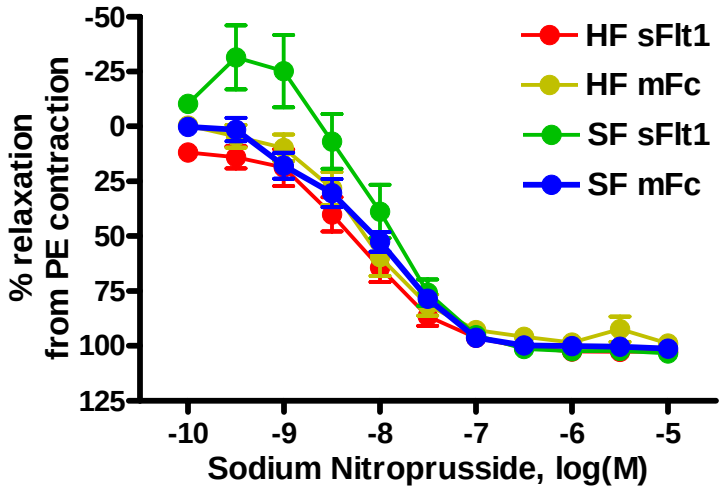

Figure 20: Relaxatory responses to sodium nitroprusside in the carotid artery from male offspring according to the study group.

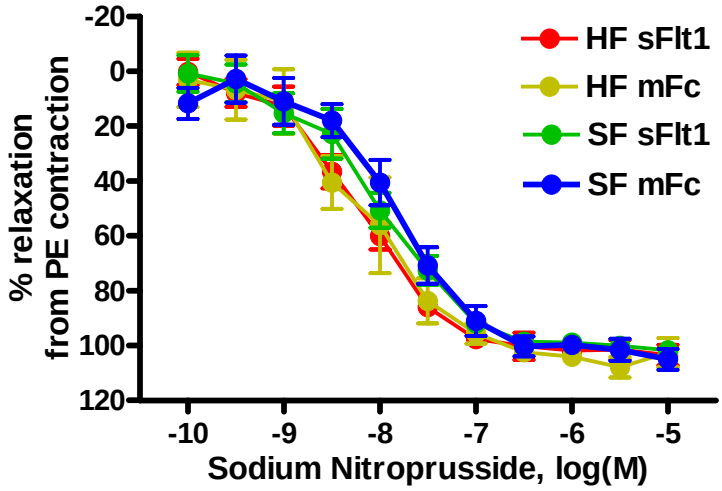

Figure 21: Relaxatory responses to sodium nitroprusside in the carotid artery from female offspring according to the study group.

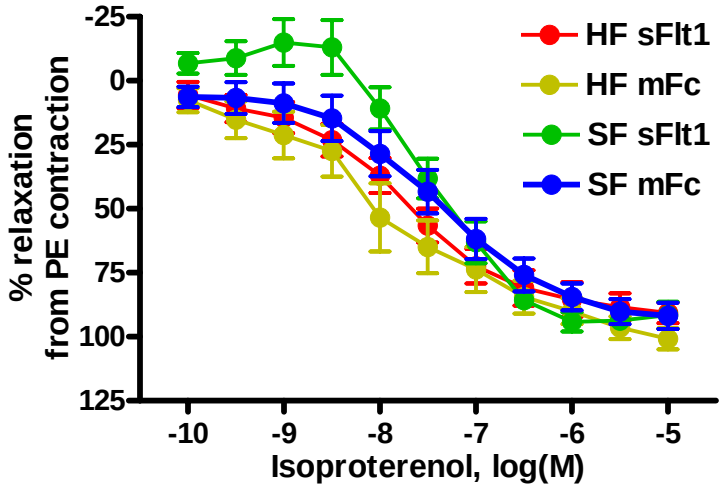

Figure 22: Relaxatory responses to isoproterenol in the carotid artery from male offspring according to the study group.

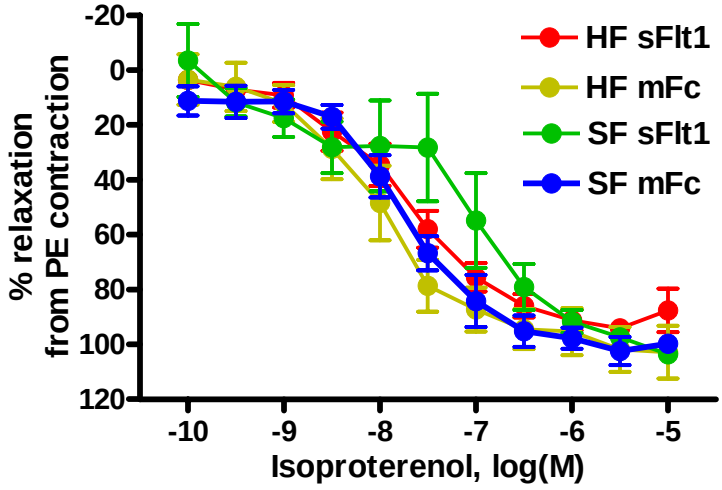

Figure 23. Relaxatory responses to isoproterenol in the carotid artery from female offspring according to the study group.

SPECIFIC AIM 1: SUMMARY

Results of experiments obtained under specific aims confirm our hypothesis that prepregnancy obesity and sFlt-1 overexpression during pregnancy plays a role in altering cardiovascular function in offspring later life. More specifically, blood pressure and vascular reactivity to contractile agents, such as KCl and serotonin are affected. Also, differences between genders were noted with increase in heart weight and impaired blood pressure observed only males. Where vascular function and heart weight is concerned, there were no differences in offspring born to obese mothers and exposed or not to sFlt1-induced preeclampsia during pregnancy, suggesting that detrimental effects of maternal obesity has more potent consequences than pregnancy complications.

SPECIFIC HYPOTHESIS 2

Prepregnancy obesity superimposed over sFlt-1-preeclampsia causes metabolic, inflammatory, and atherosclerotic syndromes in offspring later in life.

SPECIFIC AIM 2

To investigate blood concentrations of fasting glucose, total cholesterol, triglycerides, insulin, leptin, adiponectin (for metabolic syndrome), C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor α (TNF-α) for inflammatory changes, and soluble intercellular adhesion molecular-1 (sICAM-1) for atherosclerotic abnormalities of offspring using commercially available ELISAs and immunoassays.

Introduction

We hypothesize that exposure to maternal obesity and sFlt-1 overexpression during prenatal development leads to metabolic, inflammatory, and atherosclerotic alterations, which in turn modifies cardiovascular function in adult.

Experimental design

At 6 months of age, food and water was withdrawn at around 4 pm from mice in the study groups. On the next day, at 9 am, fasting glucose in blood was measured using a Glucometer Elite blood glucose meter (Ascensia; Bayer HealthCare).

Also, blood was collected via cardiac puncture after sacrifice, was allowed to coagulate for 20 min, then centrifuged, and obtained serum was -80ºC until ready for further analysis. Circulating levels of leptin, adiponectin, triglycerides, total cholesterol, CRP, IL-6, and sICAM-1 were determined in serum collected from each individual animal using commercially available kits designed specifically for mice.

SPECIFIC AIM 2: RESULTS

Blood Fasting Glucose Levels

Fasting glucose levels were significantly higher in male and female offspring in the HF sFlt1 group than in both SF group animals (Figures 24, 25).

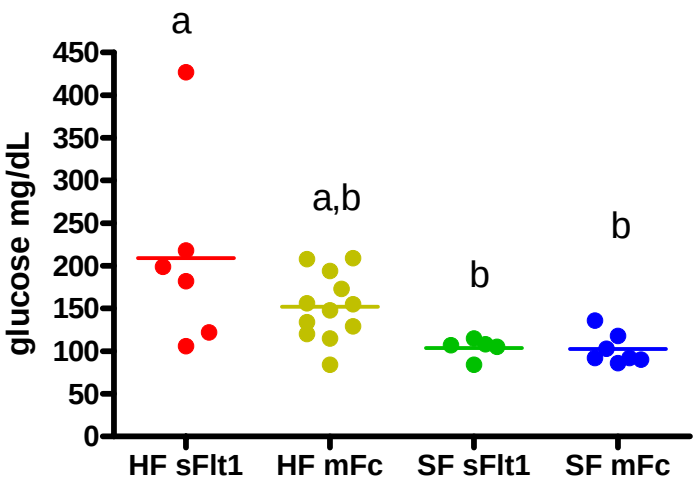

Figure 24: Fasting glucose levels in male offspring at 6 months of age according to study group: HF sFlt1 n=6, HF mFc n=5, SF sFlt1 n=12, SF mFc n=7. Each dot represents an individual animal. Line is mean value. [a,b] superscripts indicate statistically significant differences.

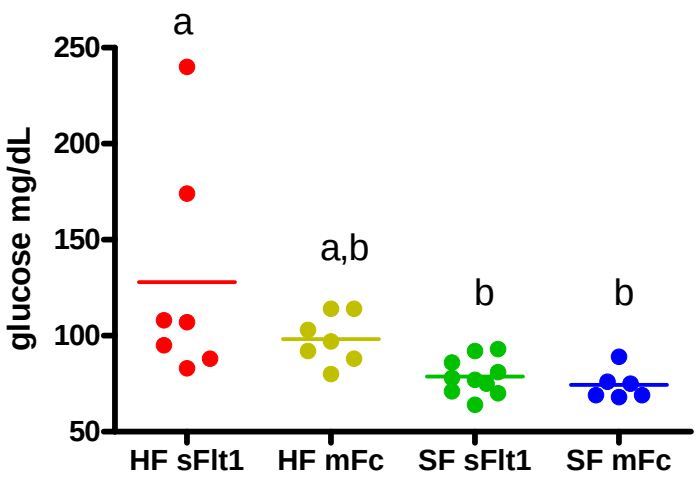

Figure 25: Fasting glucose levels in female offspring at 6 months of age according to study group: HF sFlt1 n=7, HF mFc n=7, SF sFlt1 n=10, SF mFc n=6. Each dot represents an individual animal. Line is mean value. [a,b] superscripts indicate statistically significant differences.

Serum Total Cholesterol Levels

Total cholesterol levels in males were significantly higher in the HF sFlt1 group animals than both SF groups (Figure 26). In females, there were no differences observed between HF groups, however, values were significantly higher from both SF groups, and even females born to mothers with sFlt-1-induced preeclampsia had significantly higher levels of total cholesterol than the ones born to mothers of the adenovirus control group (SF mFc, Figure 27).

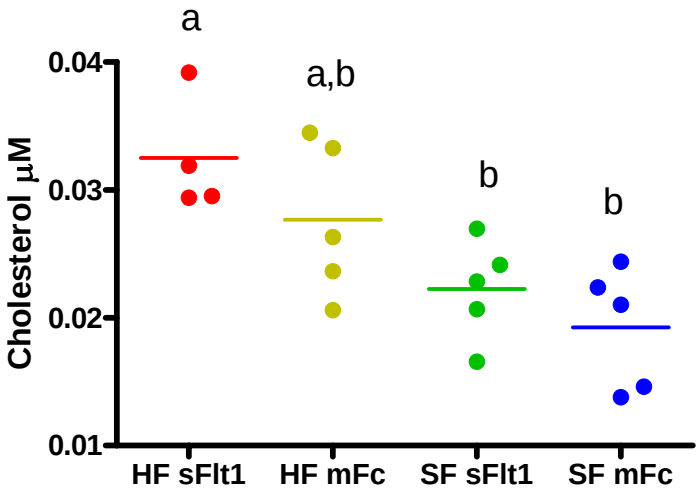

Figure 26: Total cholesterol levels in male offspring at 6 months of age according to study group: HF sFlt1 n=4, HF mFc n=5, SF sFlt1 n=5, SF mFc n=5. Each dot represents an individual animal. Line is mean value. [a,b] superscripts indicate statistically significant differences.

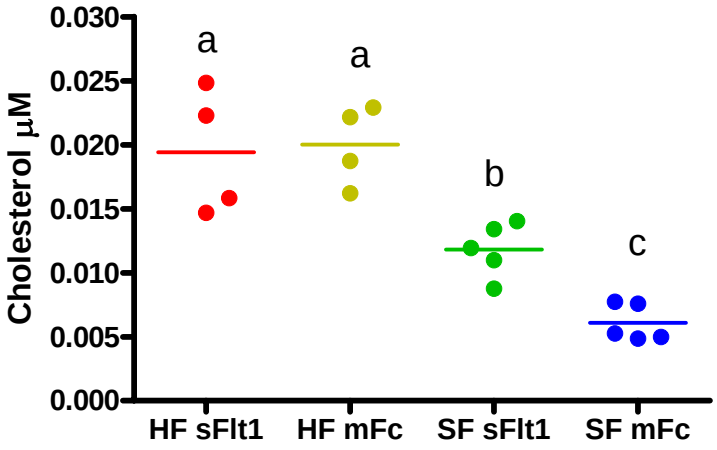

Figure 27: Total cholesterol levels in female offspring at 6 months of age according to study group: HF sFlt1 n=4, HF mFc n=4, SF sFlt1 n=5, SF mFc n=5. Each dot represents an individual animal. Line is mean value. [a,b] superscripts indicate statistically significant differences.

Serum Triglyceride Levels

Triglyceride levels had an opposite gender pattern than total cholesterol levels (Figures 28, 29). In females significantly higher levels were determined in both HF groups when evaluated against both SF groups. In males, triglyceride values, though significant higher from SF groups, were no different between HF groups. However, in SF groups, significantly higher triglyceride values were noted in sFlt1 males.

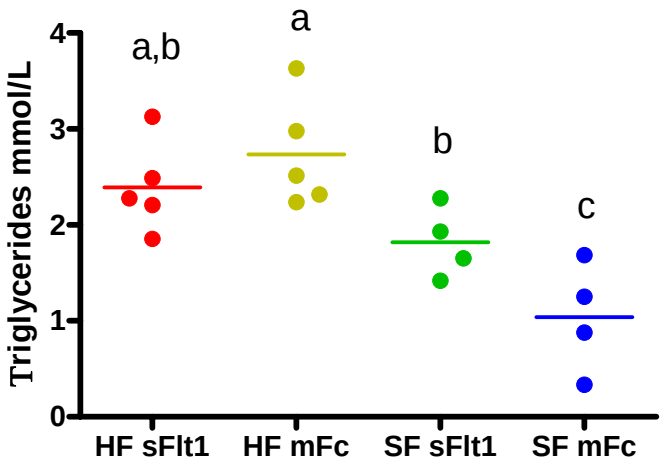

Figure 28: Triglyceride levels in male offspring at 6 months of age according to study group: HF sFlt1 n=5, HF mFc n=5, SF sFlt1 n=4, SF mFc n=4. Each dot represents an individual animal. Line is mean value. [a,b,c] superscripts indicate statistically significant differences.

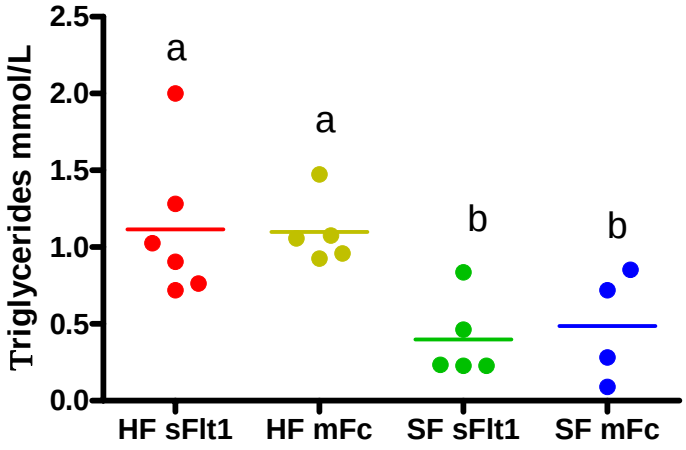

Figure 29: Triglyceride levels in female offspring at 6 months of age according to study group: HF sFlt1 n=6, HF mFc n=5, SF sFlt1 n=5, SF mFc n=4. Each dot represents an individual animal. Line is mean value. [a,b] superscripts indicate statistically significant differences.

Serum Insulin Levels

Insulin levels were significantly higher in male offspring from both HF groups in comparison to SF groups (Figure 30). There were no differences in female study groups (Figure 31).

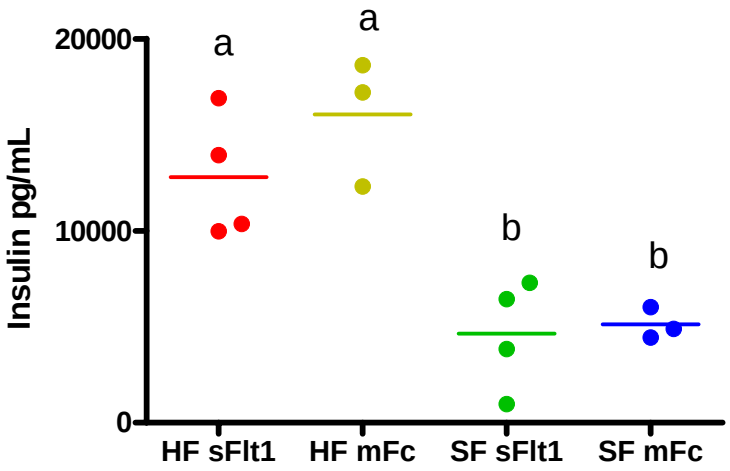

Figure 30: Insulin levels in male offspring at 6 months of age according to study group: HF sFlt1 n=4, HF mFc n=3, SF sFlt1 n=4, SF mFc n=3. Each dot represents an individual animal. Line is mean value. [a,b] superscripts indicate statistically significant differences.

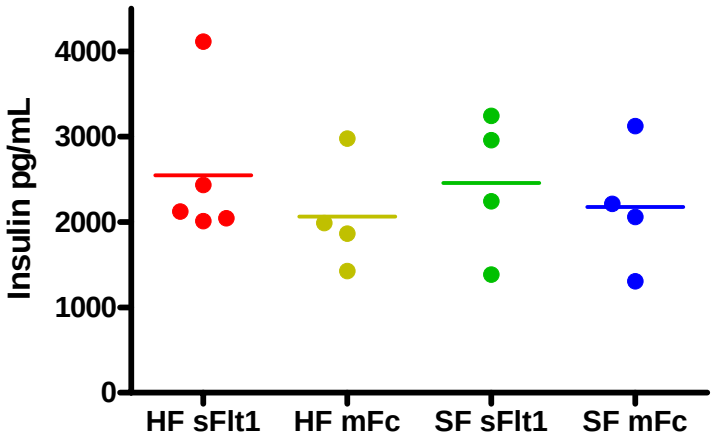

Figure 31: Insulin levels in female offspring at 6 months of age according to study group: HF sFlt1 n=4, HF mFc n=4, SF sFlt1 n=4, SF mFc n=4. Each dot represents an individual animal. Line is mean value.

Serum Leptin Levels

Leptin levels were no different between male offspring in all four study groups with a trend to be higher in HF groups (Figure 32). In females, leptin levels were significantly higher in HF group mice when compared with SF mFc group (Figure 33).

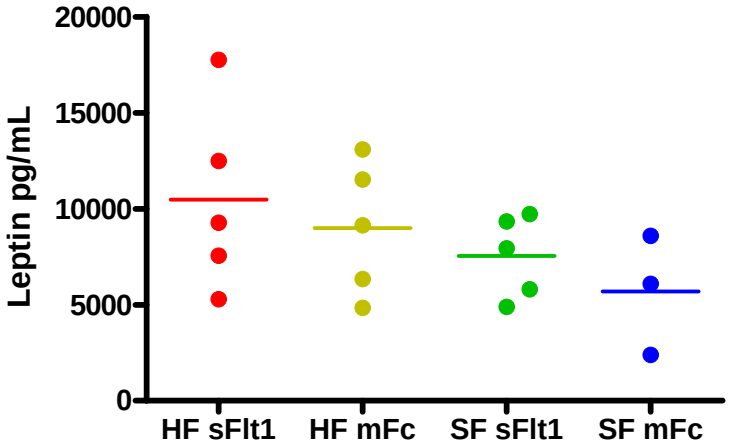

Figure 32: Leptin levels in male offspring at 6 months of age according to study group: HF sFlt1 n=5, HF mFc n=5, SF sFlt1 n=5, SF mFc n=3. Each dot represents an individual animal. Line is mean value.

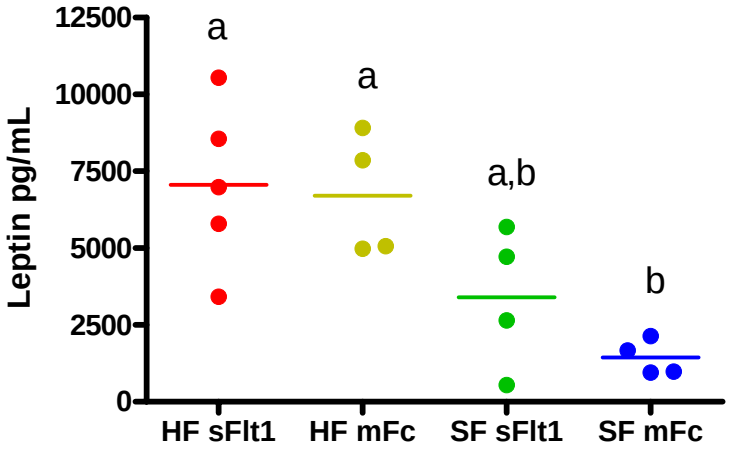

Figure 33: Leptin levels in female offspring at 6 months of age according to study group: HF sFlt1 n=5, HF mFc n=4, SF sFlt1 n=4, SF mFc n=4. Each dot represents an individual animal. Line is mean value. [a,b] superscripts indicate statistically significant differences.

Serum Adiponectin Levels

Adiponectin levels were significantly lower in males in both HF groups (Figure 34). A similar trend was observed in females; however, values were not significantly different between the groups (Figure 35).

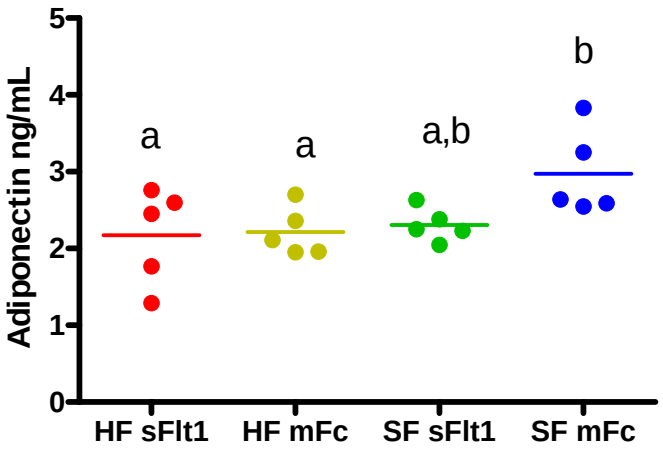

Figure 34: Adiponectin levels in male offspring at 6 months of age according to study group: HF sFlt1 n=5, HF mFc n=5, SF sFlt1 n=5, SF mFc n=5. Each dot represents an individual animal. Line is mean value. [a,b] superscripts indicate statistically significant differences.

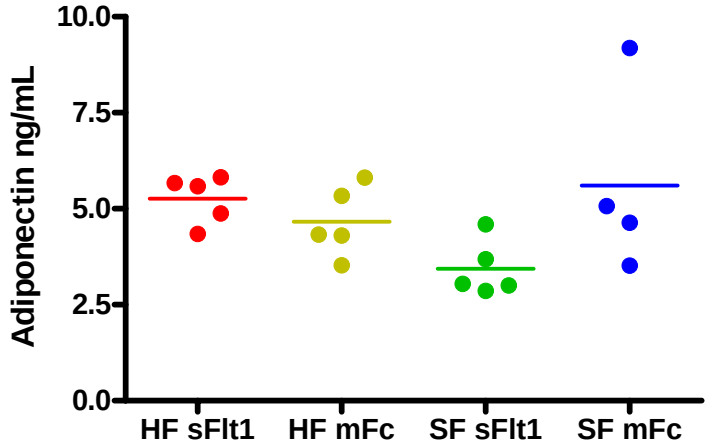

Figure 35: Adiponectin levels in female offspring at 6 months of age according to study group: HF sFlt1 n=5, HF mFc n=5, SF sFlt1 n=5, SF mFc n=4. Each dot represents an individual animal. Line is mean value.

Serum CRP Levels

CRP levels in male offspring serum were the highest in the HF sFlt1 group, followed by significantly lower levels in HF mFc group, and then both SF groups (Figure 36). In females, only the HF sFlt1 group had significantly higher levels of CRP as compared to all others (Figure 37).

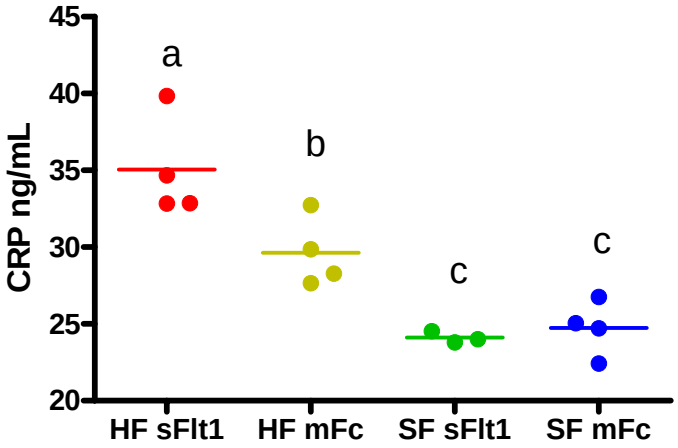

Figure 36: CRP levels in male offspring at 6 months of age according to study group: HF sFlt1 n=4, HF mFc n=4, SF sFlt1 n=3, SF mFc n=4. Each dot represents an individual animal. Line is mean value. [a,b,c] superscripts indicate statistically significant differences.

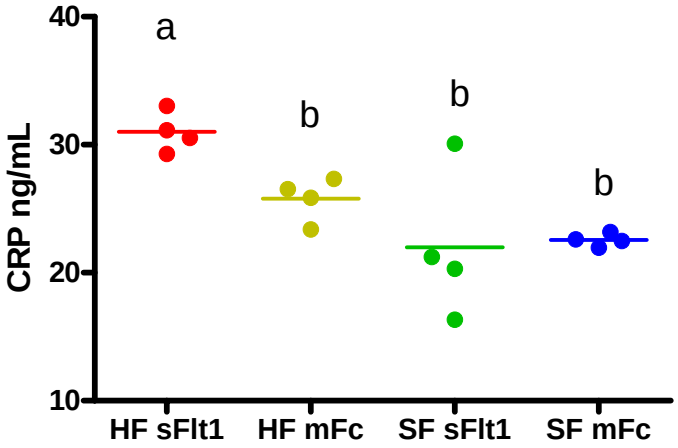

Figure 37: CRP levels in female offspring at 6 months of age according to study group: HF sFlt1 n=4, HF mFc n=4, SF sFlt1 n=4, SF mFc n=4. Each dot represents an individual animal. Line is mean value. [a,b] superscripts indicate statistically significant differences.

Serum IL-6 Levels

No statistically significant differences were determined in IL-6 levels in serum from males (Figure 38) females (Figure 39) in all four study groups.

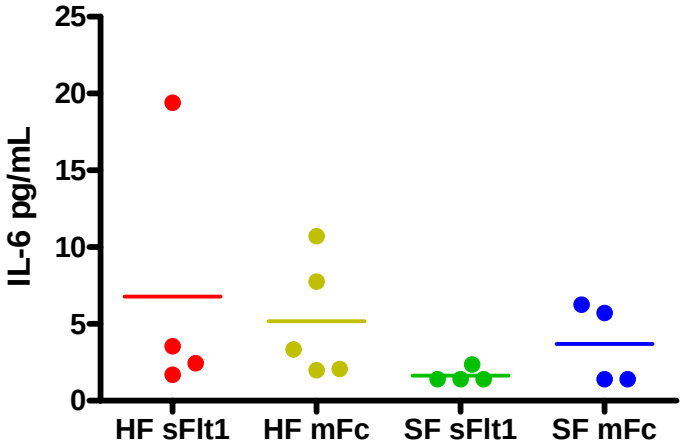

Figure 38: IL-6 levels in male offspring at 6 months of age according to study group: HF sFlt1 n=4, HF mFc n=5, SF sFlt1 n=5, SF mFc n=5. Each dot represents an individual animal. Line is mean value.

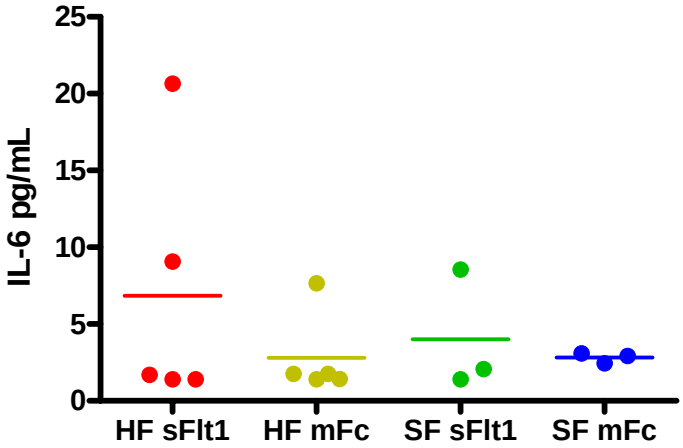

Figure 39: IL-6 levels in female offspring at 6 months of age according to study group: HF sFlt1 n=5, HF mFc n=5, SF sFlt1 n=5, SF mFc n=4. Each dot represents an individual animal. Line is mean value.

Serum sIACM-1 Levels

Soluble IACM-1 levels were similar in all groups of male offspring (Figure 40). In females, significantly higher levels were determined in the HF group when compared to the adenovirus control group (SF mFc), and only the HF mFc group had significantly higher levels when compared to SF sFlt1 females (Figure 41).

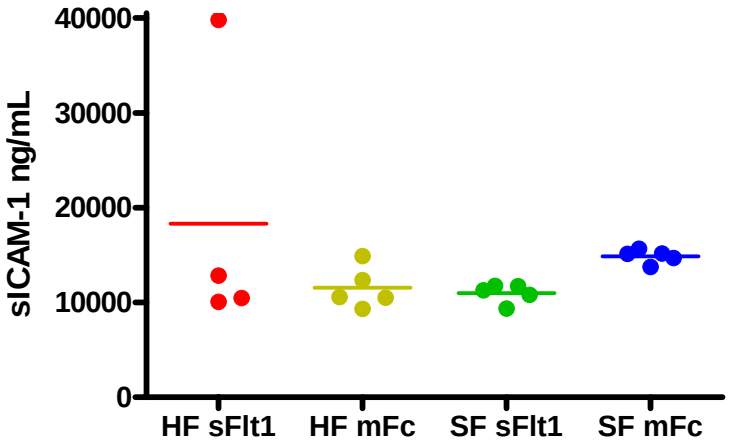

Figure 40: sICAM-1 levels in male offspring at 6 months of age according to study group: HF sFlt1 n=4, HF mFc n=5, SF sFlt1 n=5, SF mFc n=5. Each dot represents an individual animal. Line is mean value.

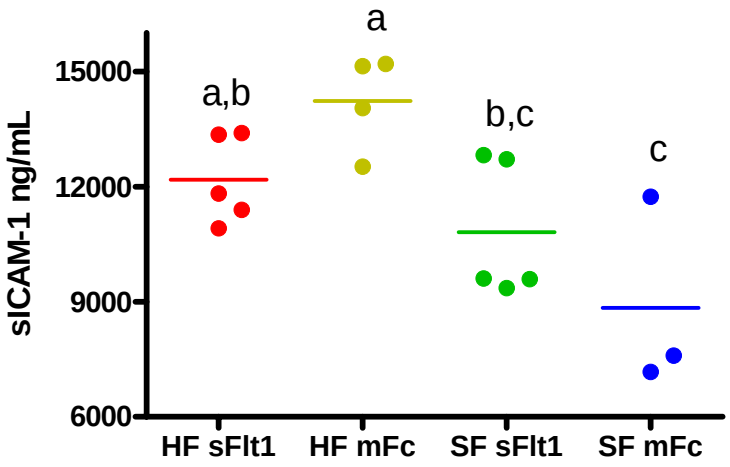

Figure 41: sICAM-1 levels in female offspring at 6 months of age according to study group: HF sFlt1 n=5, HF mFc n=4, SF sFlt1 n=5, SF mFc n=3. Each dot represents an individual animal. Line is mean value. [a,b,c] superscripts indicate statistically significant differences.

SPECIFIC AIM 2: SUMMARY

Exposure to maternal prepregnancy obesity and sFlt1-induced preeclampsia during pregnancy alter offspring metabolic, inflammatory and atherosclerotic profiles later in life as shown in results of Specific Aim 2 experiments. While obesity seems to have more detrimental effect on serum levels of the substances studied, introduction of sFtl1 also influence health of offspring later in life.

SPECIFIC HYPOTHESIS 3

Prepregnancy obesity superimposed over sFlt-1-preeclampsia leads to the changes in rennin-angiotensin system that in turn affects cardiovascular system in offspring.

SPECIFIC AIM 3

To determine the protein levels of angiotensin II (Ang II), angiotensin receptor 1 and 2 (AT1 and AT2) in offspring's adipose tissue and kidneys.

Introduction

Rennin-angiotensin system (RAS) plays a major role in the control of vascular function. Therefore, we hypothesized that prenatal exposure to maternal obesity and sFlt-1 overexpression could affect the regulation in RAS, and cause overproduction of Angiotensin II and an increase in AT1 and AT2 receptor expression. Human and animal models had shown that Ang II is not only produced by liver and kidney, but by visceral adipose tissue as well (Massiera et al., 2001).

Experimental design

At 6 months of age, visceral adipose tissue and kidneys were collected from offspring after sacrifice. Proteins were extracted from 4 animals per group and used to determine Ang II, AT1 and AT2 expressions (52 kDa, 47 kDa, 46 kDa, respectively) by Western blot analysis. The densitometric intensity of each band was normalized to β-actin detected in the same membrane.

SPECIFIC AIM 3: RESULTS

Expressions of Ang II, AT1, and AT2 were not statistically significant different between the groups of experimental animals. The following is description of the trends observed in these studies.

Analysis of Ang II expression in kidney revealed no differences between males and females: increase in Ang II expression in both HF groups was observed (Figures 42-45). Down regulation of AT1 receptors were observed in SF mFc males and HF sFlt1 females. Lower expression of AT2 protein was observed in both HF groups of males when compared to SF groups with no differences between female groups.

Experiments in adipose tissue demonstrate that in males from HF sFlt1 group Ang II is up regulated in comparison with other 3 groups (Figures 46, 47). In females, interestingly, Ang II has the highest expression in SF mFc, followed in descending order by SF sFlt1, HF mFc, with the lowest expression in HF sFlt1 group (Figures 48, 49). No differences were determined in AT1 expression between groups in males and females; AT2 is notably down regulated in adipose tissue from males in both HF groups and highly expressed in both SF groups. No differences were observed in female groups in AT2 expressions from adipose tissue.

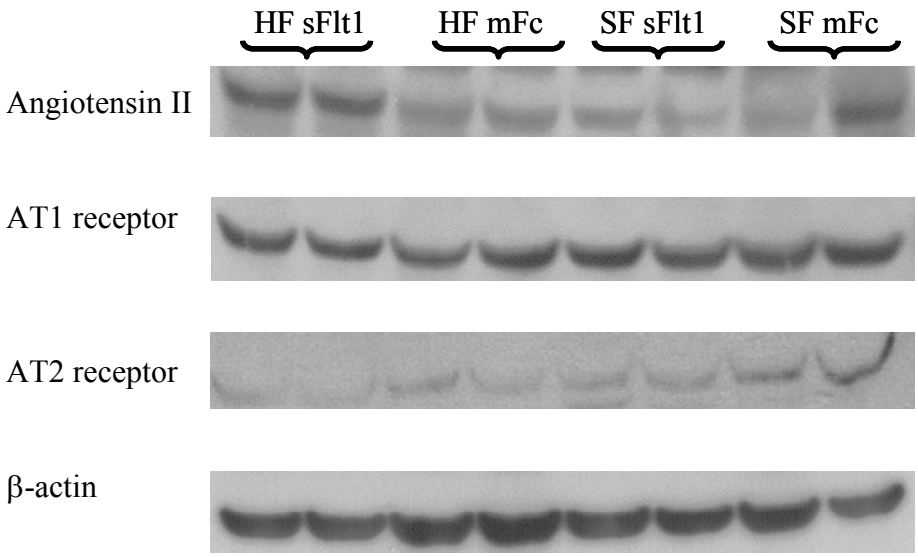

Figure 42: Expression of RAS proteins in kidney tissue of male offspring accordingly to the study groups. Representative Western blots of Ang II, AT1 and AT2 proteins are shown. A β-actin is included as an internal control.

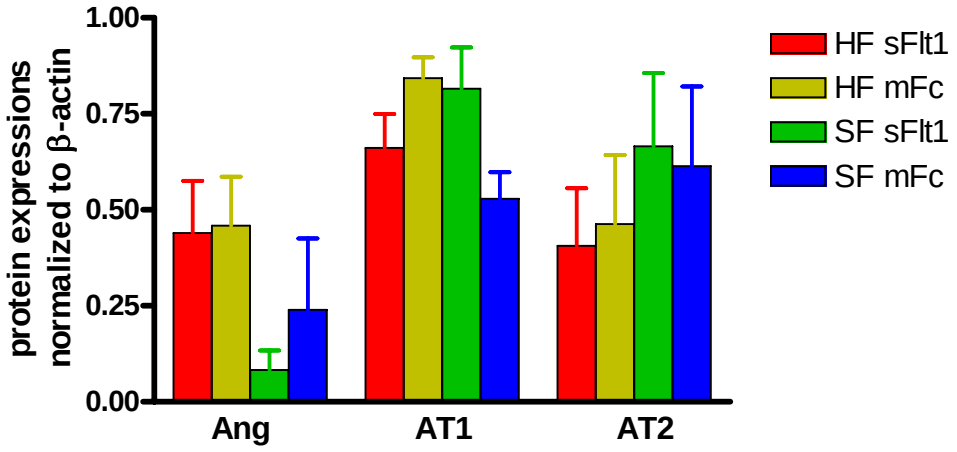

Figure 43: Densitometric analysis of Ang II, AT1, AT2 bands proteins in kidney tissue of male offspring accordingly to the study groups. Data are mean ± SEM.

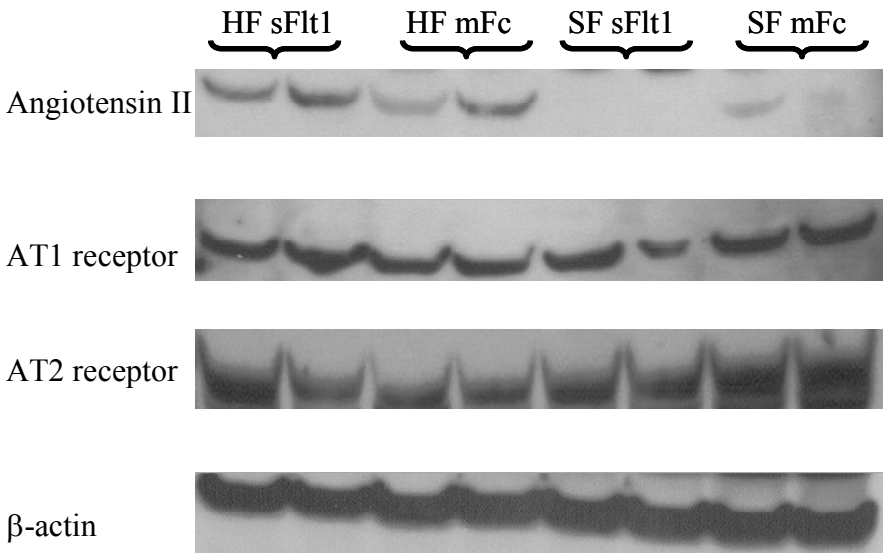

Figure 44: Expression of RAS proteins in kidney tissue of female offspring accordingly to the study groups. Representative Western blots of Ang II, AT1 and AT2 proteins are shown. A β-actin is included as an internal control.

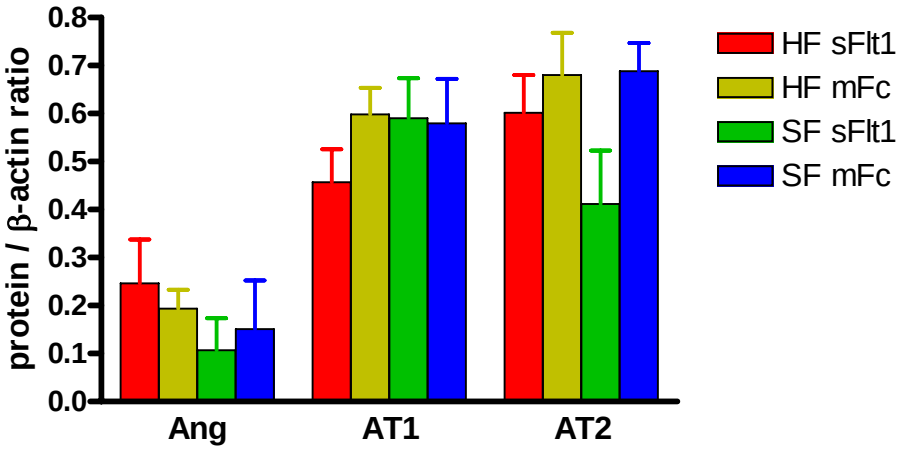

Figure 45: Densitometric band analysis of Ang II, AT1 and AT2 proteins in kidney tissue of female offspring accordingly to the study groups. Data are mean ± SEM.

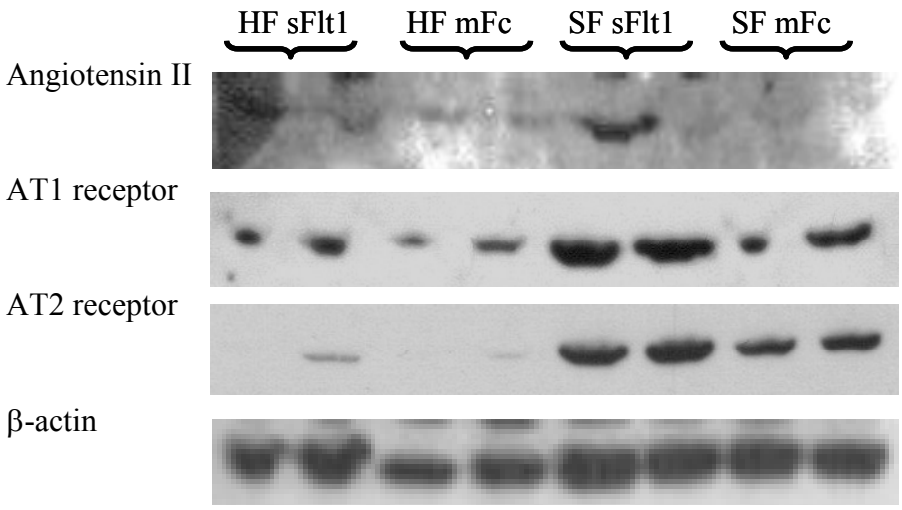

Figure 46: Expression of RAS proteins in adipose tissue of male offspring accordingly to the study groups. Representative Western blots of Ang II, AT1 and AT2 proteins are shown. A β-actin is included as an internal control.

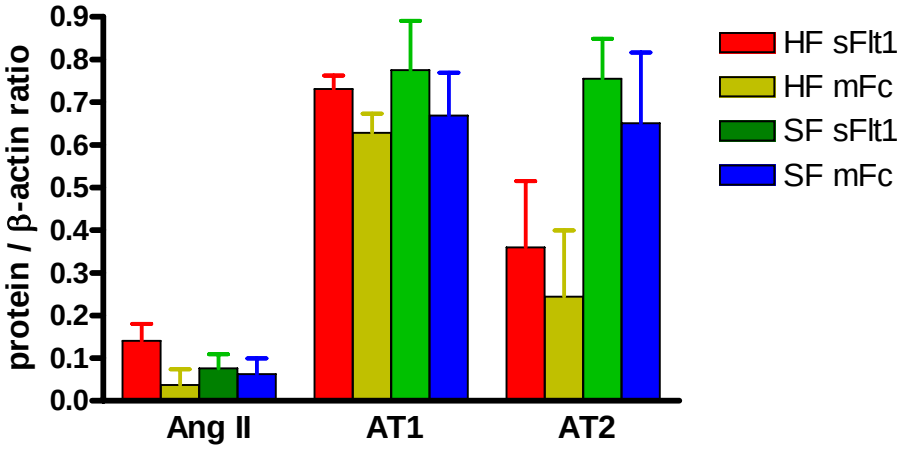

Figure 47: Densitometric band analysis of Ang II, AT1 and AT2 proteins in adipose tissue of male offspring accordingly to the study groups. Data are mean ± SEM.

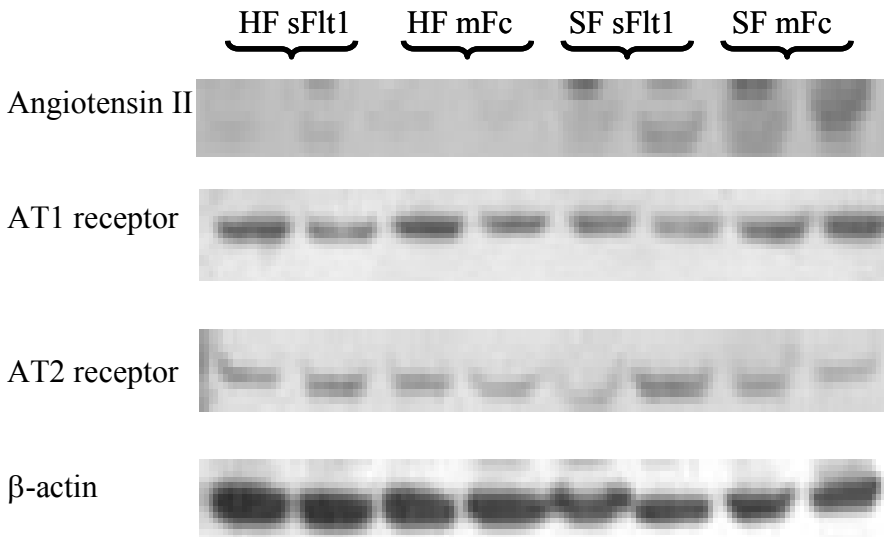

Figure 48: Expression of RAS proteins in adipose tissue of female offspring accordingly to the study groups. Representative Western blots of Ang II, AT1 and AT2 proteins are shown. A β-actin is included as an internal control.

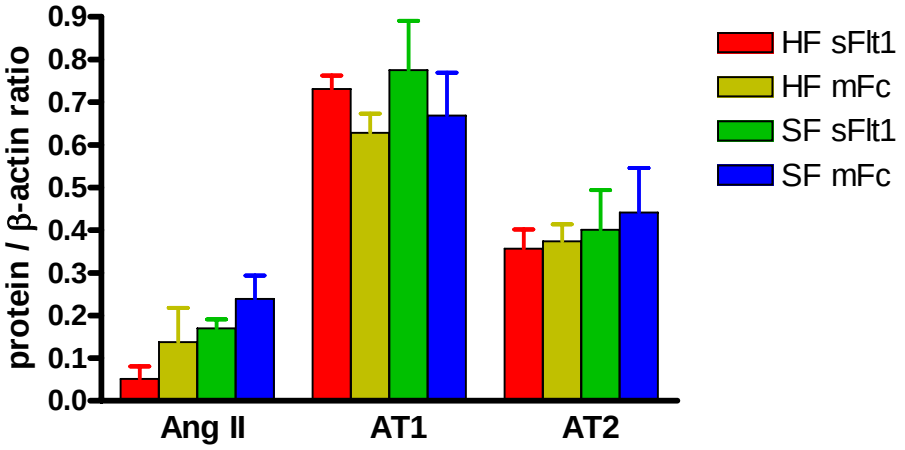

Figure 49: Densitometric analysis of Ang II, AT1 and AT2 proteins bands in adipose tissue of female offspring accordingly to the study groups. Data are mean ± SEM.

SPECIFIC AIM 3: SUMMARY

Exposure to pregnancy obesity and sFlt1-induced preeclampsia affects expression of RAS proteins. Offspring born to mothers fed high fat diet demonstrated higher levels of Ang II expression in kidneys. The most notable changes were determined in adipose tissue. Up regulation of Ang II in males from HF sFlt1 group could explain significantly higher blood pressure in this group as found in experiments of Specific Aim1. Some previous studies demonstrate that AT2 has an opposing effect to AT1 receptor on blood pressure. Our results on AT2 expressions are in line with this implication demonstrating a down regulation of AT2 in adipose tissue in males from HF groups with no differences in AT1. This observation could also explain a higher blood pressure in HF sFlt1 group males. In summary, studies in Specific Aim 3 revealed interesting trends about the role of RAS in the development of cardiovascular dysfunction in offspring later in life, which, certainly, needs further consideration.

Chapter 4: Discussion

OVERALL SUMMARY

In this study, we have tested the hypothesis that exposure to prepregnancy obesity and sFlt1-induced preeclampsia leads to alterations in the cardiovascular system in offspring later in life. This general hypothesis was tested in a mouse model. Increased blood pressure, changes in vascular reactivity and metabolic and inflammatory blood profiles, as well as modifications in expressions of angiotensin II and its receptors, indicate that a high fat maternal diet and exposure to sFlt1 overexpression during pregnancy have a negative effect on offspring cardiovascular health. The results of the study confirm that our general and specific hypotheses were correct. To our knowledge, this is the first experimental study that explores the impact of preeclampsia and prepregnancy obesity on offspring's long-term vascular function. The same animal model could be further utilized to develop potential treatments and/or interventions.

LIMITATIONS OF THE STUDY

Several limitations of the project need to be addressed: The results are limited to just one age group. Food and calorimetric intake was not recorded. In vascular reactivity experiments (Specific Aim 1), we investigated the contractile and relaxant properties of the carotid arteries, which represent conduit vessels and which control blood flow to the brain. Abnormalities may occur in resistance vessels, such as mesenteric arteries, and contribute to increased blood pressure, as well as endothelium dysfunction, which is a highlight of obesity, and which was not noted in our experiments in carotid arteries, representing conduit vessels.

And as always with animal models, a major limitation is extrapolation of findings to humans, especially since there are no published studies addressing the double burden of maternal obesity and exposure to preeclampsia on human offspring.

FEASIBLE MECHANISMS OF PREGRAVIDA OBESITY AND PRENATAL SFLT-1 OVEREXPRESSION INDUCED CARDIOVASCULAR DYSFUNCTION IN OFFSPRING

To summarize the potential mechanisms observed in this study, development of cardiovascular function impairment in the offspring exposed to prepregnancy obesity and sFlt-1 overexpression during prenatal development, we developed the scheme shown in Figure 50.

Cardiovascular alterations determined during our experiments could develop due to impairments in metabolic, inflammatory, and atherosclerotic profiles and rennin-angiotensin system.

Metabolic

Numerous models of maternal dietary imbalance have been developed in order to study the effect of maternal nutrient restriction. After exposure to a maternal fat-rich diet, offspring developed metabolic syndrome, manifested as hyperglycemia, hyperinsulinemia, glucose intolerance, elevated plasma concentrations of triglyceride, cholesterol, leptin and adiponectin, increased body weight and adiposity (Khan et al., 2003; Khan et al., 2004; Samuelsson et al., 2008). All of these factors affect the cardiovascular system by impairing endothelial function. In our study, maternal obesity and sFlt1 overexpression resulted in overweight and obese offspring with increased cholesterol, triglyceride, insulin, leptin, and decreased adiponectin levels in the blood and hypertension detected in males. We did not observe alterations in endothelial function, the most emphasized consequence of obesity causing hypertension. This could be

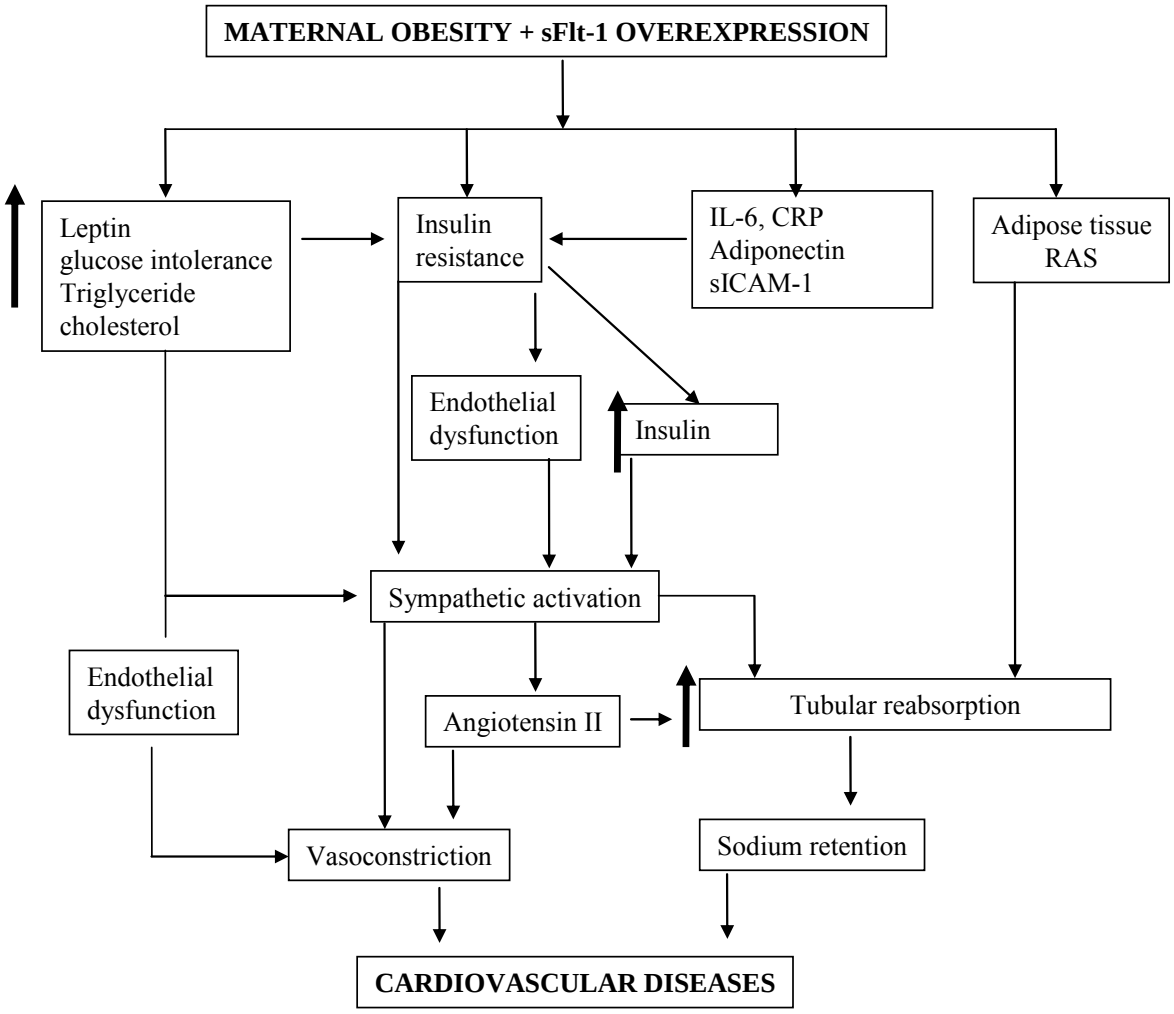

Figure 50. Proposed pathway for the development of the cardiovascular dysfunction in described model.

explained by differences in the vascular beds studied – we studied carotid artery reactivity, while others examined mesenteric arteries. Raised blood pressure, though, in our model, could be attributed to increased fat mass due to circulating hyperleptinemia, causing increased sympathetic renal activity and affecting arterial pressure (Rahmouni et al., 2005). There is one other important cause of hypertension – exposure to sFlt1 during

pregnancy. Previous studies from our lab demonstrated that males, but not females, born to sFlt1-injected mothers, had a significantly higher blood pressure, possibly, due to vascular endothelial and smooth muscle contraction impairments (Lu et al., 2007b, c). The current study revealed an impairment of vessel walls with lower reactivity to thromboxane and serotonin, which might reflect an increased arterial stiffness due to obesity-related sympathetic overactivity and hyperleptinemia (Grassi & Giannattasio, 2005). Therefore, increased blood pressure in our model could be attributed to several factors affected by exposure of the fetus to maternal obesity and sFlt1 overexpression.

Inflammatory

Overall, inflammation of the vascular cell wall is the key problem in cardiovascular diseases, and proinflammatory cytokines and chemokines play a great role in it. Inflammatory biomarkers, including C-reactive protein (CRP) and IL-6, are independent predictors of CVD (Ridker et al., 2000a, b, c; Pearson et al., 2003). Interleukin-6 is multifunctional cytokine acting on many cells and tissues. Increased levels of IL-6 have been observed in adipose tissue from obese humans.(Bastard et al., 2006) IL-6 may induce endothelial expression of chemokines and adhesion molecules (Romano et al., 1997). IL-6 induces hepatic CRP production. IL-6 has been proposed to play a central role in the link between obesity, inflammation, and coronary heart diseases (Yudkin et al., 2000). IL-6 stimulates the hypothalamic-pituitary-adrenal axis, activation of which is associated with central obesity, hypertension and insulin resistance (Yudkin et al., 2000). IL-6 also interferes with insulin signaling in adipose tissue, thus promoting insulin resistance (Rotter et al., 2003).

CRP is considered as one of the strongest predictors of future cardiovascular risk. CRP is primarily produced by the liver in response to other inflammatory cytokines,

particularly, IL-6. Therefore, in obesity, increased production of IL-6 would induce hepathic CRP synthesis. Data suggesting that CRP may play a direct role in atherogenesis have been observed in arterial plaque and causes alterations to cultured endothelial cells (Pasceri et al., 2000; Torzewski et al., 2000). CRP directly affects endothelial function by changing NO availability – exposure to CRP leads to a marked downregulation of eNOS mRNA and protein expression. Diminished NO bioactivity, in turn, inhibits angiogenesis, an important compensatory mechanism in chronic ischemia. Through decreasing NO synthesis, CRP may facilitate the development of diverse cardiovascular diseases (Verma et al., 2002).

We observed an overall decreasing trend as follows: HFsFlt1>HF mFc> SF sFlt1> SF mFc in serum levels of IL-6 and CRP, with CRP demonstrating statistically significant difference. Therefore, inflammatory mechanisms are involved in the development of cardiovascular dysfunction in offspring in the described animal model. The data demonstrate that maternal obesity, in combination with exposure to sFlt1-induced preeclampsia, leads to potentially detrimental consequences in offspring.

Atherosclerotic

Recruitment of inflammatory cells and their transendothelial migration occur during early phases of atherosclerosis. This process predominantly is mediated by cellular adhesion molecules, such as ICAM-1 and VCAM-1, which are expressed on the endothelium. It has been reported that baseline soluble ICAM-1 levels correlate with risk of cardiovascular events in apparently healthy men and women (Ridker et al., 1998; Ridker et al., 2000b). sICAM-1 levels are also increased during obesity and are positively correlated with central adiposity and insulin resistance, two conditions which also

increase the risk of cardiovascular diseases (Straczkowski et al., 2002; Leinonen et al., 2003;).

The results of sIAM-1 experiments in our study demonstrating that an increase in sICAM-1 levels is seen in both HF groups of females, with no differences between male groups, lead to the conclusion that maternal obesity, rather than sFlt1, is responsible for atherosclerotic alterations in this animal model.

Rennin-Angiotensin System

Rennin-angiotensin system (RAS) is an important mechanism in the control of vascular function. Angiotensin II, the central product of RAS, causes an increase in systemic and local blood pressure via its vasoconstrictive effects, influences renal tubules to retain sodium and water, and stimulates aldosterone release from the adrenal gland. Ang II directly causes cell growth, regulated gene expression of vasoactive hormones, growth factors, cytokines, and so on, and activates multiple intracellular signaling cascades in cardiovascular and renal cells (Kim & Iwao, 2000). Ang II also stimulates lipogenesis, leading to increased adiposity and obesity (Kim et al., 2006). Molecular and cellular actions of Ang II in cardiovascular diseases are mediated by the AT1 receptor. The role of the other Ang II receptor AT2 remains conflicting – some studies demonstrate that AT2 has an opposing effect to the AT1 receptor on blood pressure, while others report that vascular hypertrophy and remodeling by Ang II in vivo may be mediated by AT2 rather than AT1 (Kim & Iwao, 2000).

Ang II is produced by the liver, kidney, and adipose tissue. Up-regulation of Ang II and down-regulation of AT2 in kidneys and in adipose tissue from HF males was observed in our experiments. Especially notable is the higher expression of Ang II in

adipose tissue from HF sFlt1 males than in HF mFc, suggesting an explanation for significantly increased blood pressure observed in HF sFlt1 males.

FUTURE STUDIES

Several different approaches could be taken from here onward. One would be to further characterize the mouse animal by studying the reactivity of resistance vessels in order to establish if endothelial dysfunction is present, testing for insulin resistance, studying the development of adypocytes, and determining the role of free fatty acids, just to name few. The other direction would be to modify offspring's immediate postnatal environment by either changing maternal diet immediately after delivery, or by exposing newborn to lactation from mice on different diets. And finally, treating mothers during pregnancy (as well as offspring after birth) with cardiovascular risk-lowering medications, such as antioxidants, statins, and other similar agents, could guide the further development of procedures that would be appropriate for human use.

PUBLIC HEALTH ASPECT

This investigation provided a new insight into the role of pre-existing maternal obesity and sFlt-1-induced preeclampsia in the pathogenesis of the developmental origins of adult vascular disease. Maternal obesity and sFlt1 overexpression could affect different systems and pathways during fetal development, and could lead to the development of cardiovascular impairment in offspring later in life.

The findings from this study could improve our understanding and prevention of adverse cardiovascular outcomes in offspring. This model may be used to further investigate the underlying pathophysiological and molecular pathways, including modulation of relevant gene expression, leading to altered cardiovascular function due to prenatal exposure to obesity and preeclampsia.

The study could serve as an example in obesity awareness campaign, because, even we still do not know how to prevent preeclampsia, obesity is an easy modifiable variable.

Given that the proportion of overweight and obese women has doubled since the 1960s, and that preeclampsia complicates 10% of pregnancies, the potential impact on health and disease in adult offspring could be tremendous.

References

American Heart Association. Statistical fact Sheet. Women and Cardiovascular Diseases: Statistics. (2006). http://www.americanheart.org/downloadable/heart/1136818052118Females06.pdf [On-line].

Alexander, B. T. (2003). Placental Insufficiency Leads to Development of Hypertension in Growth-Restricted Offspring. *Hypertension, 41,* 457-462.

Banks, W. A. & Farrell, C. L. (2003). Impaired transport of leptin across the blood-brain barrier in obesity is acquired and reversible. *American Journal of Physiology - Endocrinology and Metabolism, 285,* E10-E15.

Barker, D. J. (1998). In utero programming of chronic disease. *Clin.Sci.(Lond), 95,* 115-128.

Barker, D. J. & Osmond, C. (1986). Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. *Lancet, 1,* 1077-1081.

Bastard, J. P., Maachi, M., Lagathu, C., Kim, M. J., Caron, M., Vidal, H. et al. (2006). Recent advances in the relationship between obesity, inflammation, and insulin resistance. *Eur.Cytokine Netw., 17,* 4-12.

Bodnar, L. M., Catov, J. M., Klebanoff, M. A., Ness, R. B., & Roberts, J. M. (2007). Prepregnancy body mass index and the occurrence of severe hypertensive disorders of pregnancy. *Epidemiology, 18,* 234-239.

Bodnar, L. M., Ness, R. B., Markovic, N., & Roberts, J. M. (2005). The Risk of Preeclampsia Rises with Increasing Prepregnancy Body Mass Index. *Ann Epidemiol, 15,* 475-482.

Boney, C. M., Verma, A., Tucker, R., & Vohr, B. R. (2005). Metabolic Syndrome in Childhood: Association With Birth Weight, Maternal Obesity, and Gestational Diabetes Mellitus. *Pediatrics, 115,* e290-e296.

Butz, G. M. & Davisson, R. L. (2001). Long-term telemetric measurement of cardiovascular parameters in awake mice: a physiological genomics tool. *Physiological Genomics, 5,* 89-97.

Carlson, S. H. & Wyss, J. M. (2000). Long-term telemetric recording of arterial pressure and heart rate in mice fed basal and high NaCl diets. *Hypertension, 35,* E1-E5.

Catalano, P. M. & Ehrenberg, H. M. (2006). The short- and long-term implications of maternal obesity on the mother and her offspring. *BJOG: An International Journal of Obstetrics and Gynaecology, 113,* 1126-1133.

Cooke, C. L. & Davidge, S. T. (2003). Pregnancy-induced alterations of vascular function in mouse mesenteric and uterine arteries. *Biology of Reproduction, 68,* 1072-1077.

Forsen, T., Eriksson, J. G., Tuomilehto, J., Teramo, K., Osmond, C., & Barker, D. J. P. (1997). Mother's weight in pregnancy and coronary heart disease in a cohort of finnish men: follow up study. *BMJ, 315,* 837-840.

Grassi, G. & Giannattasio, C. (2005). Obesity and vascular stiffness: when body fat has an adverse impact on arterial dynamics. *J Hypertens., 23,* 1789-1791.

Khan, I. Y., Dekou, V., Douglas, G., Jensen, R., Hanson, M. A., Poston, L. et al. (2005). A high-fat diet during rat pregnancy or suckling induces cardiovascular dysfunction in adult offspring. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology, 288,* R127-R133.

Khan, I., Dekou, V., Hanson, M., Poston, L., & Taylor, P. (2004). Predictive Adaptive Responses to Maternal High-Fat Diet Prevent Endothelial Dysfunction but Not Hypertension in Adult Rat Offspring. *Circulation, 110,* 1097-1102.

Khan, I. Y., Taylor, P. D., Dekou, V., Seed, P. T., Lakasing, L., Graham, D. et al. (2003). Gender-Linked Hypertension in Offspring of Lard-Fed Pregnant Rats. *Hypertension, 41,* 168-175.

Kim, S., Soltani-Bejnood, M., Quignard-Boulange, A., Massiera, F., Teboul, M., Ailhaud, G. et al. (2006). The adipose renin-angiotensin system modulates systemic markers of insulin sensitivity and activates the intrarenal renin-angiotensin system. *J Biomed Biotechnol, 2006,* 1-6.

Kim, S. & Iwao, H. (2000). Molecular and Cellular Mechanisms of Angiotensin II-Mediated Cardiovascular and Renal Diseases. *Pharmacol Revs, 52,* 11-34.

Kushner, R. F. (2002). Medical management of obesity. *Semin.Gastrointest.Dis., 13,* 123-132.

Kushner, R. F. & Foster, G. D. (2000). Obesity and quality of life. *Nutrition, 16,* 947-952.

Leinonen, E., Hurt-Camejo, E., Wiklund, O., Hultθn, L. M., Hiukka, A., & Taskinen, M. R. (2003). Insulin resistance and adiposity correlate with acute-phase reaction and soluble cell adhesion molecules in type 2 diabetes. *Atherosclerosis, 166,* 387-394.

Levine, R. J., Maynard, S. E., Qian, C., Lim, K. H., England, L. J., Yu, K. F. et al. (2004). Circulating angiogenic factors and the risk of preeclampsia. *N.Engl.J Med, 350,* 672-683.

Lu, F., Longo, M., Tamayo, E., Maner, W., Al-Hendy, A., Anderson, G. D. et al. (2007a). The Effect of Over-expression of SFlt-1 on Blood Pressure and the Occurrence of Other Manifestations of Preeclampsia in Unrestrained Conscious Pregnant Mice. *Am J Obstet Gynecol, 196,* 396.e1-396.e7.

Lu, F., Bytautiene, E., Tamayo, E., Betancourt, A., Hankins, G. D. V., Anderson, G. D. et al. (2006). Mechanisms of the developmental origin of adult hypertension in an animal model of preeclampsia. *Am J Obstet Gynecol*, 195[6, Supplement 1], S153.

Lu, F., Bytautiene, E., Tamayo, E., Gamble, P., Anderson, G. D., Hankins, G. D. V. et al. (2007b). Gender-specific effect of overexpression of sFlt-1 in pregnant mice on fetal programming of blood pressure in the offspring later in life. *Am J Obstet Gynecol, 197,* 418.

Lu, F., Goharkhay, N., Bytautiene, E., Tamayo, E., Orise, P., Anderson, G. D. et al. (2007c). Fetal programming of adult vascular function in a preeclampsia-like animal model is gender-specific. *Am J Obstet Gynecol,* 197[6, Supplement 1], S141.

Maizel, J. V., Jr., White, D. O., & Scharff, M. D. (1968). The polypeptides of adenovirus. I. Evidence for multiple protein components in the virion and a comparison of types 2, 7A, and 12. *Virology, 36,* 115-125.

Massiera, F., Bloch-Faure, M., Ceiler, D., Murakami, K., Fukamizu, A., Gasc, J. M. et al. (2001). Adipose angiotensinogen is involved in adipose tissue growth and blood pressure regulation. *The FASEB Journal, 15,* 2727-2729.

Mills, P. A., Huetteman, D. A., Brockway, B. P., Zwiers, L. M., Gelsema, A. J., Schwartz, R. S. et al. (2000). A new method for measurement of blood pressure, heart rate, and activity in the mouse by radiotelemetry. *J Appl.Physiol, 88,* 1537-1544.

Palti, H. & Rothschild, E. (1989). Blood pressure and growth at 6 years of age among offsprings of mothers with hypertension of pregnancy. *Early Hum.Dev., 19,* 263-269.

Pasceri, V., Willerson, J. T., & Yeh, E. T. H. (2000). Direct Proinflammatory Effect of C-Reactive Protein on Human Endothelial Cells. *Circulation, 102,* 2165-2168.

Payne, J. A., Alexander, B. T., & Khalil, R. A. (2003). Reduced Endothelial Vascular Relaxation in Growth-Restricted Offspring of Pregnant Rats With Reduced Uterine Perfusion. *Hypertension, 42,* 768-774.

Pearson, T. A., Mensah, G. A., Alexander, R. W., Anderson, J. L., Cannon, R. O., III, Criqui, M. et al. (2003). Markers of Inflammation and Cardiovascular Disease: Application to Clinical and Public Health Practice: A Statement for Healthcare Professionals From the Centers for Disease Control and Prevention and the American Heart Association. *Circulation, 107,* 499-511.

Pelleymounter, M. A., Cullen, M. J., Healy, D., Hecht, R., Winters, D., & McCaleb, M. (1998). Efficacy of exogenous recombinant murine leptin in lean and obese 10- to 12-mo-old female CD-1 mice. *AJP - Regulatory, Integrative and Comparative Physiology, 275,* R950-R959.

Rahmouni, K., Morgan, D. A., Morgan, G. M., Mark, A. L., & Haynes, W. G. (2005). Role of Selective Leptin Resistance in Diet-Induced Obesity Hypertension. *Diabetes, 54,* 2012-2018.

Ramsay, J. E., Ferrell, W. R., Crawford, L., Wallace, A. M., Greer, I. A., & Sattar, N. (2002). Maternal Obesity Is Associated with Dysregulation of Metabolic, Vascular, and Inflammatory Pathways. *J Clin Endocrinol Metab, 87,* 4231-4237.

Raygada, M., Cho, E., & Hilakivi-Clarke, L. (1998). High Maternal Intake of Polyunsaturated Fatty Acids During Pregnancy in Mice Alters Offsprings' Aggressive Behavior, Immobility in the Swim Test, Locomotor Activity and Brain Protein Kinase C Activity. *J Nutr, 128,* 2505-2511.

Ridker, P. M., Rifai, N., Stampfer, M. J., & Hennekens, C. H. (2000a). Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation, 101,* 1767-1772.

Ridker, P. M., Hennekens, C. H., Buring, J. E., & Rifai, N. (2000b). C-Reactive Protein and Other Markers of Inflammation in the Prediction of Cardiovascular Disease in Women. *N Engl J Med, 342,* 836-843.

Ridker, P. M., Hennekens, C. H., Roitman-Johnson, B., Stampfer, M. J., & Allen, J. (1998). Plasma concentration of soluble intercellular adhesion molecule 1 and risks of future myocardial infarction in apparently healthy men. *The Lancet, 351,* 88-92.

Ridker, P. M., Rifai, N., Pfeffer, M., Sacks, F., Lepage, S., & Braunwald, E. (2000c). Elevation of Tumor Necrosis Factor-{alpha} and Increased Risk of Recurrent Coronary Events After Myocardial Infarction. *Circulation, 101,* 2149-2153.

Roberts, J. M. & Lain, K. Y. (2002). Recent Insights into the Pathogenesis of Pre-eclampsia. *Placenta, 23,* 359-372.

Romano, M., Sironi, M., Toniatti, C., Polentarutti, N., Fruscella, P., Ghezzi, P. et al. (1997). Role of IL-6 and Its Soluble Receptor in Induction of Chemokines and Leukocyte Recruitment. *Immunity, 6,* 315-325.

Rosamond, W., Flegal, K., Furie, K., Go, A., Greenlund, K., Haase, N. et al. (2008). Heart Disease and Stroke Statistics--2008 Update: A Report From the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation, 117,* e25-146.

Rossant, J. & Cross, J. C. (2001). Placental development: lessons from mouse mutants. *Nat Rev Genet., 2,* 538-548.

Rotter, V., Nagaev, I., & Smith, U. (2003). Interleukin-6 (IL-6) induces insulin resistance in 3T3-L1 adipocytes and is, like IL-8 and tumor necrosis factor-alpha, overexpressed in human fat cells from insulin-resistant subjects. *J Biol Chem., 278,* 45777-45784.

Russell, A. & Watts, S. (2000). Vascular reactivity of isolated thoracic aorta of the C57BL/6J mouse. *J Pharmacol Exp Ther, 294,* 598-604.

Samuelsson, A. M., Matthews, P. A., Argenton, M., Christie, M. R., McConnell, J. M., Jansen, E. H. J. et al. (2008). Diet-Induced Obesity in Female Mice Leads to Offspring Hyperphagia, Adiposity, Hypertension, and Insulin Resistance: A Novel Murine Model of Developmental Programming. *Hypertension, 51,* 383-392.

Seidman, D. S., Laor, A., Gale, R., Stevenson, D. K., Mashiach, S., & Danon, Y. L. (1991). Pre-eclampsia and offspring's blood pressure, cognitive ability and physical development at 17-years-of-age. *Br J Obstet Gynaecol, 98,* 1009-1014.

Sharma, A., Satyam, A., & Sharma, J. B. (2007). Leptin, IL-10 and Inflammatory Markers (TNF-alpha, IL-6 and IL-8) in Pre-Eclamptic, Normotensive Pregnant and Healthy Non-Pregnant Women. *Am J Reprod Immunol, 58,* 21-30.

Sibai, B. M. & Frangieh, A. (1995). Maternal adaptation to pregnancy. *Curr.Opin.Obstet.Gynecol, 7,* 420-426.

Straczkowski, M., Lewczuk, P., Dzienis-Strazkowska, S., Kowalska, I., Stepien, A., & Kinalska, I. (2002). Elevated soluble intercellular adhesion molecule-1 levels in obesity: Relationship to insulin resistance and tumor necrosis factor-[alpha] system activity. *Metabolism, 51,* 75-78.

Tenhola, S., Rahiala, E., Halonen, P., Vanninen, E., & Voutilainen, R. (2006). Maternal preeclampsia predicts elevated blood pressure in 12-year-old children: evaluation by ambulatory blood pressure monitoring. *Pediatr.Res, 59,* 320-324.

Tenhola, S., Rahiala, E., Martikainen, A., Halonen, P., & Voutilainen, R. (2003). Blood Pressure, Serum Lipids, Fasting Insulin, and Adrenal Hormones in 12-Year-Old Children Born with Maternal Preeclampsia. *J Clin Endocrinol Metab, 88,* 1217-1222.

Thom, T., Haase, N., Rosamond, W., Howard, V. J., Rumsfeld, J., Manolio, T. et al. (2006). Heart Disease and Stroke Statistics--2006 Update: A Report From the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation, 113,* e85-151.

Tortoriello, D. V., McMinn, J., & Chua, S. C. (2004). Dietary-Induced Obesity and Hypothalamic Infertility in Female DBA/2J Mice. *Endocrinology, 145,* 1238-1247.

Torzewski, M., Rist, C., Mortensen, R. F., Zwaka, T. P., Bienek, M., Waltenberger, J. et al. (2000). C-Reactive Protein in the Arterial Intima : Role of C-Reactive Protein Receptor-Dependent Monocyte Recruitment in Atherogenesis. *Arterioscler Thromb Vascr Biol, 20,* 2094-2099.

Van Vliet, B. N., Chafe, L. L., & Montani, J. P. (2003). Characteristics of 24 h telemetered blood pressure in eNOS-knockout and C57Bl/6J control mice. *J Physiol, 549,* 313-325.

Verma, S., Wang, C. H., Li, S. H., Dumont, A. S., Fedak, P. W. M., Badiwala, M. V. et al. (2002). A Self-Fulfilling Prophecy: C-Reactive Protein Attenuates Nitric Oxide Production and Inhibits Angiogenesis. *Circulation, 106,* 913-919.

Visser, M., Bouter, L. M., McQuillan, G. M., Wener, M. H., & Harris, T. B. (1999). Elevated C-Reactive Protein Levels in Overweight and Obese Adults. *JAMA, 282,* 2131-2135.

Whitaker, R. C. (2004). Predicting preschooler obesity at birth: the role of maternal obesity in early pregnancy. *Pediatrics, 114,* e29-e36.

Wolf, M., Kettyle, E., Sandler, L., Ecker, J. L., Roberts, J., & Thadhani, R. (2001). Obesity and preeclampsia: the potential role of inflammation. *Obstet Gynecol, 98,* 757-762.

Wong, A. Y., Kulandavelu, S., Whiteley, K. J., Qu, D., Langille, B. L., & Adamson, S. L. (2002). Maternal cardiovascular changes during pregnancy and postpartum in mice. *AJP - Heart and Circulatory Physiology, 282,* H918-H925.

Yudkin, J. S., Kumari, M., Humphries, S. E., & Mohamed-Ali, V. (2000). Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? *Atherosclerosis, 148,* 209-214.

Vita

Egle Bytautiene was born in Moscow, Russia, on November 22, 1965. At 3 months of age she arrived to her mother's home country – Lithuania. After attending 22nd High School in Vilnius, Egle graduated from Vilnius University with a degree of Medical Doctor in 1991. During her medical education Egle was already involved in research and chaired a Medical Student's Scientific Society of Psychology and Psychotherapy. After moving to US, Nashville, TN, she joined a private cardiology practice, where she worked as research assistant in creating and collecting data from patients seen in practice for various databases. In 1997, Egle became a Research Fellow at Department of Obstetrics & Gynecology, University of Texas Medical Branch, Galveston, TX. Here she rose to the rank of Faculty by 2006, currently serving as Instructor. In 2006 she entered the Preventive Medicine and Community Health Program at the University of Texas Medical Branch as a graduate student. Egle has received several awards, including funding for NIH R03 grant application, has given presentations at the national and international meeting, as well as invited lectures.

Education

MD, 1991, Medicine Faculty of Vilnius University, Vilnius, Lithuania

Publications

Original Articles:

Vedernikov Y, Bolanos S, **Bytautiene E,** Fulep E, Saade GR, Garfield RE. Effect of fluoxetine on contractile activity of pregnant rat uterine rings. *Am J Obstet Gynecol.* 2000;182:296–299.

Garfield RE, **Bytautiene E,** Vedernikov YP, Marshall JS, Romero R. Modulation of rat uterine contractility by mast cells and their mediators. *Am J Obstet Gynecol.* 2000;183:118–125.

Fittkow CT, Shi SQ, **Bytautiene E,** Olson G, Saade GR, Garfield RE. Changes in light-induced fluorescence of cervical collagen in guinea pigs during gestation and after sodium nitroprusside treatment. *J Perinat Med.* 2001;29:535–543.

Bytautiene E, Vedernikov YP, Saade GR, Romero R, Garfield RE. Endogenous mast cell degranulation modulates cervical contractility in the guinea pig. *Am J Obstet Gynecol.* 2002;186:438–445.

Bytautiene E, Vedernikov YP, Saade GR, Romero RR, Garfield RE. Effect of histamine on phasic and tonic contractions of isolated uterine tissue from pregnant women. *Am J Obstet Gynecol.* 2003;188:774-778.

Maul H, **Bytautiene E,** Vedernikov Y, Garfield RE, Saade GR. Proteinase-activated receptor-2 activation induces uterine contractility in term pregnant rats that is not dependent on mast cell activation and cyclo-oxygenase products. *Am J Obstet Gynecol.* 2003;188:1498–1503.

Bytautiene E, Vedernikov YP, Saade GR, Romero RR, Garfield RE. Degranulation of uterine mast cell modifies contractility of isolated myometrium from pregnant women. *Am J Obstet Gynecol.* 2004;191:1705–1710.

Bytautiene E, Romero RR, Vedernikov YP, El-Zeky F, Saade GR, Garfield RE. Induction of premature labor and delivery by allergic reaction and prevention by histamine H_1 receptor antagonist. *Am J Obstet Gynecol.* 2004;191:1356–1361.

Garfield RE, Irani AM, Schwartz LB, **Bytautiene E,** Romero R. Structural and functional comparison of mast cells in the pregnant versus nonpregnant human uterus. *Am J Obstet Gynecol.* 2006;194:261–267.

Lu F, **Bytautiene E,** Tamayo E, Gamble P, Anderson G, Hakins G, Longo M, Saade G. Gender-specific effect of over-expression of sFlt-1 in pregnant mice on fetal

programming of blood pressure in the offspring later in life. *Am J Obstet Gynecol.* 2007;197:418.e1–5.

Bytautiene E, Vedernikov YP, Saade GR, Romero R, Garfield RE. Effect of mast cell degranulating agent on vascular resistance in human placental vascular bed and on the tone of isolated placental vessels. *Reprod Sci. Reprod Sci.* 2008;15:26-32.

Langenveld J, Lu F, **Bytautiene E,** Anderson GD, Saade GR, Longo M. In utero programming of adult vascular function in transgenic mice lacking low-density lipoprotein receptor. *Am J Obstet Gynecol.* 2008 Aug;199(2):165.e1-5.

Bytautiene E, Vedernikov YP, Saade GR, Romero R, Garfield RE. IgE-Independent Mast Cell Activation Augments Contractility of Nonpregnant and Pregnant Guinea Pig Myometrium. *Int Arch Allergy Immunol* 2008;147:140-146.

Bytautiene E, Vedernikov YP, Maner WL, Saade GR, Romero R, Garfield RE. Challenge with ovalbumin antigen increases uterine and cervical contractile activity in sensitized guinea pigs. *Am J Obstet Gynecol* 2008 Dec;199(6):658.e1-6.

Byers BD, Betancourt A, Lu F, Hankins GDV, Longo M, Saade GR, **Bytautiene E**. The effect of prepregnancy obesity and sflt-1-induced preeclampsia-like syndrome on fetal programming of adult vascular function in a mouse model. *Am J Obstet Gynecol.* 2009 Apr;200(4): 432.e1-432.e7

Romero R, Kusanovic JP, Gomez R, Lamont R, **Bytautiene E**, Garfield RE, Mittal P, Hassan SS, Yeo L. The clinical significance of eosinophils in the amniotic fluid in preterm labor. J Matern Fetal Neonatal Med. 2009 Oct 22. [Epub ahead of print]

Bytautiene E, Lu F, Tamayo EH, Hankins GD, Longo M, Kublickiene K, Saade GR. Long-term maternal cardiovascular function in a mouse model of sFlt-1-induced preeclampsia. Am J Physiol Heart Circ Physiol. 2010 Jan;298(1):H189-93.

Chapter:

Bytautiene E., Romero R., Garfield R "Uterine Mast Cells and their Role in Control of Allergy-Induced Premature Labor" in "Mast Cells: Roles, Interactions and Disorders", ed. Jonas F. Jung and Luca T. Scholz. Novapublishers, 2008.

Abstracts

Oral Presentations:

Effect of endogenous mast cell degranulation on uterine and cervical contractility in pregnant guinea pigs. *6th Annual Texas Forum on Female Reproduction,* Houston, TX May 18-19, 2000.

Cyclooxygenase and lipoxygenase involvement in increased uterine contractility due to endogenous mast cell degranulation. *22nd Annual Meeting of the Society for Maternal-Fetal Medicine*, New Orleans, LS, January 2002.

An allergic reaction can induce premature labor and delivery, which can be prevented by treatment with anithistaminics and cromolyn sodium. *24th Annual Meeting of the Society for Maternal-Fetal Medicine*, New Orleans, LS, January 2004.

An allergic reaction can induce premature labor and delivery, which can be prevented by treatment with anithistamines. *10th Annual Texas Forum on Female Reproduction*, April 22, 2004.

Fetal programming of type I hypersensitivity reaction: the roles of leukotriene D4, prostaglandin D2, and thromboxane A2. *26th Annual Meeting of the Society for Maternal-Fetal Medicine*, Miami Beach, FL, February 2006.

Effect of Prenatal Sensitization on the Reproductive Tract. Seminar at the Center for Interdisplinary Research in Women's Health. UTMB, Galveston, TX, November 2006.

Prenatal Sensitization and the Development of Asthma Later in Life in a Guinea Pig Model. National Student Research Forum. UTMB, Galveston, TX, April, 2007.

The Effect of Dietary Fat Intake on Long Term Vascular Function in an Animal Model of Gestational Diabetes. National Student Research Forum. UTMB, Galveston, TX, April, 2007.

Prenatal Sensitization and the Development of Asthma Later in Life in a Guinea Pig Model. South Central Conference on Perinatal Research. Austin, TX, October, 2007.

Long-Term Maternal Vascular Function in sFIt-1 Over-expression-induced Preeclampsia Model. 16th World Congress of the International Society for the Study of Hypertension in Pregnancy (ISSHP), Washington, DC, September, 2008.

Vascular Function in the Offspring Later in Life in a Mouse Model of Maternal Obesity and Preeclampsia, CAOG, New Orleans, LA, October, 2008.

Poster Presentations **(selected from 94 published abstracts):**

Bytautiene E, Vedernikov Y, Fulep E, Saade G, Romero R, Garfield R. The effects of histamine, 5-Hydroxytryptamine, and bradykinin on contractility of uterine strips from pregnant and nonpregnant guinea pigs. *Am J Obstet Gynecol.* 2000;182:S97.

Bytautiene E, Vedernikov Y, Saade G, Romero R, Garfield RE. Role of lipoxygenase and cyclooxygenase in the response of pregnant guinea pig cervix to mast cell degranulation. *J Soc Gynecol Investig.* 2000;7:269A.

Bytautiene E, Vedernikov Y, Saade G, Romero R, Garfield RE. Effect of endogenous mast cell degranulation on uterine contractility in pregnant guinea pigs. *J Soc Gynecol Investig.* 2000;7:273A.

Bytautiene E, Vedernikov YP, Saade GR, Romero R, Garfield RE. Endogenous mast cell degranulation stimulates uterine and cervical contractility in pregnant guinea pigs. 2nd Intern Conf Exp Clin Reprod Immunobiol. November 15–18, Amsterdam, The Netherlands.

Bytautiene E, Vedernikov Y, Maner W, et al. Antigen challenge in sensitized guinea pigs increases uterine EMG and intrauterine pressure. *J Soc Gynecol Investig.* 2001;8:191A.

Bytautiene E, Vedernikov Y, Saade G, Romero R, Garfield RE. Cyclooxygenase and lipoxygenase involvement in increased uterine contractility due to endogenous mast cell degranulation. *Am J Obset Gynecol.* 2002;185:S90.

Bytautiene E, Vedernikov Y, Saade G, Romero R, Garfield RE. Effect of endogenous mast cell degranulation on uterine and cervical contractility in pregnant mice lacking a functional 5-Lipoxygenase gene. *Am J Obset Gynecol.* 2002;185:S215.

Bytautiene E, Vedernikov Y, Saade G, Romero R, Garfield RE. Challenge with ovalbumin increases contractility of uterine and cervical strips from sensitized term pregnant guinea pigs. *J Soc Gynecol Investig.* 2002;9:208A.

Bytautiene E, Vedernikov Y, Saade G, Romero R, Garfield RE, Maul H. Effect of endogenous mast cell degranulation and histamine receptor antagonists on uterine contractility of term pregnant rats. *Am J Obstet Gynecol.* 2002;187:S173.

Bytautiene E, Vedernikov Y, Saade G, Romero R, Garfield RE. Tetrahydrobiopterin or oxygen radicals do not influence contractility inhibition effect of SIN-1 on term pregnant human myometrium. *J Soc Gynecol Investig.* 2002;9:308A.

Bytautiene E, Romero R, Vedernikov Y, Saade G, Garfield RE. Effect of relaxin on perfusion pressure in the isolated human placental codyledon. *Am J Obset Gynecol.* 2003;189:S198.

Bytautiene E, Vedernikov Y, Saade G, Romero R, Garfield RE. Endogenous mast cell degranulation increases perfusion pressure in isolated human placental vascular bed. *J Soc Gynecol Investig*. 2003;10:366A.

Bytautiene E, Vedernikov Y, Saade G, Romero R, Garfield RE. Antigen challenge increases vascular and tracheal smooth muscle contraction in actively sensitized nonpregnant guinea pigs. *J Soc Gynecol Investig*. 2003;10:387A.

Bytautiene E, Vedernikov Y, Saade G, Garfield RE. Effect of oxytocin and vasopressin on perfusion pressure in human placental vascular bed. *J Soc Gynecol Investig.* 2004;11:347A.

Bytautiene E, Vedernikov Y, Saade G, Romero R, Garfield RE. Gender differences in vascular responses to an antigen challenge and histamine in guinea pigs. *J Soc Gynecol Investig.* 2004;11:381A.

Bytautiene E, Vedernikov Y, Saade G, Romero R, Garfield RE. Endogenous mast cell degranulation increases tension of isolated human chorionic plate vessels. *J Soc Gynecol Investig*. 2005;12(2S):333A.

Bytautiene E, Vedernikov Y, Saade G, Garfield RE. Fetal programming of type I hypersensitivity reactions. *J Soc Gynecol Investig*. 2005;12(2S):337A.

Longo M, **Bytautiene E,** Lu F, Tamayo E, Hankins G, Anderson G, Saade G. Blood pressure regulation and uterine environments in conscious unrestrained NOS3 knockout mice. *Am J Obset Gynecol* 2005;193:S10.

Lu F, **Bytautiene E,** Maner W, Makhlouf M, Al-Hendy A, Longo M, Saade G. Role of SFLT-1 in hypertension of pregnancy. *Am J Obset Gynecol.* 2005;193:S13.

Bytautiene E, Longo M, Orise P, Hankins G, Anderson G, Saade G. Fetal programming of type I hypersensitivity reaction: the roles of leukotriene D4, prostaglandin D2, and thromboxane A2. *Am J Obset Gynecol.* 2005;193:S25.

Longo M, Lu F. **Bytautiene E,** Tamayo E, Hankins G, Anderson G, Saade G. Fetal vascular programming in a mouse modelo of hyperternsion *J Soc Gynecol Investig.* 2006;13(2S):95A.

Goharkhay N, Lu F, **Bytautiene E,** Gamble P, Longo M, Saade G, Hankins G. Alteration in vascular response patterns in an sFlt-1 model of hypertension in pregnancy. *J Soc Gynecol Investig.* 2006;13(2S):97A.

Bytautiene E, Longo M, Orise P, Kublickiene K, Hankins G, Anderson G, Saade G. Involvement of leukotriene D4 and prostaglandin D2 in the responses of reproductive tract of the offspring exposed to allergen during prenatal period. *J Soc Gynecol Investig.* 2006;13(2S):203A.

Bytautiene E, Tamayo E, Betancourt A, Makhlouf M, Anderson G, Longo M, Saade G. The effect of dietary fat intake on long term vascular function in an animal model of gestational diabetes. *J Obset Gynecol.* 2006;195:S155.

Bytautiene E, Vedernikov Y, Maner W, Longo M, Hankins G, Anderson D, Saade G. Prenatal sensitization and the development of asthma later in life in a guinea pig model. *J Obset Gynecol.* 2006;195:S175.

Bytautiene E, Gamble P, Longo M, Hankins G, Anderson D, Saade G. Outcomes of sensitization are expressed differently in prenatally and actively sensitized male, but not female guinea pig. *Reprod Sci*, 2007;14(S):99A.

Bytautiene E, Lu F, Tamayo E, Betancourt A, Hankins G, Anderson D, Longo M, Saade G. Maternal long-term cardiovascular function in an animal model of preeclampsia. *Am J Obstet Gynecol.* 2007;197:S135.

Bytautiene E, Tamayo E, Lu F, Hankins GDV, Longo M, Saade GR. Fetal programming of adult blood pressure in a mouse model of pre-pregnancy obesity and preeclampsia. *Am J Obstet Gynecol.* 2008;199:S85

Bytautiene E, Betancourt A, Byers BD, Hankins GDV, Longo M, Saade GR. Role of pre-pregnancy maternal weight on tracheal reactivity in offspring later in life. *Reprod Sci*, 2009;16 (3S):241A.

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