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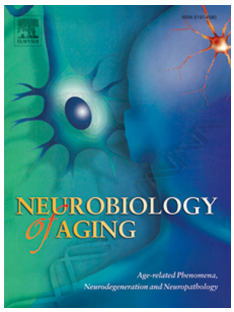

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Late life brain perfusion after prenatal famine exposure

Running head: Prenatal famine and brain perfusion

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Abstract

Early nutritional deprivation may cause irreversible damage to the brain and seems to affect cognitive function in older age. We investigated whether prenatal undernutrition was associated with brain perfusion differences in older age. We acquired Arterial spin labelling scans in 118 Dutch famine birth cohort members. Using linear regression analyses, cerebral blood flow (CBF) was compared between exposed and unexposed groups in grey and white matter, perfusion territories, the neurodegeneration-related regions anterior and posterior cingulate cortex and precuneus. Furthermore, we compared the GM/WM-ratio and the spatial coefficient of variation (CoV) as a proxy of overall cerebrovascular health. The WM ASL signal and the GM/WM-ratio were significantly lower and higher respectively among exposed participants (-2.5 mL/100g/min [95%CI: -4.3 to -0.8; p = 0.01] and 0.48 [0.19 to 0.76; p = 0.002] respectively). Exposed men had lower CBF in anterior and posterior cingulate cortices (-8.0 mL/100g/min [-15.1 to -0.9; p = 0.03]; -11.4 mL/100g/min [-19.6 to -3.2; p = 0.02]) and higher spatial CoV (0.05 [0.00 to 0.09; p = 0.05]). The latter seemed largely mediated by higher 2h-glucose levels at age 50. Our findings suggest that prenatal undernutrition affects brain perfusion parameters providing further evidence for life-long effects of undernutrition during early brain development.

Keywords: brain perfusion; prenatal famine; fetal programming;

1. Introduction

Development of the fetal brain depends heavily on the availability of nutrients. Fetal undernutrition therefore poses a serious threat to normal fetal brain development (Ramel and Georgieff, 2014). Direct evidence for detrimental effects of fetal undernutrition on the developing central nervous system (CNS) was demonstrated in a study from 1975, which showed that babies conceived during the 1944-1945 Dutch famine had higher rates of CNS congenital anomalies (Stein et al., 1975).

We have recently shown that the effects of prenatal famine exposure on the CNS can still be detected decades later. At age 68 years, men who had been exposed to the Dutch famine in early gestation had smaller intracranial volume and total brain volume compared to men who had not been exposed to the famine in utero (de Rooij et al., 2016). They also had smaller volumes of total cortical gray matter, cortical white matter, cerebellar gray matter, thalamus, caudate nucleus and accumbens area and a large number of more specific cortical gray and white matter areas. These volume differences disappeared after correction for intracranial volume, suggesting that prenatal famine exposure had an overall diminishing effect on the size of the brain in men. Exposed men also showed altered brain structure resembling premature brain ageing (Franke et al., 2018). BrainAGE, a non-invasive *in vivo* MRI biomarker using pattern recognition to analyze structural brain aging, was shown to be increased by over 4 years in exposed compared to unexposed men. Cognitive function was also affected in those exposed to famine in early gestation, as shown by worse performance on a computerized Stroop-task, a measure of selective attention (de Rooij et al., 2010). On a society level, exposed men participated less in the labor market, which may well be a consequence of the effects on brain and cognitive ageing (Scholte et al., 2015). Similar findings in relation to cognitive ageing were shown in people exposed to the Chinese famine in early life, who also exhibited higher rates of dementia in one study (He et al., 2018; Kang et al., 2017; Xu et al., 2018). In addition to these effects on the brain, exposure to famine in early gestation has effects on cardio-metabolic outcomes such as a higher BMI in exposed women and higher levels of glucose, cholesterol, clotting factors, diabetes, micro-albuminuria and heart disease in men and women (Lumey et al., 2011; Roseboom et al., 2011).

Adequate cerebral blood flow (CBF) is of vital importance for the health, growth and repair of brain tissue (de la Torre, 2012; Iturria-Medina et al., 2016). Brain perfusion changes are observed with ageing, as well as in cerebrovascular and neurodegenerative disease (Asllani et al., 2009; Chen et al., 2011; Mutsaerts et al., 2015a). These changes are believed to reflect a combination of changes in blood flow supply and in blood flow demand (de la Torre, 2012; Iturria-Medina et al., 2016). In the first case, vascular disease disrupts the supply of blood, leading to a lower supply of oxygen and nutrients (de la Torre, 2012; Wagner et al., 2012). In the second case, reductions in neuronal activity, hence reduced neuronal metabolism, leading in turn to decreases in blood flow demand and decrease of perfusion (Binnewijzend et al., 2013; Wierenga et al., 2014). Therefore, brain perfusion measurements provide a hemodynamic signature of the status of the ageing brain.

We studied perfusion measures in a subsample of the Dutch famine birth cohort to assess the effects of undernutrition during early gestation on brain perfusion in late life. Given the demonstrated effects of prenatal famine exposure on cardiovascular outcomes including blood pressure and coronary heart disease, we expected famine exposure in early gestation to be associated with decreased brain perfusion due to vascular effects (Roseboom et al., 2000; Painter et al., 2006; Stein et al., 2006). In addition, given the increasing evidence that prenatal undernutrition is associated with cognitive decline and dementia, we aimed to explore potential neurodegenerative effects on brain perfusion (de Rooij et al., 2010; He et al., 2018; Kang et al., 2017a; Xu et al., 2018). Finally, as prenatal famine exposure has been shown to markedly affect metabolic outcomes and these factors have a role in both vascular and neurodegenerative pathology, we aimed to investigate whether potential prenatal famine effects on brain perfusion are mediated by metabolic factors at younger ages (Clement et al., 2017; Lumey et al., 2011; Pinto et al., 2004; Roseboom et al., 2011; Tolppanen et al., 2012). As previously demonstrated effects of prenatal undernutrition on the brain were sex-specific, we studied men and women separately (de Rooij et al., 2016; Franke et al., 2018).

We acquired Arterial spin labelling (ASL) scans in members of the Dutch famine birth cohort, from which we computed perfusion metrics. We included whole brain CBF changes in grey matter (GM) and in white matter (WM), the GM/WM-ratio and CBF in perfusion territories as measures of

brain vascular sufficiency. In addition, we used the spatial Coefficient of Variation (CoV) as a proof-of-concept proxy biomarker of overall vascular insufficiency (Mutsaerts et al., 2017). The spatial distribution of the ASL label signal on a single time point CBF image can be used to infer between-participant arterial transit time (ATT) differences. Prolonged transit of the ASL label to tissue – leading to higher spatial CoV - is expected in individuals with cerebrovascular pathology (Mutsaerts et al., 2017). GM CBF in specific brain areas that were previously associated with dementia disorders was included as measure for neurodegenerative effects on brain perfusion and included CBF in the anterior and posterior cingulate cortex (ACC, PCC) as well as the precuneus (Mutsaerts et al., 2015b; Sierra-Marcos, 2017).

2. Materials and Methods

2.1 The Dutch famine birth cohort

The Dutch famine birth cohort consists of 2414 men and women who were born as term singletons during the period 1 November 1943 and 28 February 1947 in the Wilhelmina Gasthuis in Amsterdam, the Netherlands. The selection procedure of the cohort has been described in detail elsewhere (Ravelli et al., 1998). The study was approved by the local medical ethics committee and carried out in accordance with the Declaration of Helsinki. All participants gave written informed consent.

2.2 Experimental design

The (historical) records of official daily food-rations for the general population of 21 years and older were used to define exposure to famine. A person was considered to be prenatally exposed to famine if the average daily food-ration of the mother during any 13-week period of gestation contained less than 1000 calories.

It has previously been shown that the effect of famine exposure on congenital anomalies of the CNS affected only those exposed during early gestation (Stein et al., 1975). Also, the majority of effects of prenatal famine exposure on later life health affected participants exposed in early gestation (Roseboom et al., 2011). Therefore, we focused the current imaging study on the group exposed to famine in early gestation and did not measure those exposed to famine in late or mid gestation. Babies born between 19 August and 8 December 1945 were considered as exposed to famine in utero in the early gestational period (Ravelli et al., 1998). People born before 7 January 1945 and people conceived after the famine, born after 8 December 1945, were considered as unexposed to famine in utero and acted as control groups.

2.3 Sample selection and eligibility criteria

At the start of the present imaging study in 2012, 1307 (54%) cohort members were eligible for participation. They were alive, still living in the Netherlands and their current address was known to the investigators. Birth weight and head circumference at birth did not differ between these eligible

and non-eligible cohort members (3357 vs 3333 g, p = 0.22 and 32.8 vs 32.9 cm, p = 0.22 respectively).

We aimed to include a total of 150 people: 50 of those born before the famine, 50 of those exposed to famine in early gestation and 50 of those conceived and born after the famine. We randomly drew equal samples from each of the groups until the number of 50 people agreeing to participate was reached. A total number of 151 participants of an eligible group of 268 cohort members (56%) were visited at home (mean follow-up time after previous visit was 10 years). Participation rates were similar in the born before famine and exposed in early gestation groups (54% vs 51%) and higher in the conceived after famine group (66%). All 151 participants were invited to the MRI part of the study. A total of eight of the 151 refrained from further participation due to MRI claustrophobia. Another 15 participants were excluded from MR scanning because of the presence of metal in their bodies and nine participants declined to visit the hospital. Of one person who participated in the MRI protocol, data had accidentally not been stored. The total inclusion was 118 MRI participants of which 30% were born before the famine, 35% were exposed to famine in early gestation and 35% were conceived and born after the famine. Of the 33 excluded participants, 52% had been born before the famine, 24% were prenatally exposed to famine and 24% were conceived after the famine.

2.4 Study parameters at age 68

Maternal characteristics and birth outcomes were extracted from medical birth records. Participants were visited at home, where a trained research assistant conducted a standardized interview and took anthropometric measurements. The interview yielded information about work, education, socio-economic status (SES), lifestyle parameters, medical history and use of medication. Educational level was measured on a 10-point scale (1 = primary education not completed, 10 = university completed). SES was defined according to the International Index of Occupational Status-92, which is based on the participant's or their partner's occupation, whichever status is highest. We asked the participants about current smoking, whether he or she consumed alcohol (we considered drinking at least one glass of

alcohol per week a positive answer) and about physical activity (cycling, hiking etc. for at least 3 months per year and at least 1h per 14 days). Further, height was measured with a portable stadiometer, weight with a portable Tefal scale and waist and hip circumferences with a flexible tape measure. Blood pressure was measured twice using an automated device with arm cuff and the mean was calculated for further analysis. Finally, at the time of the MRI measurements, blood was drawn following standard procedures in a non-fasting state and concentrations of glucose and lipids (LDL and HDL cholesterol) were assessed.

2.5 Study parameters at ages 50 and 58

At age 50, a total of 702 cohort members (of whom 80 participated in the present sub-study) visited the clinic between March 1995 and August 1996 (Ravelli et al., 1998). Trained nurses took anthropometric measurements and blood pressure was measured in the same fashion as at age 68 years. Blood was drawn following a standard 75g oral glucose tolerance test and concentrations of glucose and lipids (LDL and HDL cholesterol) were assessed. At age 58, 810 cohort members (100 participating in the present sub-study) participated in a clinical study performed between August 2002 and September 2004 (de Rooij et al., 2007). Trained nurses followed the same procedures as at age 50, resulting in information on anthropometrics, blood pressure, glucose and lipid levels.

2.6 MRI imaging

MRI scans were performed on a 3T MRI scanner (Philips Ingenia, Best, the Netherlands) equipped with a 16-channel DStream Head-Spine coil. Foam padding was applied to restrict head motion. The pseudo-continuous ASL-sequence was performed with a single-shot echo-planar imaging readout with the following parameters: matrix size = 80x80, voxel-size = 3 x 3 mm^2, 17 axial slices with 7 mm thickness and a 0.7 mm slice-gap, echo time/repetition time = 14.4/3865 ms, SENSE = 2.5, initial post-label delay = 1525 ms; slice readout time = 34.9 ms; resulting post-label delay range for 17 slices = 1525-2215 ms, labelling duration = 1650 ms and two background suppression pulses played at 1710 and 2860 ms after a pre-labelling saturation pulse. Thirty control-label pairs were acquired for each scan, for a total scan duration of 3:52 min. The labelling plane was positioned parallel and 90 mm

inferior to the centre of the imaging volume (the anterior-commissure - posterior-commissure line). A 1x1x1 mm^3 3D T1-weighted (T1w) scan was included in the imaging protocol for segmentation and registration purposes. Image processing was performed with ExploreASL (www.ExploreASL.com) as described in (Mutsaerts et al., 2018). ExploreASL is a Matlab toolbox based on Statistical Parametric Mapping (SPM).

In short, a 3D FLAIR scan was added to segment white matter hyperintensities, which were used to correct the T1w segmentation using the Lesion Segmentation Toolbox (de Rooij et al., 2016; Steenwijk et al., 2013). The Computational Anatomy Toolbox 12 (CAT12) was used to segment the GM and WM partial volume (PV) maps from the WMH-filled T1-weighted images (Gaser, 2009) and register them to standard space using Diffeomorphic Anatomical Registration analysis using Exponentiated Lie algebra (DARTEL) (Ashburner, 2007). Subsequently, control and labeled images were motion corrected and motion outliers were removed, after which the mean subtracted perfusion-weighted image was registered to the GM PV map from the T1w image (Mutsaerts et al. 2018). CBF was quantified, using the single-compartment quantification model (Alsop et al., 2015). ASL analyses were repeated without and with partial volume correction (PVC). For the first, the analysis was performed with a GM region-of-interest (ROI) obtained by thresholding the GM map at 70%. For the second, we applied voxelwise PV correction by linear regression (Asllani et al., 2008).

2.7 CBF variables

Grey matter (GM) and white matter (WM) masks were defined as pGM>0.7 and pWM>0.7, where the WM mask was eroded with a 6 mm sphere to isolate the deep WM, avoiding signal mixing between GM and WM (Mutsaerts et al., 2014). We compared the following parameters between exposed and unexposed groups: CBF for GM and WM, GM/WM-ratio and CBF for the GM in the perfusion territories anterior and middle cerebral artery (AMCA) and posterior cerebral artery (PCA). In addition, we compared CBF in the regions ACC and PCC as well as in the precuneus. Within the GM ROI, we defined the following ROIs by intersecting their atlas ROIs with the subject-specific GM ROI. The ACC, PCC and precuneus ROIs were obtained from the Harvard-Oxford atlas (Desikan et

al., 2006), whereas the AMCA and PCA ROIs were obtained from a previous vascular atlas (Tatu et al., 1998). A deep WM atlas was created by eroding the SPM12 WM tissue class by a 4 voxel sphere (i.e. 6 mm), to avoid signal contamination from the GM (Mutsaerts et al., 2014), which was intersected with the subject-wise WM segmentation. The spatial CoV was calculated as the ratio between the standard deviation of CBF and the mean CBF within GM (Mutsaerts et al., 2017). Spatial CoV has previously been shown to correlate with the ATT and may serve as a proxy measure of vascular insufficiency.

2.8 Statistical analysis

In line with previous publications on this cohort, we compared those exposed to the famine during gestation to those unexposed to famine during gestation (born before the famine or conceived after the famine). Before we combined those born before the famine and those conceived after the famine into one control group, we tested for group differences (two sexes combined as well as separately) on all of the CBF dependent variables outcomes. No statistically significant differences were found between these two control sub-groups. We used linear regression analyses to compare continuous maternal, birth, and general adult characteristics between the exposed and unexposed groups and logistic regression analyses to compare binary characteristics between these groups.

We used linear regression analyses to test associations between CBF variables (dependent variables) and adult characteristics as well as metabolic outcomes (independent variables), of which the models were adjusted for sex. Linear regression analyses were also used to compare CBF measures between the exposed and unexposed groups (adjusted for sex). As there are known sex differences in brain volumes and perfusion, as well as in effects of fetal programming and prenatal famine exposure specifically, we also ran our models for men and women separately.(de Rooij et al., 2016; Franke et al., 2018) To test for mediation by metabolic parameters, we added these parameters to the regression models and in addition applied the mediation model by Preacher and Hayes using the PROCESS macro for SPSS (Hayes, 2013; Preacher and Hayes, 2004). This procedure estimates the indirect effect of a potential mediator on an outcome and provides a confidence interval for this effect using

bootstrapping. In these mediation analyses, we included participants who also participated at age 50 (n = 80) or age 58 (n = 100). In all regression models, we considered group differences to be statistically significant if p-values were <= 0.05. Statistical analyses were performed with IBM SPSS Statistics version 24.

3. Results

3.1 Study group characteristics

A total of 118 cohort members underwent MRI scanning. The ASL scan of one person was excluded due to insufficient quality. A further ASL scan was excluded as the spatial CoV value constituted an extreme outlier (around 5 SD above the mean) and CBF measures become unreliable in such cases. Data of the remaining 116 participants were analyzed. We further discarded the GM/WM ratio of one person that was > 5 SD above the mean. Of the 116 participants, 50 were men (43%) and 66 were women (57%). Mean age for the total sample was 67.5 (SD 0.9) years. A total of 35 (30%) persons were born before the famine, 40 (35%) were exposed to the famine during early gestation and 41 (35%) were conceived after the famine. Table 1 shows the mean maternal, birth and adult characteristics according to exposure group. There were no significant differences between the exposed and unexposed groups in any of these variables.

3.2 CBF outcomes and covariates

Table 2 shows the mean CBF values and Figure 1 displays the average CBF for the total study group. CBF in WM was higher for women compared to men (2.0 mL/100 g/min [95%CI: 0.2 to 3.7], p = 0.03), while both the GM/WM-ratio (-0.50 [-0.79 to -0.22], p = 0.001) and spatial CoV (-0.06% [-0.09 to -0.04], p < 0.001) were lower in women. Education, SES, current smoking, alcohol consumption, physical activity, antihypertensive, lipid lowering or antidiabetic medication were not associated with CBF parameters. A history of CVA or TIA (n = 5) was associated with higher CBF in GM (15 mL/100 g/min), the ACC, PCC and AMCA (all p <= 0.02), which could potentially be explained by the anticlotting agents these individuals all used.

CBF values were not associated with metabolic variables measured at age 68 years (glucose, cholesterol, blood pressure and BMI). However, 2-h glucose levels at age 58 were significantly negatively associated with CBF in GM (-0.9 mL/100 g/min [-1.9 to 0.0], p = 0.05,). In the same manner, total cholesterol levels at age 58 were negatively associated with CBF in GM (-1.8 mL/100 g/min [-3.5 to -0.2], p = 0.03), the ACC (-2.4 mL/100 g/min [-4.3 to -0.6], p = 0.01), and AMCA (-1.9

mL/100 g/min [-3.6 to -0.3], p = 0.02), and showed a trend towards a negative association with CBF in the PCC (-2.2 mL/100 g/min [-4.8 to 0.3], p = 0.08). Systolic blood pressure and diastolic blood pressure at age 58 were positively associated with spatial CoV (0.001% [0.0 to 0.002], p = 0.02); 0.002% [0.001 to 0.004], p = 0.01 respectively). Levels of 2-h glucose at age 50 showed a trend towards a negative association with CBF in WM (-0.7 mL/100 g/min [-1.4 to 0.0], p = 0.07) and a positive association with spatial CoV (0.01% [0.0 to 0.02], p = 0.07).

3.3 CBF outcomes and famine exposure

Table 2 shows mean outcomes for the CBF measures according to famine exposure status. CBF in WM was -2.5 mL/100g/min [95%CI: -4.3 to -0.8; p = 0.01] lower for those prenatally exposed compared to those unexposed to famine. The GM/WM-CBF ratio was higher for those exposed (0.48 [0.19 to 0.76; p = 0.002]), whereas the spatial CoV showed a trend towards a positive association with famine exposure (0.03% [0.00 to 0.05, p = 0.08]). The other CBF measures did not differ between the exposed and unexposed groups. Exclusion of the subjects with a history of CVA or TIA from the dataset, also did not change the results.

3.4 CBF outcomes and famine exposure in men

Sex-specific outcomes in CBF measures after prenatal famine exposure are shown in Table 3a (effect sizes and 95% CI, unadjusted outcomes) and Supplemental Table 1a (effect sizes and 95% CI, outcomes adjusted for partial volume effects). In both uncorrected and corrected analyses, prenatally famine exposed men had significantly lower CBF in the ACC and PCC and trended towards lower CBF in WM, while spatial CoV was higher compared to unexposed men (see Figure 2 fore illustrative examples). In all cases, the uncorrected models rendered the same results as the models corrected for partial volume effects or only slightly attenuated the effects.

There was little evidence for mediation of the effects of prenatal famine exposure on CBF measures by metabolic outcomes at earlier ages, with one exception. The effect of famine exposure on the spatial CoV in men seemed for a large part to be mediated by 2-h glucose levels at the age of 50, which were higher among exposed men (effect change from B = 0.05% [95%CI: 0.0 to 1.0; p = 0.05 to

B = 0.02% [-0.04 to 0.08; p = 0.49]). Figure 3 depicts the mediation model as computed by the Preacher & Hayes model.

3.5 CBF outcomes and famine exposure in women

In exposed women, CBF in WM and the GM/WM ratio were respectively lower and higher compared to unexposed women (see Table 3b and Supplemental Table 1b) . As was shown in the men, the models corrected for partial volume effects showed similar outcomes as the uncorrected models. Notably though, the effect size of the difference in GM/WM ratio between the groups increased after correction. Effects of prenatal famine exposure on CBF in WM and GM/WM ratio were not mediated by metabolic parameters at earlier ages.

3.6 Adding spatial CoV to CBF models

Pearson's correlations between GM and WM and spatial CoV were -0.44 ($p < 0.001$) and -.60 ($p < 0.001$) respectively. Adding spatial CoV to the famine exposure models decreased, but not completely explained, the effects of exposure on the CBF in WM and the sex-specific effects of CBF in the ACC, PCC and WM (WM in all exposed: -1.6 mL/100g/min [95%CI: -3.1 to -0.2; $p = 0.03$]; ACC in exposed men: -6.7 mL/100g/min [-14.2 to 0.9; $p = 0.08$]; PCC in exposed men: -8.7 mL/100g/min [-17.2 to -0.1; $p = 0.05$]; WM in exposed women: -2.1 mL/100g/min [-4.0 to -0.2; $p = 0.03$]).

Discussion

Our findings suggest that in this relatively healthy group of older adults, overall brain perfusion was worse in participants who had been exposed to undernutrition during the first months of fetal life. This was especially the case for exposed men, who had a significantly higher spatial CoV, which we postulate indicates higher vascular insufficiency, but also indications of potential neurodegenerative effects as demonstrated by lower CBF in the ACC and PCC, areas where CBF was previously demonstrated to be lower in those with dementia disorders (Mutsaerts et al., 2015b; Sierra-Marcos, 2017). These findings are in line with previous studies in this cohort, in which we demonstrated sex-specific associations between prenatal famine exposure and brain outcomes, with smaller intracranial volumes and increased ageing of the brain in men only (de Rooij et al., 2016; Franke et al., 2018).

Due to the fact that WM has relatively low CBF and high ATT, the calculated WM CBF probably does not reflect accurate WM perfusion values (van Osch et al., 2009). Nevertheless, the ASL signal in WM may still contain meaningful information about WM hemodynamics. For this reason, we refer to the computed WM CBF as WM ASL signal throughout the discussion. The lower WM ASL signal and higher GM/WM-CBF ratio among those with early gestation famine exposure without clear differences for GM CBF are interesting. This finding fits with the spatial CoV differences that we found between the exposed and unexposed groups, although this was mostly the case in exposed men. Changes in ATT and vascular health – as supposedly reflected by the spatial CoV - could make the difference between being able to measure WM CBF on an ROI level. A previous study in healthy freedivers showed that breath-hold-induced hypercapnia lead to concomitant CBF increase and spatial CoV decrease, which led to a higher increase in the measured WM ASL signal than in the GM ASL signal, likely since the ATT decreased to a point where the WM CBF was measurable (Keil et al., 2018). This would mean that the ASL WM signal difference between exposed and unexposed participants simply reflects a difference in the ability of WM CBF to be measured in those with higher or lower vascular burden. In this context, one would also have expected differences in the burden of white matter hyperintensities (WMH), which we could however previously not demonstrate in this group of participants (de Rooij et al, 2016). Given the associations between

prenatal famine exposure and reduced brain perfusion we have shown here, a higher burden of WMH may be expected with increasing age.

The difference in spatial CoV between exposed and unexposed men appeared for a large part to be mediated by higher 2-h glucose levels at age 50 years. Based on our data, we cannot say whether these two are causally connected, as parts of the same pathway, or simply both a separate but related result of famine exposure. Type 2 diabetes has been associated with regional hypo perfusion in some studies but other studies failed to demonstrate effects on brain perfusion (van Bussel et al., 2017). The higher glucose levels observed in famine exposed men at the age of 50 years may also be closely related to other adverse (metabolic) parameters which may explain the association with lower brain perfusion, although blood pressure and cholesterol did not seem to mediate the association.

In addition to indirect effects of high glucose levels on reduced brain perfusion in those exposed to famine in early gestation, prenatal famine exposure may also have had a direct effect on brain perfusion through influences on the vascular system. Nutritional deprivation during the first gestational trimester may have compromised the development of the cerebrovasculature affecting its function into older age. Although the cerebrovascular system undergoes many changes in the years following fetal life, a basic system is completely laid down during the first two months after conception (Scher, 2012). Rodent studies have demonstrated that maternal under- as well as overnutrition during pregnancy can lastingly alter the structure and function of the brain vasculature. Food restriction in female rats during gestation led to increased artery stiffness and wall thickness as well as decreased contractility in the 8-month old offspring (Durrant et al., 2014). Feeding female mice a high fat diet during pregnancy and lactation caused long-term changes in the expression and/or activity of several components of the neurovascular unit and dramatically changed the morphology of the basement membranes of the cerebral vasculature in the offspring (Hawkes et al., 2015). The cerebrovasculature of people exposed to the Dutch famine during early gestation may have been affected in similar ways.

In prenatally exposed men, CBF was also reduced in the ACC as well as the PCC. Hypo perfusion of these areas has previously been shown in patients with Alzheimer's Disease (AD) or mild cognition impairment (MCI) and it has been related to amyloid-beta load in both MCI and AD patients (for review of studies see (Zhang et al., 2017)). One study showed that reduced CBF in the PCC was associated with the deterioration of cognitive function in healthy older adults over an 18-month follow-up period, suggesting that it may serve as a risk factor for developing MCI or even AD (Xekardaki et al., 2015). Taken together with previous findings in our cohort demonstrating reduced performance on a Stroop task at age 58 years (de Rooij et al., 2010), smaller intracranial volume and altered brain structure resembling premature brain ageing at age 68 years (de Rooij et al., 2016; Franke et al., 2018), men exposed to undernutrition in early gestation may be at a higher risk to develop AD compared to unexposed men. A study in individuals born around the time of the Great Chinese famine of 1959-1961, suggests that this is indeed the case by showing a higher prevalence of both MCI and dementia in those exposed to undernutrition in early life (Kang et al., 2017a). However, results of this Chinese famine study have to be interpreted with caution as exposed participants were older than unexposed participants and results may have been confounded by age. Also, it has to be noted that we measured perfusion, which is not a direct reflection of metabolism, limiting our interpretation that the association we found between reduced CBF in ACC and PCC in exposed men is due to recued metabolism as a consequence of neurodegeneration.

Our differential findings in men and women can be explained in two different ways. Firstly, selective participation of more healthy exposed women may have taken place. We have previously shown that women exposed to famine in early gestation suffered higher mortality up to the age of 63 years resulting in the stronger, surviving women participating in the present study (van Abeelen et al., 2012). This seems to be confirmed by the fact that at age 50, exposed women in the present sample had 2h-glucose levels that were similar to levels of unexposed women (results not shown). Secondly, outcomes such as decreased brain volumes and increased mental disorders after deprivation in early life have been shown to predominantly affect men suggesting that sex differences may exist in the

vulnerability for early life programming effects (Brown and Susser, 1997; de Rooij et al., 2011; Franzek et al., 2008).

A number of limitations to our study have to be taken into account. Effects were overall small. Also, our study had an exploratory focus and we did not correct for multiple testing. Furthermore, we have observed an increase of spatial CoV in many participants. As said previously, increase of spatial CoV related to increased ATT indicates a presence of labelled bolus in the vessels, leading to underestimation of CBF in the distal brain tissue (Alsop et al., 2015; Mutsaerts et al., 2017). This fact may explain part of the apparent CBF decreases in the ASL WM signal, the CBF decrease in the ACC and PCC after prenatal famine exposure, and could also explain our relatively high GM/WM CBF ratio and high GM CBF after PVC. This was further aggravated by the used post labeling delay (PLD) range (1525ms - 2215 ms, average PLD 1870 ms), which was lower than 2000 ms PLD recommended for older adults by the ASL consensus paper (Alsop et al., 2015). Spatial CoV is a more effective parameter with shorter PLDs, which may have increased the group difference of spatial CoV relative to the group difference in CBF. Indeed, adding spatial CoV to the models made the effects of exposure on CBF in WM, ACC and PCC somewhat smaller, but it did not completely explain the effects. A future more detailed study of the interplay between the arrival time, spatial CoV, and its effect on measured CBF would be useful to address the magnitude of this problem. Another limitation is the lack of blood hematocrit measurements. Hematocrit changes are associated with CBF under- or overestimations and it would have been good if we could have taken this into account, although we have no reason to expect that hematocrit levels differ between those exposed and those unexposed to prenatal famine (Henriksen et al., 1981; Vaclavu et al., 2016). A final limitation is that we did not have repeated ASL measurements over time, which could have given us an idea about the timing of the effects of prenatal famine exposure on brain perfusion, i.e. whether perfusion has been worse from the start or has declined with increasing age.

In conclusion, our study suggests that exposure to undernutrition in early gestation can affect late life cerebrovascular health and that there may potentially also be neurodegenerative effects of

prenatal undernutrition in men. These findings encourage future studies to investigate whether these can lead to an increased risk for vascular or neurodegenerative disorders.

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Competing interests

The authors declare no conflicts of interest.

Author Contributions

Conception and design of the study (SRdR, TJB, MS, AJN, MC); acquisition and analysis of data (SRdR, HJMMM, IA, JP, PG, MWAC); drafting the manuscript (all authors).

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Figure legends

Figure 1. Average population cerebral blood flow projected on the average population T1w image. ((Note the underestimated CBF due to susceptibility artifacts at regions with air-tissue proximity: the orbitofrontal cortex (close to ethmoidal sinus), and the inferior medial temporal cortex (close to mastoid).))

Data are adjusted for partial volume effects; β = unstandardized beta coefficient; CBF Spatial CoV = cerebral blood flow Spatial Coefficient of Variation; CI = Confidence Interval.

Figure 2. The spatial Coefficient of Variation (CoV): on the left side a participant from the control group prenatally unexposed to famine with very low spatial CoV and on the right side a participant from the group exposed to famine during early gestation with very high spatial CoV. The spatial CoV provides an indication of the transit of the ASL signal to tissue, with higher CoV values indicating transit delay.

Figure 3. Depiction of mediation of the effect of famine exposure in early gestation on CBF Spatial CoV in men by 2-h glucose levels measured at age 50 years.

Table 1. Maternal, birth, and adult characteristics according to famine exposure status.

		Exposure to famine				
	n	Born before	Early gestation	Conceived after	Total	*p*
General characteristics						
N		35	40	41	116	
Age (years)	116	68.7 ± 0.5	67.4 ± 0.4	66.7± 0.2	67.5 ± 0.9	0.22
Female (%)	116	60.0	55.0	56.1	56.9	0.77
Maternal and birth characteristics						
Occupation head household manual (%)	94	76.0	63.9	63.6	67.0	0.80
Maternal weight last antenatal visit (kg)	98	64.7 ± 9.3	69.1 ± 9.7	70.7 ± 8.2	68.3 ± 9.3	0.50
Gestational age (days)	101	285 ± 11	287 ± 10	286 ± 14	286 ± 12	0.58
Birth weight (g)	116	3375 ± 492	3469 ± 508	3393 ± 518	3414 ± 504	0.39
Adult characteristics						
Education [a]	116	4.7 ± 2.5	4.6 ± 1.8	4.5 ± 2.1	4.6 ± 2.1	0.89
Socio-economic status	116	50 ± 13	48 ± 13	49 ± 15	49 ± 14	0.58
Current smokers (%)	116	2.9	15.0	12.2	10.3	0.24
Alcohol consumers >1 glass p/wk (%)	116	65.7	77.5	68.3	70.7	0.26
Physically active (%)	116	85.7	75.0	80.5	80.2	0.31
Hypertension medication (%)	116	40.0	35.0	41.5	38.8	0.54
Hypocholesteraemia medication (%)	116	31.4	32.5	31.7	31.9	0.92
Diabetes medication (%)	116	17.1	20.0	14.6	17.2	0.57
History of CVA or TIA (%)	116	2.9	2.5	7.3	4.3	0.50
BMI (kg/m^2)	116	27.4	28.3	30.3	28.7 ± 5.0	0.48
Systolic blood pressure (mmHg)	115	149	147	153	149 ± 16	0.15
Diastolic blood pressure (mmHg)	115	82	83	87	84 ± 11	0.36
Non-fasting total cholesterol (mmol/l)	114	5.7	5.4	5.7	5.6 ± 1.1	0.14
Non-fasting glucose (mmol/l)	114	6.3	6.0	6.1	6.1 ± 1.5	0.64

Data are given as means ± SD, except where given as count and percentages; *p*-values for differences between the exposed and combined unexposed groups based on regression analysis; [a] Educational level measured on a 10-point scale (1 = primary education not completed, 10 = university completed); CVA = cerebrovascular accident; TIA = transient ischemic attack.

Table 2. Cerebral blood flow measures (unadjusted for partial volume effects) according to prenatal famine exposure.

	Exposure to famine				
	Born before	Early gestation	Conceived after	Total	*p*
N	35	40	41	116	
General					
GM	56.4 ± 6.9	54.3 ± 10.3	57.3 ± 12.1	56.0 ± 10.1	0.19
WM	16.8 ± 3.4	14.1 ± 4.3	16.6 ± 5.7	15.8 ± 4.7	0.01*
GM/WM-ratio	3.46 ± 0.59	4.03 ± 0.77	3.63 ± 0.91	3.72 ± 0.81	0.002*
Spatial CoV	0.566 ± 0.069	0.595 ± 0.086	0.569 ± 0.75	0.577 ± 0.078	0.08
Dementia related					
ACC	60.6 ± 9.0	58.1 ± 11.5	62.2 ± 13.9	60.3 ± 11.8	0.15
PCC	73.7 ± 11.8	71.9 ± 14.6	76.8 ± 17.0	74.2 ± 14.8	0.23
Precuneus	62.1 ± 8.1	59.9 ± 13.2	63.0 ± 12.3	61.7 ± 12.3	0.26
Flow territories					
AMCA	55.9 ± 7.1	54.3 ± 10.2	57.5 ± 11.8	55.9 ± 10.0	0.22
PCA	60.6 ± 9.8	56.9 ± 13.1	59.0 ± 12.9	58.8 ± 12.9	0.26

Data are given as means ± SD in mL/100 g/min; *p*-values for differences exposed versus combined unexposed groups adjusted for sex, where * indicates $p <= 0.05$; GM = Grey matter; WM = White matter; CoV Coefficient of Variation; ACC = anterior cingulate cortex; PCC = posterior cingulate cortex; AMCA = anterior and middle cerebral artery; PCA = posterior cerebral artery.

Table 3a. Cerebral blood flow measures according to prenatal famine exposure in men.

	Exposure to famine				
	Born before	Early gestation	Conceived after	**ES (95% CI)**	***p***
Men N	*14*	*18*	*18*		
General					
GM	54.6	52.8	59.6	-4.6 (-10.9 to 1.6)	*0.14*
WM	15.3	13.0	15.8	-2.5 (-5.2 to 1.0)	*0.06*
GM/WM ratio	3.69	4.22	4.02	0.35 (-0.15 to 0.85)	*0.16*
Spatial CoV	0.60	0.64	0.59	0.05 (0.00 to 0.09)	*0.05**
Dementia related					
ACC	60.5	56.2	67.4	-8.0 (-15.1 to -0.9)	*0.03**
PCC	80.0	68.6	80.3	-11.4 (-19.6 to -3.2)	*0.02**
Precuneus	59.2	57.5	64.0	-6.0 (-14.2 to 2.1)	*0.27*
Flow territories					
AMCA	54.9	52.8	60.1	-5.0 (-11.1 to 1.2)	*0.11*
PCA	55.4	54.6	60.0	-3.4 (-11.3 to 4.6)	*0.40*

Table 3b. Cerebral blood flow measures according to prenatal famine exposure in women.

	Exposure to famine				
	Born before	Early gestation	Conceived after	**ES (95% CI)**	***p***
Women N	*21*	*22*	*23*		
General					
GM	57.7	55.6	55.5	-1.0 (-6.1 to 4.2)	*0.71*
WM	17.8	15.0	17.2	-2.5 (-4.9 to -0.1)	*0.04**
GM/WM ratio	3.30	3.88	3.53	0.46 (-0.03 to 0.94)	*0.06*
Spatial CoV	0.54	0.56	0.55	0.01 (-0.03 to 0.04)	*0.61*
Dementia related					
ACC	60.7	59.7	58.1	0.4 (-5.4 to 6.2)	*0.90*
PCC	71.5	74.5	74.1	1.7 (-6.2 to 9.5)	*0.67*
Precuneus	64.1	61.8	62.3	-1.3 (-7.3 to 4.7)	*0.66*
Flow territories					
AMCA	56.5	55.5	55.4	-0.4 (-5.6 to 4.7)	*0.86*
PCA	64.1	58.8	58.2	-2.2 (-8.7 to 4.3)	*0.50*

Data are given as means ± SD in mL/100 g/min; Effect sizes (ES) (95% Confidence interval [CI]) and *p*-values for differences exposed versus combined unexposed groups, where *indicates $p <= 0.05$; GM = Grey matter; WM = White matter; CoV Coefficient of Variation; ACC = anterior cingulate cortex; PCC = posterior cingulate cortex; AMCA = anterior and middle cerebral artery; PCA = posterior cerebral artery.

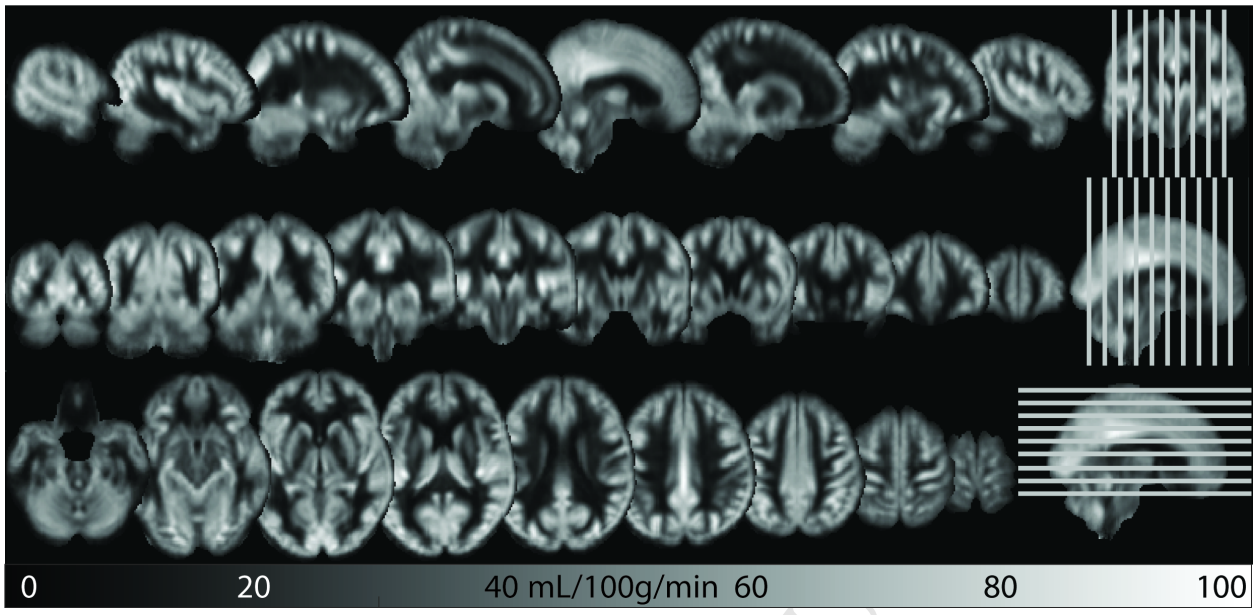
Supplemental Table 1a. Cerebral blood flow measures according to prenatal famine exposure in men adjusted for partial volume effects.

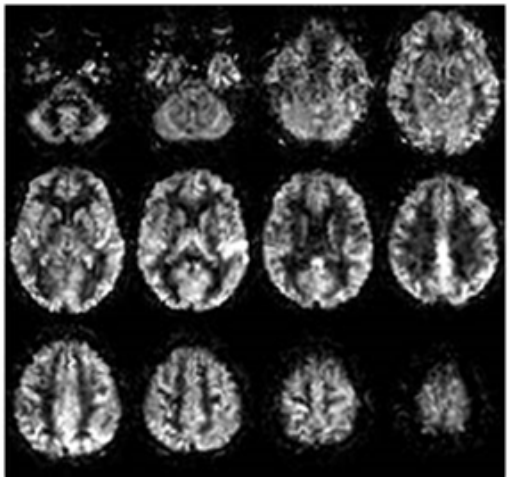
	Exposure to famine				
	Born before	Early gestation	Conceived after	**ES (95% CI)**	***p***
Men N	*14*	*18*	*18*		
General					
GM	86.2	84.2	94.7	-6.8 (-16.6 to 2.9)	*0.17*
WM	15.3	13.6	16.2	-2.2 (-4.6 to 0.3)	*0.08*
GM/WM ratio	5.80	6.37	6.13	0.39 (-0.31 to 1.08)	*0.27*
Spatial CoV	0.60	0.64	0.59	0.05 (0.00 to 0.09)	*0.05**
Dementia related					
ACC	89.9	85.7	103.1	-11.6 (-23.1 to -0.5)	*0.05**
PCC	108.7	101.1	118.1	-12.9 (-24.6 to -1.1)	*0.03**
Precuneus	99.8	99.0	109.5	-6.3 (-19.1 to 6.6)	*0.33*
Flow territories					
AMCA	94.4	92.1	104.3	-8.0 (-18.5 to 2.5)	*0.13*
PCA	79.9	80.0	87.5	-4.2 (-14.6 to 6.3)	*0.43*

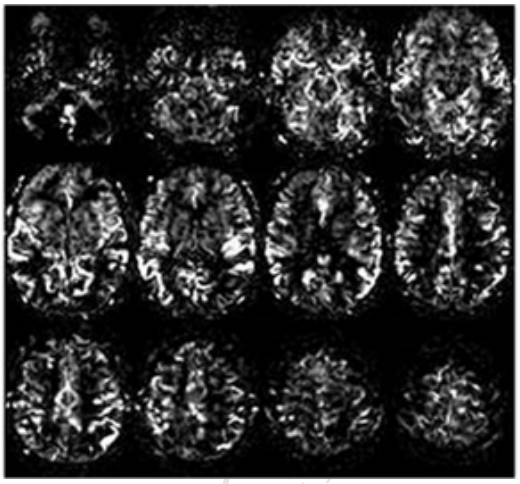
Supplemental Table 1b. Cerebral blood flow measures according to prenatal famine exposure in men adjusted for partial volume effects.

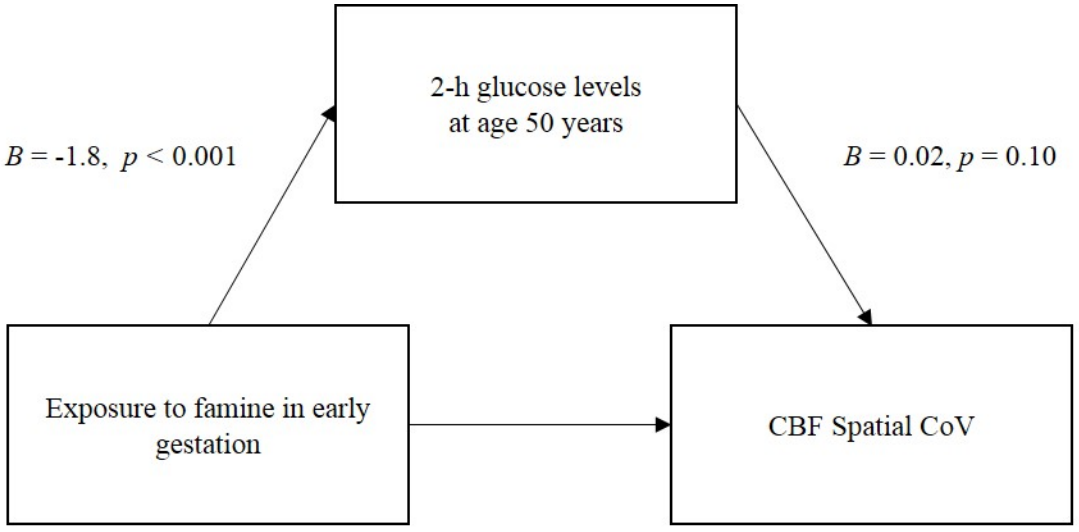
	Exposure to famine				
	Born before	Early gestation	Conceived after	**ES (95% CI)**	***p***
Women N	*21*	*22*	*23*		
General					
GM	89.7	87.3	85.1	0.0 (-7.4 to 7.3)	*0.99*
WM	17.7	15.1	17.5	-2.5 (-4.6 to -0.3)	*0.03**
GM/WM ratio	5.14	5.93	4.88	0.93 (0.58 to 1.27)	*<0.001**
Spatial CoV	0.54	0.56	0.55	0.01 (-0.03 to 0.04)	*0.61*
Dementia related					
ACC	86.7	87.6	84.5	2.0 (-6.1 to 10.2)	*0.62*
PCC	104.4	106.3	104.0	2.1 (-8.3 to 12.6)	*0.69*
Precuneus	107.2	103.3	101.0	-1.1 (-10.1 to 8.0)	*0.81*
Flow territories					
AMCA	95.0	94.9	92.3	1.3 (-6.5 to 9.0)	*0.74*
PCA	89.3	83.0	80.9	-1.9 (-9.9 to 6.1)	*0.63*

Data are given as means ± SD in mL/100 g/min; Effect sizes (ES) (95% Confidence interval [CI]) and *p*-values for differences exposed versus combined unexposed groups, where * indicates $p <= 0.05$; GM = Grey matter; WM = White matter; CoV Coefficient of Variation; ACC = anterior cingulate cortex; PCC = posterior cingulate cortex; AMCA = anterior and middle cerebral artery; PCA = posterior cerebral artery.









Total effect: $B = 0.05$, $p = 0.05$
Direct effect: $B = 0.02$, $p = 0.49$
Indirect effect: $B = 0.03$, 95% CI [-0.01 to 0.07]

Highlights

- Prenatal famine exposure is associated with altered brain perfusion parameters in older age.
- In exposed men, cerebral blood flow was lower in the anterior and cingulate cortices.
- Early exposed men also had a higher spatial coefficient of variation (CoV).
- The higher spatial CoV was largely mediated by higher glucose levels at age 50.