

Decoding the Female Brain:

Investigating Cerebral White Matter Changes
throughout the Menopausal Transition,
using diffusion MRI

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Kato Herregods

Patricia Clement

Preface

Welcome to my thesis, the product of countless hours of writing, searching, thinking and late-night debugging. As I would never have been able to create this work alone, I would like to express my gratitude to some amazing people, supporting me throughout this process.

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Summary

Due to the historical underrepresentation of women in medical research, the structural, functional and metabolic brain changes occurring during menopause transition remain poorly understood. However, many menopause-related symptoms are neurological in nature. Moreover, female sex has recently emerged as a major risk factor for Alzheimer's Disease. The metabolic brain changes happening during menopause transition are therefore thought to create a vulnerable brain state, increasing women's risk of developing the disease later in life. To investigate whether white matter is impacted in the hypometabolic, perimenopausal brain, diffusion-weighted imaging (DWI) was used to assess measures of white matter macro- and microstructure. For this, fractional anisotropy (FA), fiber density (FC), fiber cross-section (FC) and the combined measure FDC were compared between pre-, peri-, and postmenopausal women. Whole-brain analysis revealed a significant increase in log-transformed fiber cross-section (log (FC)) in the anterior corpus callosum of perimenopausal women compared to premenopausal controls. Given that the anterior corpus callosum is a region known to be vulnerable to neurodegenerative processes, the increase in FC is interpreted not as a reflection of reduced structural compactness. These findings suggest that perimenopause is associated with subtle but detectable changes in white matter architecture, particularly in regions susceptible to neurodegeneration.

Layman summary with societal impact

Women make up half of the global population and spend nearly one third of their lives in peri- or postmenopause. This period is marked by major hormonal changes that not only impact the reproductive system, the brain is greatly affected too. Despite this, the female brain remains understudied, and relatively little is known about how menopause influences brain health. Previous research has shown that during the menopause transition, the brain becomes less efficient at using glucose, its main energy source under normal circumstances. These metabolic changes may alter brain structure and function, potentially contributing to common menopause symptoms, such as hot flushes, mood changes or memory problems. They may also partly explain why women have a higher risk of developing Alzheimer's disease later in life. This thesis contributes to a better understanding of the menopausal brain by using advanced medical imaging techniques to investigate changes in the brain's white matter. In the long term, this research may contribute to improved public health and women's healthcare, by advancing our understanding of menopause-related brain changes and female brain aging. Findings may positively impact healthcare professionals, public health organizations, researchers in neuroscience and women's health, pharmaceutical and biotech companies. Most importantly, this research may eventually improve patients' quality of life by supporting better management of menopausal symptoms and informing earlier detection, prevention, and treatment of neurodegenerative diseases such as Alzheimer's disease.

1.Introduction

Historically, women have been underrepresented in both clinical and experimental research. The reasons for this are multifold and have changed over the course of history. One major factor is the misconception that the male body serves as a universal standard, while the female body is simply a smaller version of it.¹ This misconception has had large implications: Standard drugs such as beta-blockers and aspirin are less effective in women than in men.² Additionally women experience nearly twice as much adverse drug reactions compared to men. This disparity is attributed to the fact that many drugs prescribed to women, were primarily tested on men.³

Although this gender bias goes back to the early days of medical research, the exclusion of women exacerbated after the thalidomide crisis: In the 1960, thousands of children were born with severe birth defects after their pregnant mothers used thalidomide, a drug against nausea.⁴ In response to concerns about fetal safety and female fertility, the FDA issued guidelines for 'women of childbearing potential' to be excluded from the early phases of most clinical trials.⁵ However, growing awareness of the consequences of this systematic exclusion gradually shifted public and scientific opinion. Consequently, in 1993, the National Institute of Health (NIH) required the inclusion of women in the research population for a project to receive NIH funding.⁶ In 2016, an additional policy was implemented by the NIH, urging for sex to be included as a biological variable. Not only did these changes lead to an increased inclusion of women in clinical trials and general biomedical research, but it also sparked interest into previously overlooked aspects of female physiology.

In recent years, more research attention has gone to different female-specific neuroendocrinological transition states, such as puberty, pregnancy and menopause transition. During these life phases, not only the reproductive system adapts, the brain is greatly affected as well. A better understanding of the mechanisms underlying these hormonally induced brain changes, could also help understand associated pathologies, such as premenstrual dysphoric disorder (PMDD), post-natal depression or perimenopause associated neurological disturbances such as hot flashes, mood disturbances, forgetfulness and insomnia. Recently, it has been hypothesized that brain changes occurring during the menopause transition may increase women's risk of developing Alzheimer Disease (AD) later in life.^{Citation} A better understanding of these neurobiological changes could help inform preventative strategies for women.

Finally, it should be mentioned that a distinction is made between 'sex' and 'gender'. The American Medical Association Journal of Ethics (AMA) defines these terms as following: "Sex refers to the biological differences between males and females. Gender refers to the continuum of complex psychosocial self-perceptions, attitudes, and expectations people have about members of both sexes". Recognizing the difference between sex and gender, it should be noted that, throughout this thesis, the terms 'female/male' and 'women/men' will mainly be used to refer to the biological sex of a person.⁷

1.1 Cerebral energy metabolism

1.1.1 The healthy brain

The brain is characterized by an exceptionally high metabolic demand. Even though this organ makes up only a fraction of the total body mass and volume, it accounts for over 20% of its overall oxygen consumption.⁸ Additionally, it consumes ~5.6 mg glucose per 100 g human brain tissue every minute.⁸ Neurons are estimated to use the largest share, consuming about 75-80% of the energy available, despite the fact that glial cells are more abundant in the human brain.⁹ Neurons mainly use this energy to restore membrane potentials, needed for action potential generation.¹⁰ Other high energy consuming, neuronal mechanisms include the generation of postsynaptic potentials, the biosynthesis of neurotransmitters and the maintenance of ion gradients.⁸

Glucose and oxygen are the primary energy substrates for the brain.¹¹ To ensure their continuous supply, blood flow in the brain is strongly coupled to neuronal activity via a mechanism called neurovascular coupling, regulated via glucose transporters (GLUTs).^{8 12 13} The GLUT1 transporter allows for glucose transport across the endothelial membranes and into astrocytes, while GLUT3 and GLUT4 enable entrance of glucose into the neurons, as illustrated in Figure 1.^{8 14}

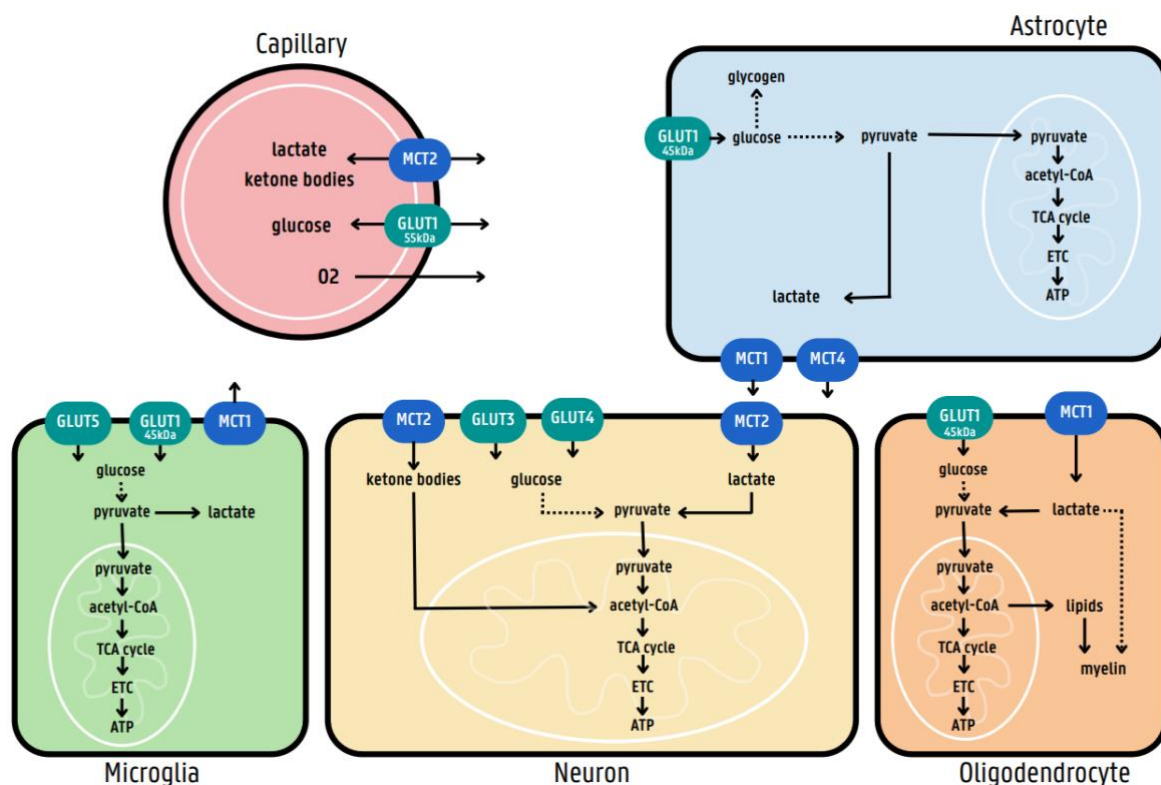


Figure 1: Bioenergetic processes in the female brain. Figure inspired by Ding, F., Yao, J., Rettberg, J. R., Chen, S. & Brinton, R. D. Early decline in glucose transport and metabolism precedes shift to ketogenic system.

As different cell types in the brain have different energetic needs, significant dissimilarities are present in their energy metabolism. Neurons mainly rely on glycolysis and subsequent mitochondrial oxidative phosphorylation of glucose for energy supply, explaining their sensitivity to hypoxia.¹⁰ Astrocytes depend on oxidative phosphorylation too, but, in contrast to neurons, will often resort to glycolysis with subsequent formation of lactate instead of oxidative phosphorylation. This phenomenon was first described in cancer cells and is known as the Warburg effect, allowing for fast and oxygen independent supply of energy and metabolites.¹⁵ In astrocytes however, this effect plays a role in the Astrocyte Neuron Lactate Shuttle (ANLS), a concept put forward in the early 90's. It suggests an alternative metabolic route whereby neurons can only meet their high energetic demands through collaboration with astrocytes. In response to heightened neuronal activity, the astrocytes increase their glycolytic activity, resulting in an enhanced lactate production and release into the extracellular space, as illustrated in Figure 2.^{12 16}

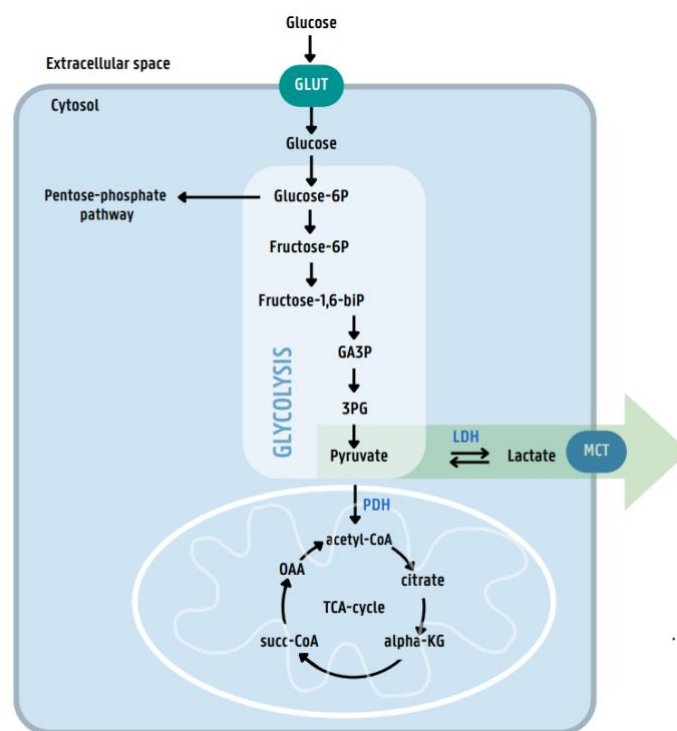


Figure 2: Besides oxidative phosphorylation in the mitochondria, astrocytes perform aerobic glycolysis with lactate production in high oxygen conditions. This mechanism is part of the Astrocyte Neuron Lactate Shuttle (ANLS) which ensures the supply of energy to neurons.

Neurons may thus use lactate to meet their acute energetic needs, because unlike glucose, which requires 10 enzymatic steps to produce pyruvate, lactate requires only a single step.¹⁶ The use of astrocyte-derived lactate is therefore suggested to be more efficient than direct glucose utilization. Additionally, neurons have shown a preference for lactate when both lactate and glucose are present.¹² Lactate has also shown to be essential for processes requiring intense neuronal activity, such as *Long Term Potentiation* (LTP) and memory formation, through which lactate acts as additional energy sources and as signaling molecule.¹³

1.1.2 The aging brain

As with all organs in the body, the brain naturally ages, leading to a series of structural and functional changes that appear over time. These changes do not necessarily lead to disease, but create a vulnerability for the development of neurological disorders, including Alzheimer Disease (AD).¹⁰ One of the mechanisms put forward as a driver of brain aging is the dysregulation of glucose homeostasis and associated mitochondrial dysfunction.^{17 10} The underlying reason here is a large metabolic shift in aging astrocytes; While aerobic glycolysis and lactate production are highly present in young astrocytes, oxidative phosphorylation becomes more important when aging occurs. Consequently, their ability to export lactate to neurons as a fast energy substrate declines. In turn, neurons seem to reduce their reliance on astrocytes for energy provision, as aging hippocampal neurons show elevated concentrations of glycogen metabolism enzymes.¹⁸

1.1.3 The role of estrogen in energy metabolism

Not only aging affects brain metabolism; hormones are highly influential as well. Estrogen, for example, plays a major role in regulating energy homeostasis throughout a women's life. Besides its well-established role in the female reproductive system, this sex hormone affects nearly all body tissues, including the musculoskeletal and cardiovascular system, the skin, adipose tissue and hair. It also has a major effect on the brain's physiology and energy metabolism.^{19 20}

Estrogen is a lipophilic steroid hormone, which can easily pass over the cell membrane and enter the cell. In women, four types of estrogens have been identified: estrone (E1), 17 β -estradiol (E2), estriol (E3), and estetrol (E4). E2 is produced by the ovaries and acts as the most potent form. It also is the principal circulating estrogen during a woman's reproductive years. E3 is almost exclusively found in pregnant women and is produced by the placenta. E1 is the main estrogen in postmenopausal women, formed by aromatase in the skin and peripheral adipose tissue.^{21 20 22}

Importantly, different subtypes can be distinguished. Estrogen receptor alpha (ER α) and beta (ER β), also named the classical estrogen receptors, are members of the nuclear receptor superfamily and can act as transcription factors. When estrogen binds ER α and ER β intracellularly, an E2-dimer complex is formed that enters the nucleus and binds to estrogen response elements (EREs). This activation typically takes multiple hours, inducing a slow, genomic effect.²¹ Importantly, mitochondrial DNA contains sequences similar to EREs found in the nucleus. As a result, ER- β can act as a transcription factor on the mitochondrial DNA too, enhancing the expression of mitochondrial genes.^{20 23} However, some of estrogen's effects occur more rapidly than can be explained by the slower genomic receptors, which lead to the discovery of membrane-bound estrogen receptors (mERs).

The first type of mERs are posttranslationally modified forms of the classical ER and are found on the cell membrane, the membrane of presynaptic terminals, in postsynaptic spines and in glial cell processes. Additionally, ER- β can be present on the mitochondrial membrane too and are referred to as mitochondrial-ER β s (mtER- β).^{24 25 26} The G-protein-coupled estrogen receptor 1 (GPER1), serves as a third type of mER.²¹ When estrogen binds this membrane-bound receptor, fast, non-genomic effects are induced, including the opening of ion channels and the mobilization of Ca²⁺ ions. Additionally, GTPase-driven signaling pathways are activated, including the cAMP, PI3K-Akt and MAPK-Erk cascades, which play major roles in cell survival, metabolism, cell motility, plasticity and cell growth. MAPK-Erk activation upon

GPER1 ligand binding can, for example, induce neurite formation in developing hippocampal neurons.^{21 27} Importantly, rapid signaling triggered by mER can also alter gene transcription further downstream. Taken together, the ER are part of an intricate network, enabling the interplay between membrane-bound, nuclear and mitochondrial receptors for the integration of both fast and slow signals over different cellular compartments.²⁶

Both the nuclear and membrane-bound ERs are found all over the brain, from the most rostral part of the forebrain, all the way to the cerebellum. However, some brain regions are more densely populated, as illustrated in

Figure 3. Preclinical evidence has localized nuclear ER α and ER β in the hypothalamus and pituitary gland, reflecting estrogen's role in regulating reproductive processes.²⁸ Within the hypothalamus, the preoptic and suprachiasmatic nucleus are especially rich in ERs, with the preoptic nucleus acting as the body's primary thermoregulator and the suprachiasmatic nucleus controlling sleep and circadian rhythms.²⁶ Other densely populated regions include the hippocampus, amygdala, prefrontal cortex, the cingulate cortex, ventral tegmental area, as well as the raphe nuclei and the nucleus coeruleus of the midbrain.^{29 30 26} High levels of membrane-bound ERs have been localized in the striatum, nucleus accumbens, prefrontal cortex and hippocampus.³⁰ Additionally, high levels of GPER1 have been observed in the olfactory bulbs, hypothalamus and a range of cortical regions including the motor, somatosensory and piriform cortices, the hippocampus, and the habenular nucleus of the epithalamus.³⁰

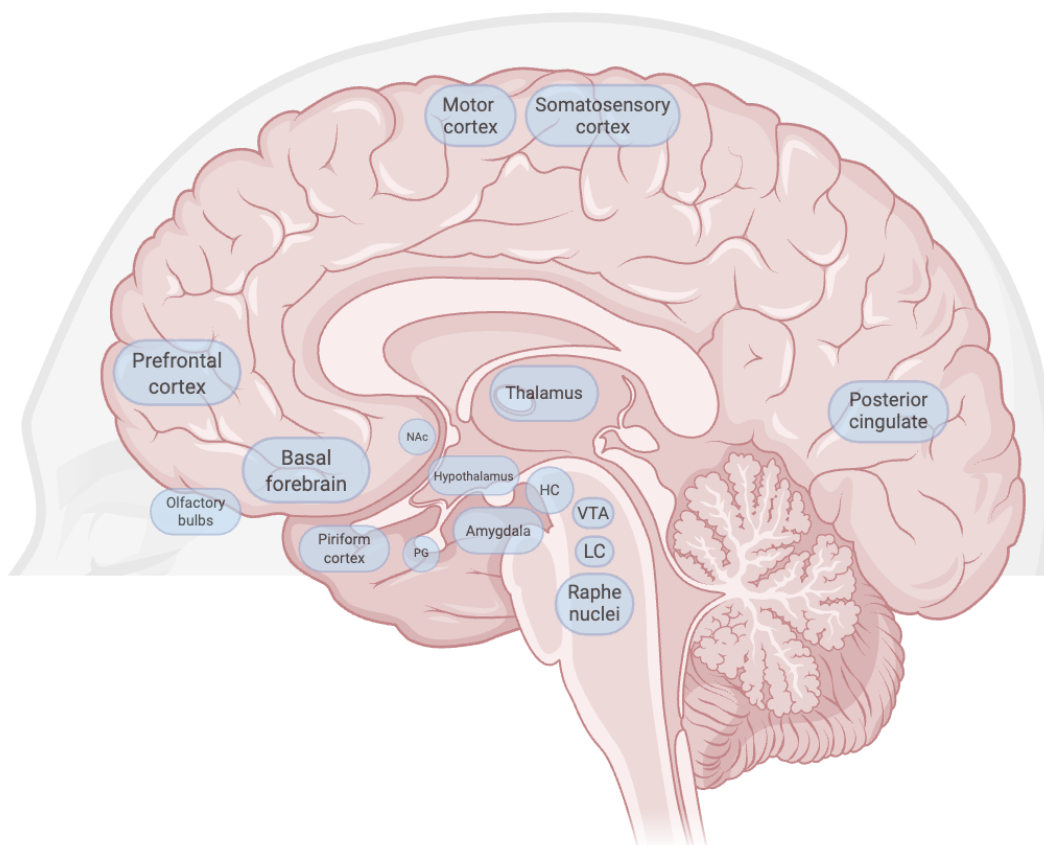


Figure 3: Brain regions densely populated with estrogen receptors (ERs). Abbreviations: PG, pituitary gland; NAc, nucleus accumbens; HC, hippocampus; VTA, ventral tegmental area; LC, locus coeruleus.

Inside the brain, estrogen acts a potent neuromodulator. By binding the nuclear, mitochondrial and membrane-bound ERs, it activates neuroprotective and neuroplastic signaling pathways in a coordinated manner.³¹ For example, the estrous cycle has a clear impact on the density of dendritic spines in CA1 pyramidal neurons, as shown in Figure 4.³² Estrogen has also been shown to enhance the expression of brain derived neurotrophic factor (BDNF), a molecule that plays a key role in regulating neurotransmitter systems and neuronal survival, promoting regeneration following injury. It has also been implicated in the stabilization of microtubules, the facilitation of glutamate transmission and cholinergic activity, synaptogenesis and myelination^{33 34} Estrogen also has antioxidant properties and fosters nonamyloidogenic metabolism of amyloid precursor protein (APP).³⁵

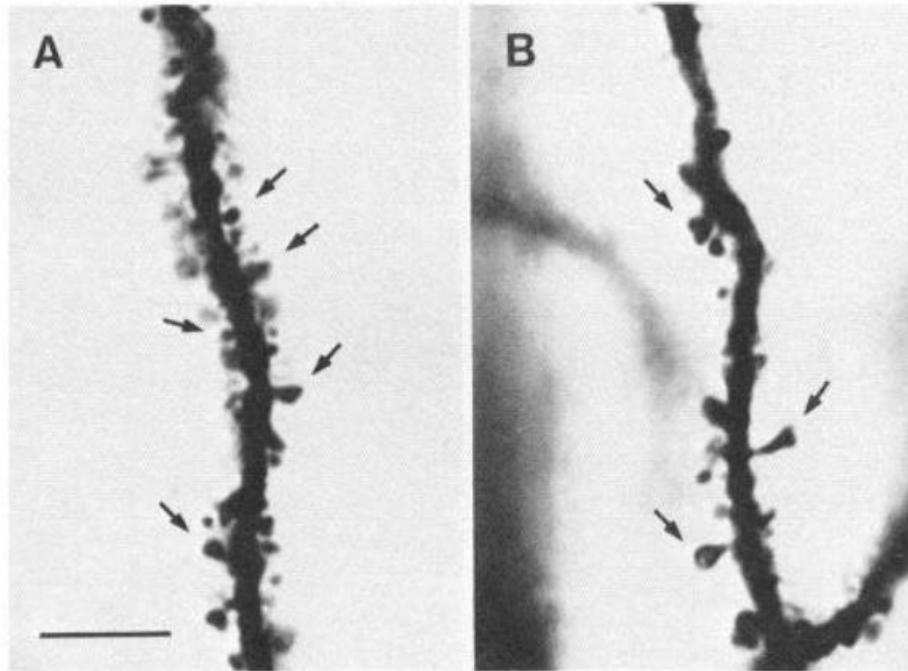


Figure 4: Photomicrographs of Golgi-impregnated apical dendrites from CA1 pyramidal cells of rats in high-estrogenic proestrus (A) or low-estrogenic estrus (B) phase of estrous cycle. The arrows indicate dendritic spines. Note the greater density of dendritic spines in A compared to B. Scale bar, 10 μ m for both A and B. Figure adopted from Woolley, C. et al 'Naturally occurring fluctuation in dendritic spine density on adult hippocampal pyramidal neurons. *J. Neurosci.* 10, 4035–4039 (1990)'.

Additionally, estrogen has a major impact on the brain's energy metabolism by protecting mitochondria and stimulating glucose metabolism. Firstly, estrogen protects the mitochondria by regulating their proteomes, enhancing the expression of the mitochondrial enzymes pyruvate dehydrogenase (PDH) and Complex IV cytochrome c oxidase (COX). Consequently, oxidative phosphorylation and ATP production increase, and beta-oxidation of fatty acids decreases. Furthermore, animal studies have shown that estrogen protects mitochondria against the accumulation of amyloid-beta and hyperphosphorylation of tau.³⁶ Secondly, estrogen stimulates glucose metabolism by enhancing the expression of glucose transporters, increasing glucose transport over the blood brain barrier (BBB) and stimulating the metabolism of glucose via mitochondrial oxidative phosphorylation.^{26 36 37} The menopause-related decline in circulating estrogen is therefore thought to be responsible for the reduction in glucose metabolism (CMRglc) in postmenopausal women.³⁸ To better understand these hormonally induced brain-changes, a closer look into the endocrinology and symptomatology of menopause is needed.

1.2 Menopause transition

Menopause transition is a natural process of endocrinological changes, shifting a women's body into the post-reproductive stage. However, hormonally induced physiological changes are not limited to the reproductive system; other organs, such as the cardiovascular system, the musculoskeletal system and the brain are greatly affected as well. This transition is initiated by a loss of ovarian follicles. Starting from puberty and proceeding during the reproductive years, these follicles are responsible for the production of the sex hormones estrogen and progesterone. When follicles are depleted, estrogen levels drop and menopause transition is initiated.

1.2.1 Endocrinology

Women are born with a finite number of follicles. During a normal menstrual cycle, follicle stimulating hormone (FSH) and luteinizing hormone (LH), produced by the anterior pituitary gland, lead to follicle development and eventually to ovulation of an oocyte.³⁹ This process of follicular activation, as well as natural follicular atresia, lead to the natural decline of follicles as women age.⁴⁰ The granulosa cell, a type of follicular cell, is responsible for the production of estrogen, anti-mullerian hormone (AMH) and inhibin B. As the number of ovarian follicles drops, a decline in inhibin B and AMH mark one of the first endocrinological changes of perimenopause. In response to the loss of negative feedback by inhibin B, FSH levels start rising roughly six years before the final menstrual period and remain elevated thereafter.^{41 42}

During the early phases of menopause transition, elevated FSH hyperstimulates the remaining follicles, accelerating folliculogenesis and contributing to menstrual cycle dysregulation. At first, cycles oftentimes become shorter and anovulatory cycles may occur. The unpredictable occurrence of either ovulatory or anovulatory cycles makes the hormonal landscapes among women at similar stages of reproductive aging highly heterogenous. As the transition progresses, cycles become longer, hormonal fluctuations get more extreme and the frequency of anovulatory cycles increases, eventually leading to the complete cessation of reproductive function.^{43 44} In postmenopausal women, hormone levels stabilize with high FSH, low E2 and undetectable inhibin B and AMH. Because estrone (E1) is mainly produced through peripheral aromatization, independent of the gonads, it becomes the predominant circulating estrogen after menopause. However, E1 is less potent than E2, and overall estrogen levels are substantially lower compared to premenopausal women. Furthermore, estrogen levels in menopausal women have been shown to drop below levels in elderly men.^{43 35}

1.2.2 Staging

1.2.2.1 Normal menopause transition

A standardized staging system for female reproductive aging, was developed during the *Stages of Reproductive Aging Workshop* (STRAW) in 2001 (STRAW staging system)⁴⁵ and updated in 2011 (STRAW +10 staging system).⁴⁶ This system puts forward nomenclature and defined stages of ovarian aging based on menstrual and qualitative criteria. STRAW +10 is considered the gold standard for characterizing reproductive aging and allows the distinction of ten distinct, as illustrated in Figure 5. Staging starts at -5 in the reproductive period and counts to +2 in the postmenopausal phase, with the final menstrual period (FMP) considered the null point.

During the **Late reproductive stage (Stage -3)**, reproductive senescence is initiated, which can be further divided into two substages (-3b and -3a). In both stages, anti-mullerian hormone (AMH) and antral follicle count (AFC) are low. Starting from stage -3a, subtle changes in cycle characteristics start occurring.⁴⁴

The **Early menopausal transition (Stage -2)** is initiated when the length of cycles persistently differs more than 7 days in consecutive cycles. During this phase, FSH levels are variable but rising, while AMH and AFC remain low. Once amenorrhea has persisted for 60 days or longer, a woman shifts into the **Late menopausal transition (Stage -1)**. Here, cycles get longer and the hormonal fluctuations become more extreme. There is also an increase in anovulatory cycles. Late menopausal transition lasts about 1 to 3 years, with a higher chance of symptoms. FSH levels can at first be variable but will eventually rise with a blood concentration of 25 IU/L considered characteristic for late menopausal transition.⁴⁴ These two phases together are also called perimenopause.

Menopause can be diagnosed retrospectively after twelve consecutive months of amenorrhea.⁴⁷ The final menstrual period (FMP) is considered the null point in the STRAW staging system, with postmenopause starting immediately after. **Early postmenopause (Stage +1)** is divided in three substages (Stage +1a, +1b, +1c) with Stage +1 overlapping with the 12-month period of amenorrhea, needed to define FMP. Both Stages +1a and +1b last one year and are characterized by a continued increase in FSH and decrease in estradiol. From stage +1c onwards, the hormone levels stabilize with high FSH and low estradiol. This stage is estimated to last 3 to 6 years. Compared to other stages, symptoms are most likely to occur during Stage +1a and +1b, when hormone levels are still changing. **Late postmenopause (Stage +2)** represents the remaining lifespan with stable reproductive hormone levels.⁴⁴

	Menarche				FMP (0)					
Stage	-5	-4	-3b	-3a	-2	-1	+1 a	+1b	+1c	+2
Terminology	REPRODUCTIVE				MENOPAUSAL TRANSITION		POSTMENOPAUSE			
	Early	Peak	Late		Early	Late	Early			Late
					Perimenopause					
Duration	variable				variable	1-3 years	2 years (1+1)	3-6 years	Remaining lifespan	
PRINCIPAL CRITERIA										
Menstrual Cycle	Variable to regular	Regular	Regular	Subtle changes in Flow/ Length	Variable Length Persistent ≥7- day difference in length of consecutive cycles	Interval of amenorrhea of ≥60 days				
SUPPORTIVE CRITERIA										
Endocrine FSH AMH Inhibin B			Low Low	Variable* Low Low	↑ Variable* Low Low	↑ >25 IU/L** Low Low	↑ Variable Low Low	Stabilizes Very Low Very Low		
Antral Follicle Count			Low	Low	Low	Low	Very Low	Very Low		
DESCRIPTIVE CHARACTERISTICS										
Symptoms						Vasomotor symptoms Likely	Vasomotor symptoms Most Likely			Increasing symptoms of urogenital atrophy

* Blood draw on cycle days 2-5 ↑ = elevated

**Approximate expected level based on assays using current international pituitary standard⁶⁷⁻⁶⁹

Figure 5: Schematic overview of the different stages of reproductive aging and their principal and supportive criteria used for classification. Endocrine aging is subdivided in stages (-5) until (+2) with the final menstrual period (FMP) as the null point. Adopted from Siobán D. Harlow et al. 'Executive summary of the Stages of Reproductive Aging Workshop + 10: addressing the unfinished agenda of staging reproductive aging' ⁴⁴

1.2.2.2 Early menopause transition

Menopause before the mean age of 51 years is common. Around 10% of women will experience early natural menopause between the age of 40 and 44 years. In 2-4% of women, menopause occurs even before the age of 40 years and is diagnosed as premature ovarian insufficiency (POI)⁴⁸. An early transition has a significant impact on both the physical and psychological health of women, with a higher chance of developing type 2 diabetes, atherogenic dyslipidemia, abdominal obesity and arterial hypertension.^{49 50} As these are all cardiovascular risk factors, women with POI have a 1.5–2.4-fold higher risk of developing cardiovascular disease, especially coronary heart disease, compared to women with a normal age at menopause.⁵¹ The musculoskeletal system and central nervous system are affected as well, with a higher risk for osteoporosis, fractures and sarcopenia, as well as depression and impaired cognition, indicating the importance of the gonadal hormones.⁵¹

1.2.3 Symptoms

About 80% of women going through menopause at a normal age will experience symptoms. Most often, women report vasomotor symptoms, such as hot flushes, sleep problems and genitourinary symptoms. A smaller fraction of women report significant changes in mood, sexuality and cognition or have musculoskeletal complaints.⁵² For many of these symptoms, but especially for vasomotor symptoms, estrogen-based therapies are the most effective.⁵³ The cause of most of these symptoms is unknown, however worsening of symptoms coincides with the menopause related decrease of brain metabolism.²⁶

1.2.4 Menopause associated brain changes

Gonadal hormones are well-established neuromodulators, shaping brain development and function. Although many menopause-related symptoms are neurological in nature, brain changes occurring during this hormonal transition period remain poorly understood. Preclinical as well as emerging clinical evidence have shown major effects on brain metabolism, structure and function. It is thought that these changes are induced by the menopause related decline in estrogen.²⁶

1.2.4.1 Estrogen loss and hypometabolism

During menopause transition estrogen drops, which has major implications on overall brain metabolism. Only recently, researchers have started mapping the effects of this endocrinological shift in the human brain. The transformation can broadly be categorized into changes in glucose metabolism, mitochondrial function and the metabolic adaptations it forces the brain to make.

The transition from pre- to postmenopause is accompanied by a 19% reduction in cerebral glucose metabolism (CMR_{glc}) in women. For the transition from pre- to perimenopause a reduction of 8% is observed.⁵⁴ In preclinical research, similar observations have been made using FDG-microPET. When reproductive senescence is initiated, the female mice brain shows a significant decline in glucose transport, which is sustained thereafter and can in part be explained by a reduced expression of glucose transporters.⁵⁵ Importantly, animal studies have shown that, as glucose metabolism declines, the use and transport of lactate declines as well. It is therefore argued that lactate, mostly generated from glucose in astrocytes, cannot be the main alternative energy substrate during menopause transition.⁵⁵

When looking at brain mitochondrial function, preclinical evidence has shown a significant decline in the enzymes pyruvate dehydrogenase (PDH) and complex IV (COX) when comparing mice before and after reproductive senescence. Both enzymes are essential for oxidative phosphorylation, as PDH links glycolysis to the TCA cycle and COX is needed for ATP production via ETC.⁵⁶ In humans, a reduction in peripheral mitochondrial COX activity has been observed in women transitioning into menopause, with a 54% decline in activity when comparing pre- to postmenopausal women.⁵⁴ Additionally, mice transitioning into reproductive senescence show a decline in multiple other bioenergetic proteins.⁵⁶

Data in humans on the exact metabolic changes during menopause transition remain scarce. However, animal studies suggest that in response to cerebral glucose deprivation, the brain shifts to ketone bodies as an alternative energy substrate, as is the case in reproductively incompetent mice.^{26 55 38 57} When energy reserves are low, the liver metabolizes lipids into the ketone bodies beta-hydroxybutyrate and acetoacetate. From the bloodstream, these ketones can pass over the BBB and into the brain via the monocarboxylic acid transporter 1 (MCT1), which is also found in glial cells. The MCT2 and MCT4 are mainly found in neurons and astrocytes respectively.⁵⁸ This cerebral ketogenic metabolism is well established in neonates and in periods of long-term fasting, where ketone bodies can supply up to 60% of the brain's energetic needs. Additionally, preclinical and clinical evidence have shown a shift to ketone bodies to be characteristic for the AD brain.⁵⁷

Further, in reproductively incompetent mice, a rise in cerebral beta-hydroxybutyrate is paralleled by a drop in peripheral plasma beta-hydroxybutyrate.⁵⁷ This paradoxical finding led to the hypothesis that in an energy deprived brain, endogenous brain lipids are used for ketone body generation. The proposed mechanism is as follows: mitochondrial dysfunction leads to production of hydrogen peroxide (H₂O₂), which activates the cytosolic phospholipase A2 (cPLA2)-sphingomyelinase pathway. Upon activation, myeline is broken down to provide fatty acids for ketone body generation. These ketone bodies can then be harnessed as energy substrate in neurons, as shown in

Figure 6.⁵⁷ Myelin is the lipid-rich, multi-layer membrane structure found around axons. In the central nervous system (CNS), it is produced by the oligodendrocytes and serves as an insulator to axons, allowing for fast signal transduction between neurons.

Temporary reductions in brain myelin content have already been reported in marathon runners, using multicomponent relaxometry MRI. Once glycogen granules in the brain run low, runners increasingly depend on alternative energy sources, such as fatty acids.⁵⁹ Amounting evidence suggests that myelin may function as a fatty acid reserve, readily available as energy source when normal brain nutrients are low.⁶⁰ As menopause transition is marked by a comparable hypometabolic phenotype, stretching out over a substantially longer period, similar compensatory mechanisms may be at play.

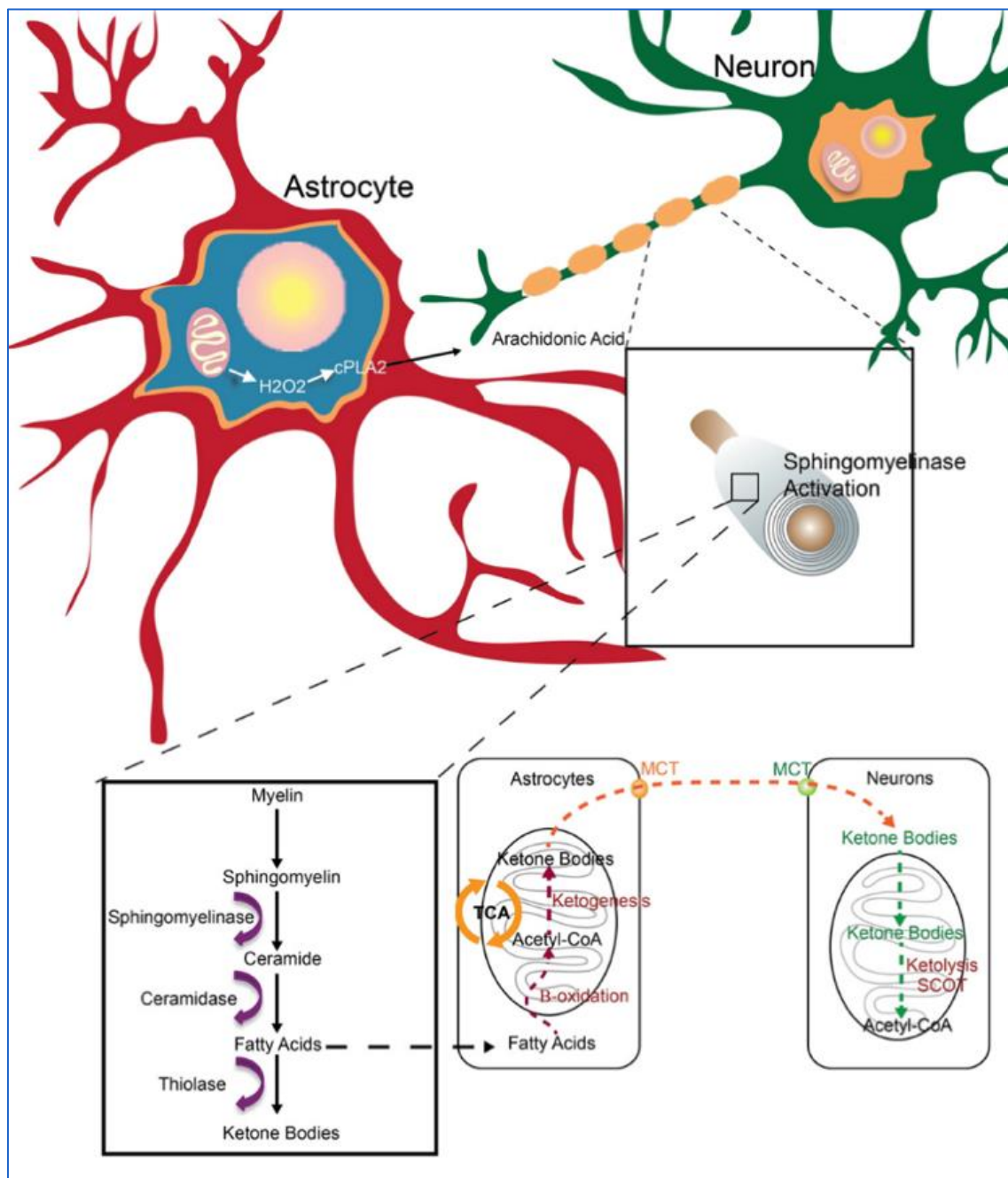


Figure 6: Schematic model of mitochondrial H_2O_2 activation of cPLA2-sphingomyelinase pathway as an adaptive response to glucose hypometabolism, providing myelin derived fatty acids as a substrate for ketone body generation. Figure adopted from Lauren P. Klosinski et al. 'White Matter Lipids as a Ketogenic Fuel Supply in Aging Female Brain: Implications for Alzheimer's Disease'

1.2.4.2 White matter changes

If myelin derived ketone bodies indeed serve as alternative energy source throughout menopause transition, a visible loss of myelin membranes may be present. Using Elektron microscopy (EM) of reproductively incompetent mice, a loss of structural myelin integrity after activation of the cPLA2-sphingomyelinase pathway, as illustrated in **Error! Reference source not found.**⁵⁷

In women transitioning through menopause, reports on white matter changes have been made as well. Both Diffusion Tensor Imaging (DTI) and anatomical MRI have been used for assessing measures of white matter microstructure and white matter volume (WMV) respectively. DTI in perimenopausal women with subsyndromal depression showed decreased fractional anisotropy (FA), a measure for the directionality of diffusion, in the left insula. Additionally, increased FA was observed bilaterally in the brainstem and in the left ventrolateral thalamus compared to controls. Furthermore, a positive correlation between E2 and FA values in the left insula was reported.⁶¹

A more recent study investigated the relationship between FA and WMV, in which postmenopausal women showed a decrease in FA in the right external capsule in comparison to perimenopausal women. When comparing to age-matched men, postmenopausal women showed increased FA, but reduced WMV in the corona radiata. An additional increase in FA was found in the fornix. Furthermore, perimenopausal women showed higher FA in the uncinate fasciculus, fornix, external capsula and corona radiata and lower FA in posterior thalamic radiation and superior longitudinal fasciculus compared to age-matched men. Lastly, the premenopausal groups showed higher FA in corona radiata and lower FA in superior longitudinal fasciculus, compared to age-matched men.³⁸

WMV was found to be lower in postmenopausal women in the anterior and posterior corona radiata compared to pre- and perimenopausal women. In comparison to age-matched men, a postmenopausal women showed lower WMV in the corona radiata, the superior longitudinal fasciculus, the cortico-spinal tracts and the posterior thalamic radiation. The perimenopausal women showed a similar pattern of lower WMV compared to age-matched men. Perimenopausal women exhibited lower WMV in the internal capsule.³⁸

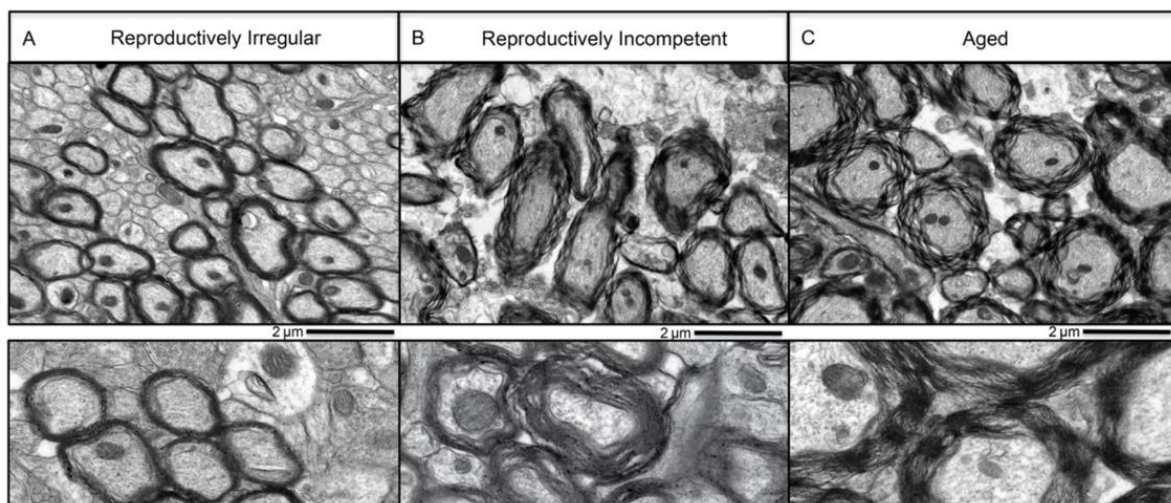


Figure 7: Elektron microscopy image of myelinated axons in the anterior commissure of reproductively aging female mice. Figure adopted from Lauren P. Klosinski et al. 'White Matter Lipids as a Ketogenic Fuel Supply in Aging Female Brain: Implications for Alzheimer's Disease'.

1.2.4.3 Alzheimer Disease in women and its relation to menopause

Women have a higher lifetime risk of developing AD. In search of an explanation for this unequal divide, this difference was oftentimes attributed to the greater longevity of women. Women tend to live longer than men and therefore, their lifetime risk of developing AD is greater as well. Even though this hypothesis is generally accepted, researchers have argued that longevity alone cannot sufficiently explain the gender gap and put forward female sex as its own major risk factor for the development of AD.^{62 63 64}

One of the arguments were repeated reports of differences in AD incidence rate between men and women. Incidence rate reflects the number of new disease cases per unit of time in the population at risk and is thus unaffected by longevity.⁶⁵ A study following 12.945 individuals reported a significant difference in AD incidence between men and women in the 80 to 84-year-olds. In this age-group, the occurrence rate of AD was more than double for women compared to men. In the 90+ group, this rate even tripled.⁶⁴ Similarly, a more recent study following 16.926 people in Sweden reported a divergence in incidence at approximately 80 years of age, with the gap between sexes widening as individuals grew older.⁶⁶ A nation-wide study based in Taiwan found a similar pattern. AD incidence rates went up rapidly with age, reflecting older age as a major risk factor, but remained significantly higher in women compared to men.⁶⁷ In a European meta-analysis, an overall AD incidence of 11.08 per 1000 person-years was found. Broken down by sex, the incidence was 13.25 per 1000 person-years in women, compared to 7.02 per 1000 person-years in men.⁶⁸ In contrast, no significant differences have been reported in the incidence rates of vascular dementia when comparing men to women.⁶⁴

The mechanisms behind the increased risk of AD in women remain poorly understood. Recently, the metabolic changes during menopause transition have been proposed as a contributing factor. Menopause is associated with hypometabolism, mitochondrial dysfunction and increased oxidative stress, creating a vulnerable brain that promotes A β plaque accumulation and neuronal dysfunction. As pathological changes in AD are thought to begin in midlife, researchers suspect that the metabolic changes during menopause put women at greater risk for developing AD later in life.⁵⁴

1.3 Magnetic Resonance Imaging (MRI)

1.3.1 General principles and modalities

1.3.1.1 Spin and net magnetization

Magnetic Resonance Imaging or MRI is a powerful, non-invasive imaging technique that leverages the magnetic properties of hydrogen nuclei to gain insight into the structural, functional and molecular nature of tissues. Hydrogen nuclei, plenty of which can be found in the human body, come with an important characteristic: the presence of a 'spin' or 'spin quantum number' of $I=1/2$, with spin being a fundamental property intrinsic to particles or nuclei, like mass or charge. Using this spin quantum number, one can calculate the number of possible spin states by $2I+1$.

When placed inside a large, external magnetic field called B_0 , as is the case in an MR scanner, the charged hydrogen nuclei have two possible states: a low-energy state aligned parallel to B_0 , and a high-energy state aligned anti-parallel to B_0 . Following the laws of classical electrodynamics, this positively charged, spinning hydrogen nucleus gives rise to a magnetic moment μ , oriented along the rotational axis, as illustrated in Figure 8. In the absence of an

external magnetic field, both states are energetically equal, and the magnetic moments of the nuclei orient themselves randomly, as shown in Figure 9. However, once placed inside an external magnetic field B_0 , the nuclei start precessing at an angular frequency known as the Larmor frequency. Additionally, the spin-up and spin-down states are separated by an energy gap. As slightly more spins will precess in the low-energy, parallel state, a net magnetization vector M_0 is generated, as shown in Figure 9.^{69 70 71}

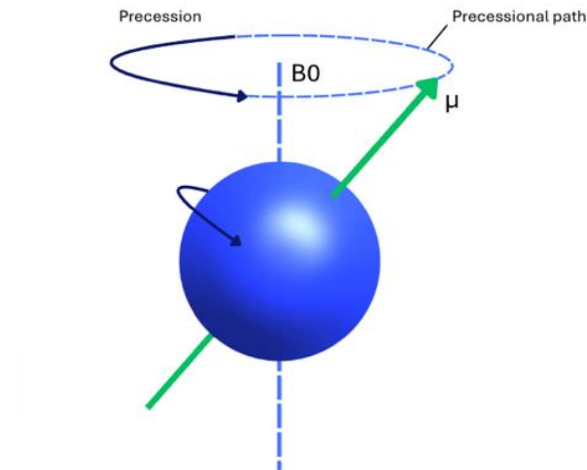


Figure 8: Precession of a hydrogen nucleus in an external magnetic field.

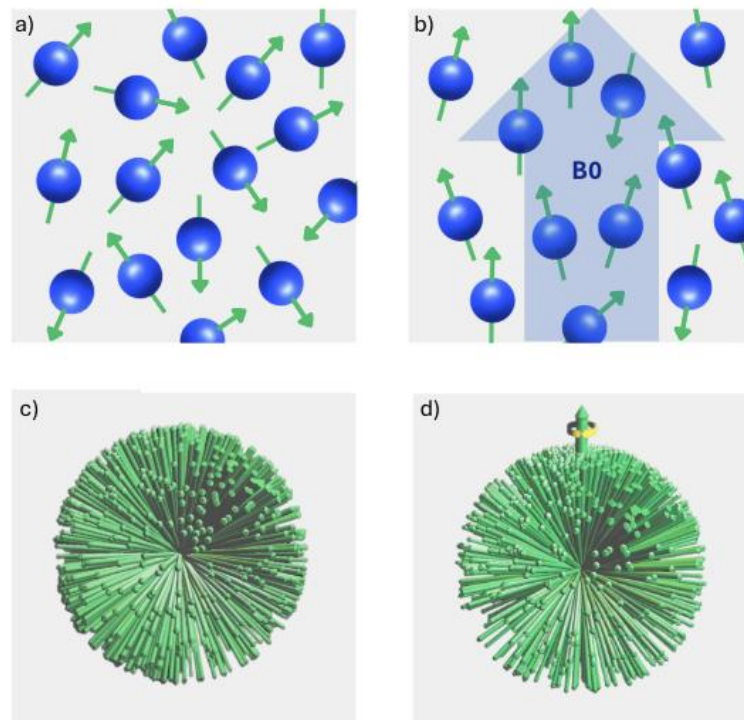


Figure 9: (a) Randomly orientated spins in the absence of an external magnetic field. (b) Parallel or anti-parallel alignment of spins to the external magnetic field B_0 . (c) Distribution of spins in the absence of an external magnetic field. (d) Distribution of spins, generating a net magnetization vector.

1.3.1.2 Signal creation and reception

Although the net magnetization vector is an important first component for creating MR images, we cannot yet detect it, as it is much smaller than B_0 and points in the same direction. To generate a signal that is detectable, a radiofrequency (RF) pulse is emitted in the Larmor frequency, tipping magnetization into the transverse plane. When the RF pulse is switched off, magnetization in the longitudinal plane gradually increases. At the same time, but independently, magnetization in the transverse plane decreases. These processes are referred to as T1 and T2 relaxation respectively. As relaxation occurs, the net magnetization vector follows a spiraling path whereby its direction and magnitude constantly change.⁷¹ Looking at the transverse plane, magnetization oscillates. This signal is known as free induction decay (FID). In the RF receiver coils, oscillating voltage changes are induced: a signal is picked up. To spatially encode this signal, magnetic field gradients are employed.⁷⁰

1.3.1.3 Contrast and weightings

T1 is the time at which 63% of longitudinal magnetization has recovered and is mainly determined by the capacity for energy exchange within a tissue. T2 is the time at which transverse magnetization has decayed to 37% of its initial value and is determined by how quickly spins lose phase coherence. In practice, the decay of transverse signal is accelerated by field inhomogeneities, resulting in a faster effective decay, referred to as T2*. As relaxation times differ across tissues, MRI can exploit this characteristic for contrast generation. Fat-rich tissues such as white matter typically have shorter T1 and T2 times, while cerebrospinal fluid (CSF) is characterized by longer relaxation times. To control the degree of T1 and T2 weighting, pulse sequence parameters such as repetition time (TR) and echo time (TE) can be chosen.^{70 71 72}

1.3.1.3 MRI modalities

Besides T1 and T2 weighting for obtaining different anatomical contrasts, advanced MR acquisition sequences, such as diffusion MRI, perfusion MRI and functional MR, allow researchers and clinicians to obtain information on different aspects of physiology and anatomy. Additionally, MR spectroscopy is used for estimating information on tissue metabolites.

1.3.2 A short introduction to Diffusion Weighted Imaging (DWI)

1.3.2.1 Basic principles

Diffusion-weighted imaging is an MRI technique that uses the diffusivity of water for contrast generation and for obtaining estimates of the structural properties of tissues. The sequence allowing the MR signal to be sensitized to motion was first introduced by Stejskal and Tanner in 1965. It is commonly referred to as the Pulsed Gradient Spin Echo (PGSE), in which a symmetrical pair of diffusion sensitizing gradients is added on either side of the 180° refocusing pulse of a standard spin-echo sequence.⁷³

At the basis of DWI lie the principles of Brownian motion and the distinction between free and restricted diffusion. Brownian motion describes the random microscopic displacement of particles, resulting from thermal collisions with other particles. Diffusion refers to the net movement of particles from regions of high concentration to regions of low concentration.⁷⁴ In the case of free, isotropic diffusion, where molecular motion is unrestricted and equal in all directions, the mean squared displacement along one spatial direction can be estimated as a function of the diffusion time (t_d) and the diffusion coefficient (D), according to Equation 1.⁷⁴

$$(1) \quad \langle x^2 \rangle = 2Dt_d$$

When diffusion is free, D is solely influenced by factors such as particle radius, temperature and medium viscosity. In biological tissue, however, water diffusion is hindered by its microstructural environment, including cell membranes, axonal fibers and macromolecules.⁷⁵ Diffusion will therefore have a preferred directionality, a phenomenon referred to as anisotropic diffusion. It is this sensitivity of diffusion to tissue microstructure that forms the basis of DWI. By applying diffusion weighting to a T2* image, the attenuation of the signal in a given pixel is affected by the amount of diffusivity in that pixel. The amount of signal loss can therefore be used to estimate the apparent diffusion coefficient (ADC) using Equation 2:

$$(2) \quad ADC = \frac{1}{b} \ln \left(\frac{S_0}{S(b)} \right)$$

This ADC value can subsequently be used for contrast generation. In equation 2, the b-value represents the amount of diffusion-weighting applied during image acquisition, which is determined by the gyromagnetic constant γ , the amplitude of the gradient G, the duration of each gradient δ and the separation of the gradients in time Δ as shown in Equation 3:

$$(3) \quad b = \gamma^2 G^2 \delta^2 \left(\Delta - \frac{\delta}{3} \right)$$

However, as ADC is a scalar measure calculated per voxel, it does not yet capture the full three-dimensional character of diffusion. It was only upon introduction of the diffusion tensor, that estimations could be made on both the magnitude and orientation of anisotropic diffusion. The tensor describes diffusion in three dimensions using a 3x3 matrix, defined by 3 eigenvectors and 3 eigenvalues, derived by applying diffusion-sensitizing gradient pulses along the X, Y and Z axes.⁷⁶

The tensor framework eventually led to applications such as tractography, which creates virtual estimates of white matter tracts in the brain, by tracking the principal diffusion directions. Additionally, diffusion tensor imaging (DTI) metrics such as fractional anisotropy (FA), axial diffusivity (AD), radial diffusivity (RD), and mean diffusivity (MD) can be computed. From these, FA is reported most often, which represents the directionality of diffusion.⁷⁷ However, the interpretation of this measure warrants caution as it does not inform on the cause of the observed changes. In voxels containing crossing fibers, FA represents the aggregate signal of the different fiber populations within that voxel. As a consequence, FA is naturally low in voxels with crossing fibers. Given that 90% of all white matter voxels are estimated to comprise crossing fibers, interpretations with regards to white matter integrity, myelination status, or axon density are discouraged, unless built upon a strong theoretical framework or complementary evidence.^{77 78 79}

In response to these limitations, the fixel was introduced, representing a single fiber population within a voxel.⁷⁹ The biggest advantage of fixel-based analyses, is its ability to separately estimate quantitative measures for different fiber populations within a voxel. For fixel estimation, the diffusion-weighted signal, capturing diffusivity from different fiber populations at once, needs to be disentangled into its components. By applying constrained spherical deconvolution (CSD), the fiber orientation distribution function (fODF) can be estimated from the diffusion-weighted signal, by deconvolving it with the response function. Next, fixels are derived by segmenting the fODFs, also called fiber orientation distributions (FOD), into their individual peaks.⁸⁰ The FODs and fixels can subsequently be used for calculating fiber-specific measures such as Fiber Density (FD), Fiber Cross-section (FC) and a combined measure, assessing both Fiber Density and Cross-section (FDC), as shown in Figure 8.

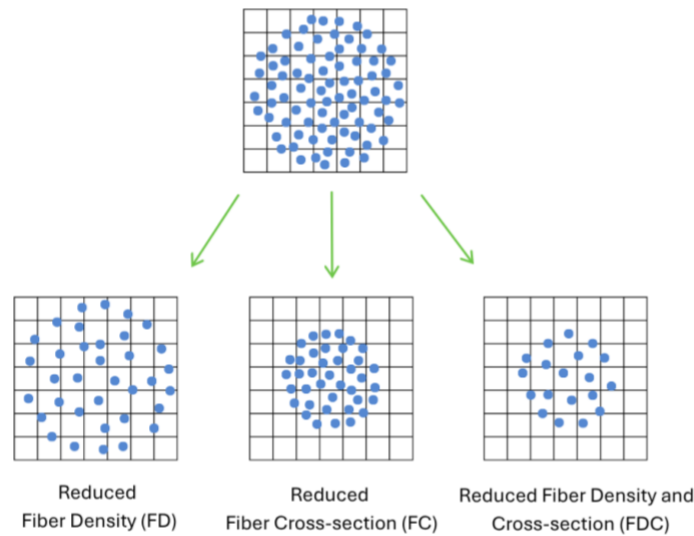


Figure 8: Schematic representation of possible alterations to intra-axonal volume. Changes may manifest as altered within-voxel fiber density, fiber bundle cross-sectional area or a combination of both fiber density and bundle cross-section. The blue dots represent individual axons within a bundle, and the grid represent imaging voxels.

FD is an estimated parameter of the intra-axonal volume of a fiber bundle. This concept was first introduced as Apparent Fiber Density (AFD) and is based upon the assumption that the diffusion of intra-axonal water is restricted in the radial direction, due to the presence of a cell membrane. Additionally, the DW signal from extra-axonal water is known to be strongly attenuated, while the DW signal from intra-axonal water is known to be strongly preserved, when using gradient pulses of ~ 30 ms. Therefore, the total radial DW signal in a voxel is mainly determined by, and proportional to, the volume of the intra-axonal compartment. As the radial DW signal translates into the amplitude of the FOD, this amplitude can then be used to derive AFD as a proxy for the volume of the intra-axonal compartment. FD was later introduced within the FBA framework and is based on the same logic. However, instead of using FOD amplitude, the angular integral of the FOD lobe is used for estimating the FD of a fixel.⁸¹

FC is an estimate of a fiber's volume perpendicular to its direction. It was introduced to account for orientation when assessing white matter volume, based on the idea that the loss of axons is mainly implied when volume is lost perpendicular to the fiber orientation. Importantly, FC is not derived directly from the DW signal, but from the Jacobian Matrix, used to spatially warp subjects to the template. Specifically, the warp performed in the plane perpendicular to the fixel direction is assessed. If volume perpendicular to the fixels' axis must be expanded to match the template, FC was smaller compared to the template, whereas contraction indicates a larger FC relative to the template. This calculation is performed for all white matter fixels and can subsequently be used in a FBA analysis. Finally, FDC represents the combined metric of FC and FD, obtained by multiplication of the two measures.⁸²

In this thesis, both a FBA and a tensor-based assessment of FA will be performed. This approach will allow for the assessment of changes in FD, FC, FDC and FA. The tensor-based model has been criticized for its limited biological interpretability, while the fixel-based framework is presumed to more accurately reflect changes in white matter micro- and macrostructure. However, applying both analyses to the same dataset will allow for the direct comparison of these two frameworks.

1.4 Research objectives

Although aging is oftentimes thought of as a linear process, it is likely comprised of dynamic transition states that can either protect against or increase vulnerability to neurodegenerative disorders. Menopause transition is seen as one of those critical phases, potentially putting women at risk for developing AD later in life. We are however, only starting to understand the exact metabolic, functional and structural changes associated with this neuroendocrinological transition. This study investigates microstructural white matter changes associated with menopause transition based on the hypothesis that the cerebral hypometabolic state during menopause transition may trigger use of myelin as an alternative energy substrate, potentially resulting in white matter damage. Diffusion weighted imaging will be used to generate whole brain estimations of white matter integrity.

2. Materials and Methods

2.1 Data acquisition

Images were acquired on a 3T MR scanner (Magnetom 3T XR, Siemens Healthcare, Erlangen, Germany) running the Numaris/X VA60A software with a 32-channel headradiofrequency coil. The MRI scanning protocol included, amongst others, a localizer, an anatomical T1 weighted image and a diffusion weighted image with PA correction. First, scout scans were acquired prior to anatomical imaging to aid in slice positioning [time repetition (TR)/time echo (TE) = 3.15/1.37ms, voxel size = $1.6 \times 1.6 \times 1.6 \text{ mm}^3$, field of view (FOV) = 260 mm, flip angle (FA) = 8°]. Next, the high-resolution anatomical image was recorded using a T1-weighted magnetization-prepared rapid gradient echo (MPRAGE) sequence, consisting of 208 sagittal slices [TR/TE = 2400.0/2.22 ms, time inversion (TI) = 1000 ms, resolution = $0.80 \times 0.80 \times 0.80 \text{ mm}$, field of view (FOV) = 256 mm, flip angle (FA) = 8°]. The diffusion weighted volumes were acquired using a high-angular-resolution diffusion-weighted imaging (HARDI) sequence and the direction of phase-encoding was anterior-posterior (AP). Diffusion weighting of $b=1200 \text{ s/mm}^2$ was applied in 74 diffusion gradient directions distributed uniformly on the unit sphere. An additional image without diffusion-sensitizing gradient was acquired ("the b0 image") as a baseline reference to compute diffusion signal attenuation. To correct for EPI distortion a PA or reverse phase encoding scan was acquired separately (TR/TE 2744.0/66.80ms).

2.2 Study population

Data collection was performed as part of the research project 'Reward and Sexual Health in Pre-, Peri-, and Post-menopausal Women' at the University Hospital for Psychiatry and Psychotherapy in Tübingen, Germany. For this project, a cohort of 88 healthy females between the age of 35 and 55 were recruited. Other inclusion criteria included a normal body-mass-index (18-28 kg/m²), Caucasian, non-smoking, German language fluency, normal or corrected-to-normal vision, and at least advanced technical college entrance qualification. Data was initially collected to investigate reward and sexual health in pre-, peri-, and post-menopausal women. Considering the impact of sexual orientation on sexual response, only heterosexual and bisexual participants were included. Next, the participants were divided into groups according to their menopausal stage, based on criteria outlined in the 'Stages of Reproductive Aging Workshop (STRAW).'⁴⁴

PRE women were included if they showed no symptoms of menopausal transition, meaning their menstrual cycle frequency was regular and they had their menses within the past two months. PRE women should not use any kind of hormonal contraception or copper IUD for at least three months and should have a natural menstrual cycle between 26-32 days, which will be confirmed by tracking the previous two cycles. PERI women are included if they are in menopausal transition, meaning they had a menstrual bleeding in the past 12 but not within the past three months. Women with a final menstrual bleeding at least 12 months ago, were included in the POST group.

A total of five participants dropped out before MR images were collected (n=5). Six participants were excluded due to either neuropathological conditions encountered in the MR images (n=4) or a history of neurological pathology (n=2). Ten additional participants were excluded due to missing or incomplete DWI data (n=10). One participant was excluded due to claustrophobia induced movement artifacts (n=1). Analysis was performed on the 66 remaining participants, with 30 women in the PRE group, 14 in the PERI group and 22 in the POST group. A total of four women reported the use of menopause hormone therapy (MHT) for at least two months. Two of these women belonged to the PERI group and two to the POST. Analyses were performed both with and without the women using MHT.

2.3 Study process

After recruitment, participants were screened for in-and exclusion criteria by phone. During the first appointment participants were informed about the study process and a written informed consent form was collected. They also filled in a questionnaire on psychometric and sociodemographic data. Additionally, a standardized clinical interview to screen for mental disorders (SCID) was performed.

All participants were divided into one of the three groups based on their menopausal transition phase, as described by the STRAW criteria: PRE, PERI or POST. All PRE women were scanned twice, once during day 2-7 of their menstrual cycle (i.e. the early follicular phase) and once ± 3 days around their ovulation. For this research project only the images taken during the PRE-follicular phase were used. PERI women were scanned a second time as well, within an interval of 6 months, to investigate their progress in the menopausal transition. For this research project, only the images taken during the first session were used. POST women were scanned only once.

Each scanning session included an MRI scan, a blood sample and multiple questionnaires. For this research project, only the MR images were used. For a more complete documentation of data acquisition, following levels of (sex) hormones and biomolecules were determined: estradiol, estriol, estrone, progesterone, hydroxyprogesterone, pregnenolone, allopregnanolone, testosterone, dihydrotestosterone, corticosterone, cortisol, cortisone as well as Anti-Müllerian hormone (AMH), kisspeptide, Neurokinin B, Follicle Stimulating Hormone (FSH), luteinizing-hormone (LH), prolactin, C-peptide, insulin, triglycerides, glucose, glucagon-like peptide-1, leptin, adiponectin, ghrelin and brain-derived neurotrophic factor.

2.4 Development and application of scripts for image processing

At the start of my research project, no pre-or postprocessing pipelines for diffusion-weighted imaging (DWI) were available within the FEMBRA or IRTG research teams in Ghent and Tübingen. Since DWI remained my topic of interest, creating the image processing pipelines was part of my project. This allowed my thesis to not only answer my own research questions but also make DWI analysis available for both research groups.

2.4.1 Image preprocessing using QSIprep

A pipeline using the containerized 1.0.1 version of QSIprep was created for the preprocessing of diffusion weighted images. Steps to successfully build the QSIprep Apptainer can be found on the QSIprep 'readthedocs' page under 'installation'.⁸³ The outline of the script was formed by combining and modifying the fMRIPrep script as written by dr. Ann-Christin Kimmig and the QSIprep script as written by dr. Amelie Rauland. To make it directly implementable at the IRTG lab, its structure was shaped similarly to scripts already in use by the research group. The full script can be found in APPENDIX A.

The pipeline operates on DWI data, structured conform to the brain imaging data structure (BIDS)⁸⁴. Based on the available metadata, QSIprep will automatically perform optimal preprocessing, using state-of-art methods. In the pipeline, three major steps can be distinguished: processing anatomical reference images, preprocessing DWI data and lastly, computation of quality assessment metrics.

During the first step, the anatomical reference images are processed, using the --anatomical-modality T1w flag to define T1w as the contrast image of choice. Subsequently, all T1w images found for a participant are used to create an average participant-specific anatomical image. These reference images are first aligned to the anterior commissure - posterior commissure (AC-PC) in the MNI152NLin2009cAsym template space, using rigid transformations only. Next, these AC-PC reoriented images undergo a full symmetric nonlinear registration (Syn) to the MNI152NLin2009cAsym template space using antsRegistration from ANTsPy, a python library for medical imaging. Subsequently, brain extraction and tissue segmentation are performed using Synthstrip and Synthseg.^{85 86} These files will later be used for visualizing susceptibility distortion correction (SDC).

In the second step, the diffusion weighted images are preprocessed in multiple sub-workflows. First, MRtrix3' dwidenoise and dwibiascorrect are used for denoising of DWI data and correction of B1 field inhomogeneities respectively.⁸¹ Second, FSL's eddy is used for head motion correction and eddy current correction. This step integrates the fieldmap estimated by FSL's TOPUP, correcting for susceptibility distortion. In the last step, framewise displacement (FD) is computed using Nipype, quantifying head motion between consecutive scans.⁸² As additional quality control metric, slice-wise cross correlation was computed. QSIprep will thus generate preprocessed DW imaging data, including metadata required for further processing (e.g. information on the transformations performed between different spaces). Additionally, it will create a visual and quantitative quality assessment (QA) report for each subject. The visual report comes as an HTML file, with images illustrating the quality of different steps in the pipeline. The file for quantitative QA is a single-line csv file and can be used for reporting on subject motion, coregistration quality and image quality.⁸⁹

2.4.2 Fixel-based analysis of Fiber Density and Cross-section

The fixel-based analysis (FBA) pipeline is based upon MRtrix3' (version 3.0.8-21-g8b3d398a) multi-shell multi-tissue constrained spherical deconvolution (MSMT-CSD) workflow as described in the official documentation and was adapted for this thesis project.⁹⁰ The full pipeline can be found in APPENDIX B. A graphical representation is shown in Figure 9.

First, group-average tissue response functions for white matter, grey matter, and cerebrospinal fluid were estimated. Next, diffusion-weighted images were upsampled, and brain masks were generated from these upsampled images. For all subjects, multi-tissue spherical deconvolution was then applied to estimate fiber orientation distributions (FODs) from the diffusion-weighted signal. Next, joint bias field correction and global intensity normalization were performed to ensure inter-subject comparability. Subsequently, a study-specific unbiased FOD template was generated, after which all subject FOD images were registered to this template.

After this, a template brain mask was computed as the intersection of all subject masks in template space, followed by the generation of a fixel mask, which defines the fixels that will later be analyzed statistically. Next, the subject FOD images were warped to template space and segmented to identify the number and orientation of fixels in each voxel. Additionally, fiber density (FD) was calculated for each fixel. Upon warping of the subjects' FOD images to template space, fixel orientations were reoriented based on the same spatial transformations. Next, subject fixels were assigned to the corresponding template fixels, matching fixels both across subjects and between subjects and the template. Subsequently, fiber cross-section (FC) and log (FC) was computed from the deformation fields, and a combined fiber density and cross-section (FDC) metric was derived.

Finally, whole-brain probabilistic tractography was performed on the FOD template, followed by tractogram filtering to reduce reconstruction biases. Lastly, a fixel-fixel connectivity matrix was generated from the filtered tractogram and used to smooth fixel-wise metrics prior to statistical analysis.⁹⁰

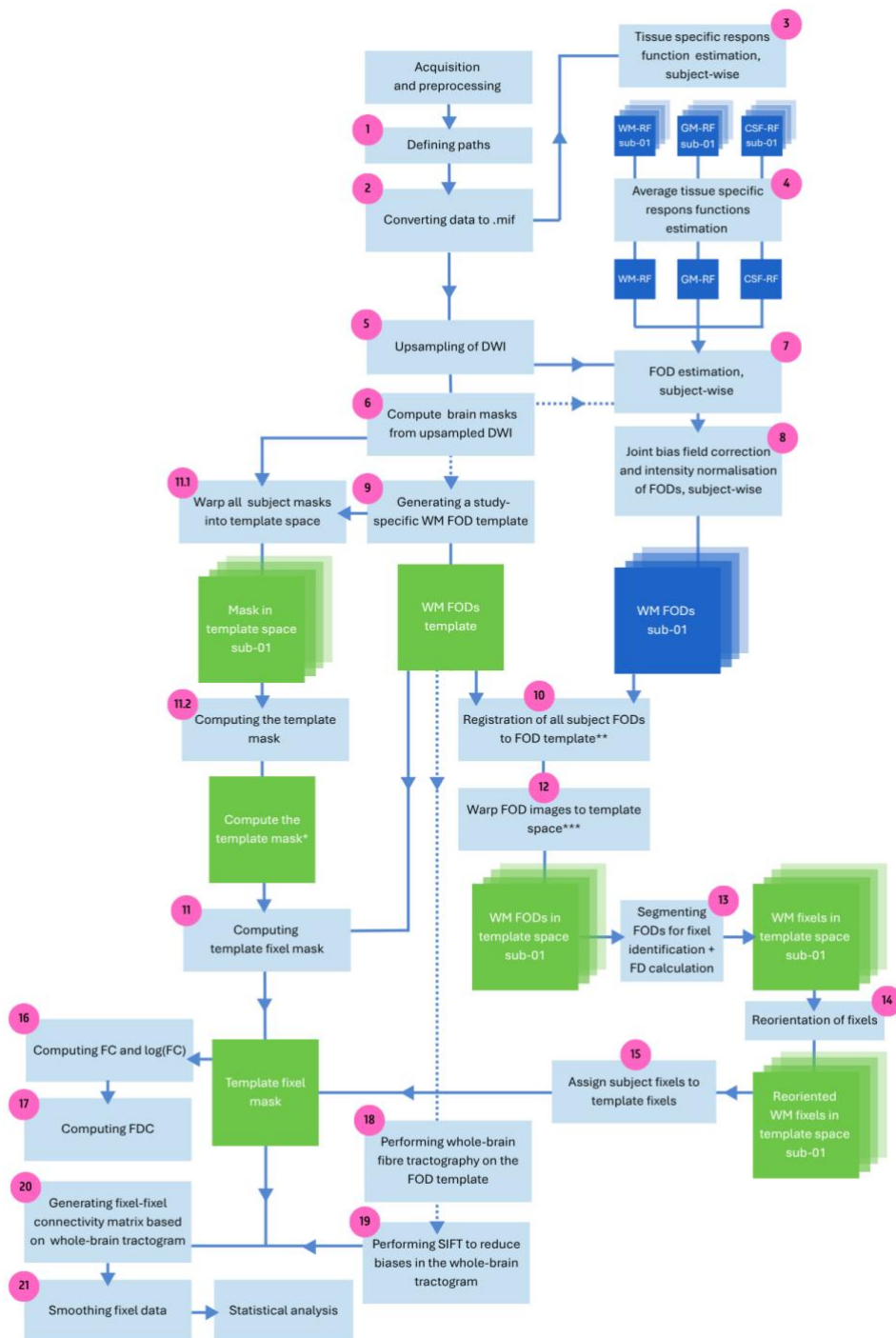


Figure 9: Workflow of the fixel-based analysis. The green squares indicate that these files are processed in template space, while blue represents processing in subject space. *The template mask is the intersection of all masks, which have just been warped into template space. **In this step, the transformation that is needed is computed. The actual warp is only executed in step 12. ***FOD images are warped, but FODs are not yet reoriented. Reorientation happens in step 14, when FOD's have already been segmented into fixels. Abbreviations: RF, respons function; FOD, fiber orientation density function; WM, white matter; DWI, diffusion weighted images; FC, fiber cross-section; FD, fiber density; FDC, fiber density and cross-section; SIFT, Spherical-deconvolution Informed Filtering of Tractograms.

2.4.3 Computation of Fractional Anisotropy using TBSS

A third pipeline was created for performing a voxelwise statistical analysis of FA data using Tract-Based Spatial Statistics (TBSS). First, FA images were created by fitting a tensor model to the raw diffusion data and extracting the FA measure. Next, FA data were converted to .nii files for further processing with TBSS. The first step in TBSS is the nonlinear registration and subsequent transformation of all FA images into a common space, upon which a mean FA image is created. This image is then used to create a mean FA skeleton, representing the parts of the tracts that are common to all subjects, as shown in Figure 10. The last step before statistical analysis is the projection of the subjects' aligned FA data onto the mean FA skeleton. The skeletonized FA data will then be fed into the voxelwise statistics.⁹¹ The full pipeline can be found in APPENDIX H.

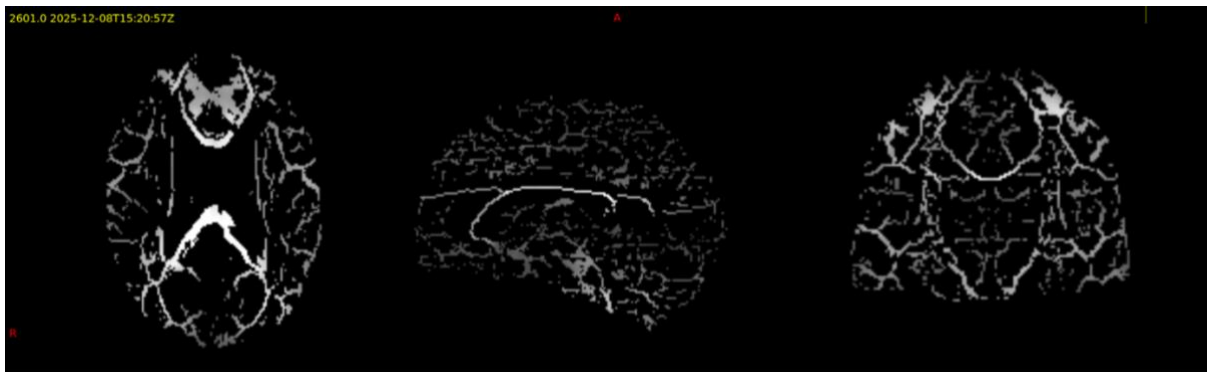


Figure 10: Mean FA skeleton computed using TBSS from FSL.

2.4.4 Statistical analysis

2.4.4.1 Whole-brain fixel-based analysis of FD, log (FC) and FDC

To identify fixels with altered fiber density (FD), fiber bundle cross-section (log (FC)), and the combined measure (FDC) between menopausal stages, a 'whole-brain fixel-based analysis (FBA)' was performed. To address the incompatibility of traditional voxel-based smoothing and cluster-based inference with a fixel-based analysis, 'connectivity-based fixel enhancement (CFE)' was introduced. This framework allows for clustering and smoothing to be performed based on fixel connectivity rather than voxel proximity.⁹² Differences in total intracranial volume (TIV) and age were assessed between the three groups using an ANOVA. When significant differences were found, a post-hoc Tukey test was performed. Age was introduced as a covariate for all analyses. In the analyses of log (FC) and FDC, log-transformed total intracranial volume (log (TIV)) was added as additional covariate, as recommended by the developers.

To perform this analysis, MRtrix3' *fixelcfestats* command was used, requiring a design and contrast matrix, as well as separate text files for each metric, containing the filename of all files to be analyzed. The design and contrast matrices were created in RStudio, for which the relevant code can be found in APPENDIX E. As the PRE, PERI and POST groups were analyzed simultaneously, an F-test was used by adding the *-ftests* option to *fixelcfestats*. Additionally, an input file that specifies the rows of the contrast matrix to be included in the F-test was supplied, which is provided in APPENDIX C. If significantly altered fixels were found for any of the three measures, a pairwise post-hoc test was performed. The script for this test can be found in APPENDIX D.

Fixelcfeats first generates a General Linear Model (GLM) for every single fixel. From this GLM, a raw, unenhanced F-statistic is computed. Subsequently, connectivity-based smoothing is performed, followed by the statistical enhancement using CFE.⁹³ To assess which fixels differ significantly, their test-statistics are assessed using non-parametric permutation testing. During this procedure, the enhanced test-statistics are compared to an empirical null distribution, which is built by performing 5000 permutations. For each permutation, only the maximum statistic is recorded and added to the null distribution, essentially creating a distribution of maximal test statistics under the null hypothesis. Importantly, CFE is performed before each permutation. Next, the Family-wise error (FWE)-corrected P-values are assigned per fixel to correct for multiple comparison, by calculating the proportion of test statistics in the maximal null-distribution that are as extreme or more extreme than the test statistic that is being assessed. Lastly, *fixelcfeats* computed the absolute effect using $\text{abs_effect} = \log(\text{FC_PERI}) - \log(\text{FC_PRE})$.

2.4.4.2 Whole-brain voxel-wise analysis of FA

Upon creation of the skeletonized FA data, a voxelwise, permutation-based (5000 permutations) nonparametric analysis was performed using FSL's *randomize*. Again, a design and contrast matrix were provided. As an f-test was performed, an additional input file was supplied to specify the rows of the contrast matrix to be included. Threshold-free cluster enhancement (TFCE) was used for multiple comparisons correction, using the $-T2$ option within *randomize* (APPENDIX G). If significantly different voxels were found, a post-hoc pairwise t-test was performed.

3. Results

3.1 Demographics

A total of 66 women were included in the final analysis. No significant differences in total intracranial volume (TIV) were found between the PRE, PERI and POST group, as illustrated in Figure 11. A significant age difference existed between the PRE and PERI group and the PRE and POST group, but not between the PERI and POST group, as shown in Figure 12.

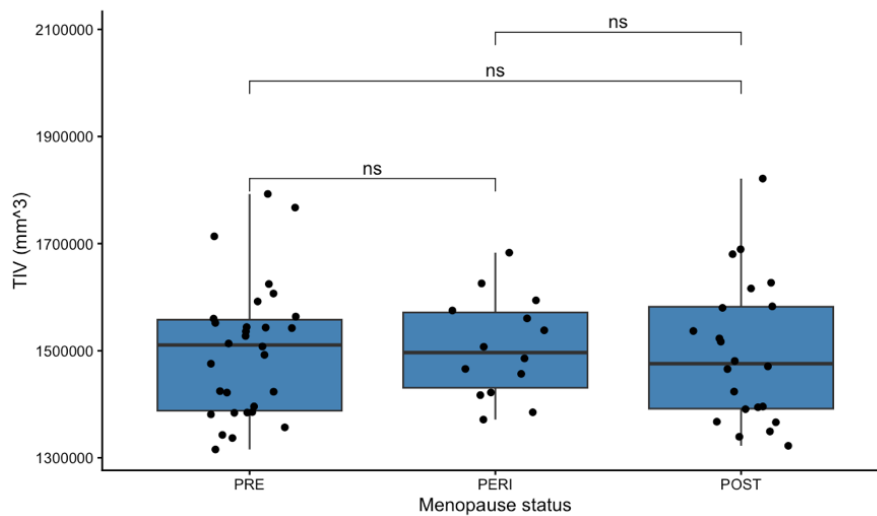


Figure 11: Boxplots of total intracranial volume in mm³ for pre-, peri- and postmenopausal women included in the final analysis. An ANOVA showed no significant differences between the three groups were present.

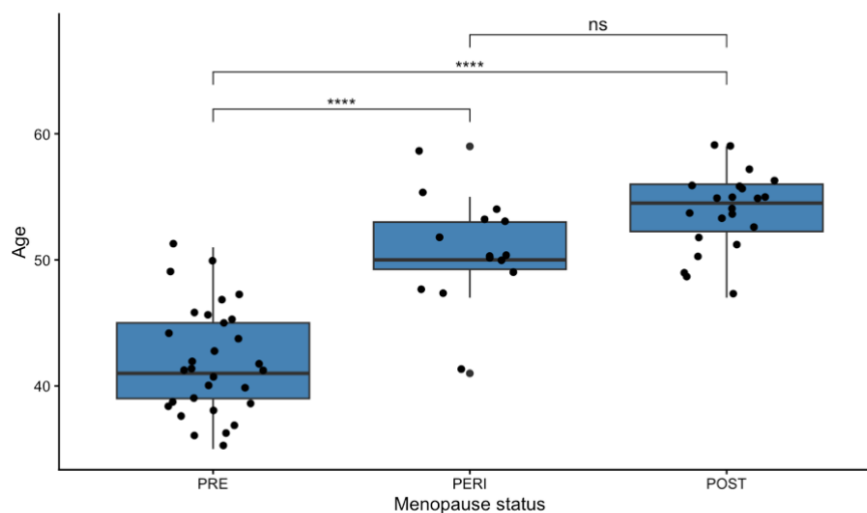


Figure 12: Boxplots of the age distribution of the pre-, peri- and postmenopausal women included in the final analysis. ANOVA showed a significant difference in age between the pre- and peri-group and between the pre- and post-group. No significant differences between the peri- and post-group was found.

3.2 Fixel based analysis

Whole-brain fixel-wise comparison across the three groups revealed significantly different log (FC) in fixels located in the genu of the Corpus Callosum (CC), as shown in Figure 13 and Figure 14, after correcting for age and TIV. Post-hoc analysis showed fixels with significantly different FC between the PRE and PERI group, passing from left to right. The absolute effect associated with these fixels is positive, which corresponds to an increase in log (FC) in PERI compared to PRE as shown in Figure 15.

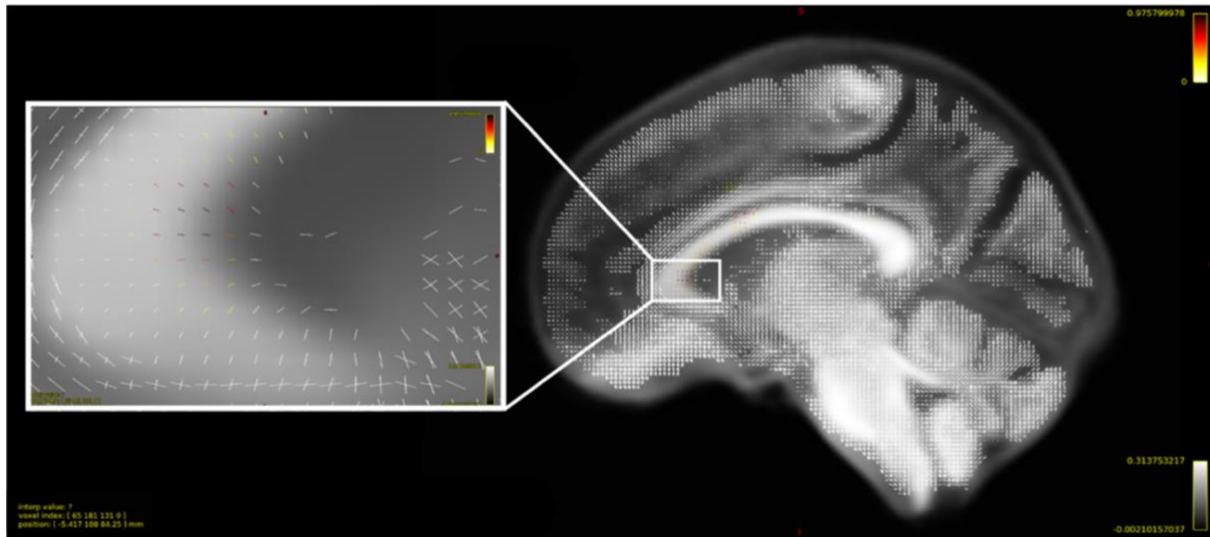


Figure 13: Whole-brain fixel-wise comparison of pre-, peri- and postmenopausal women shows fixels with significantly altered fiber cross-section (log (FC)) in the Genu of the Corpus Callosum.

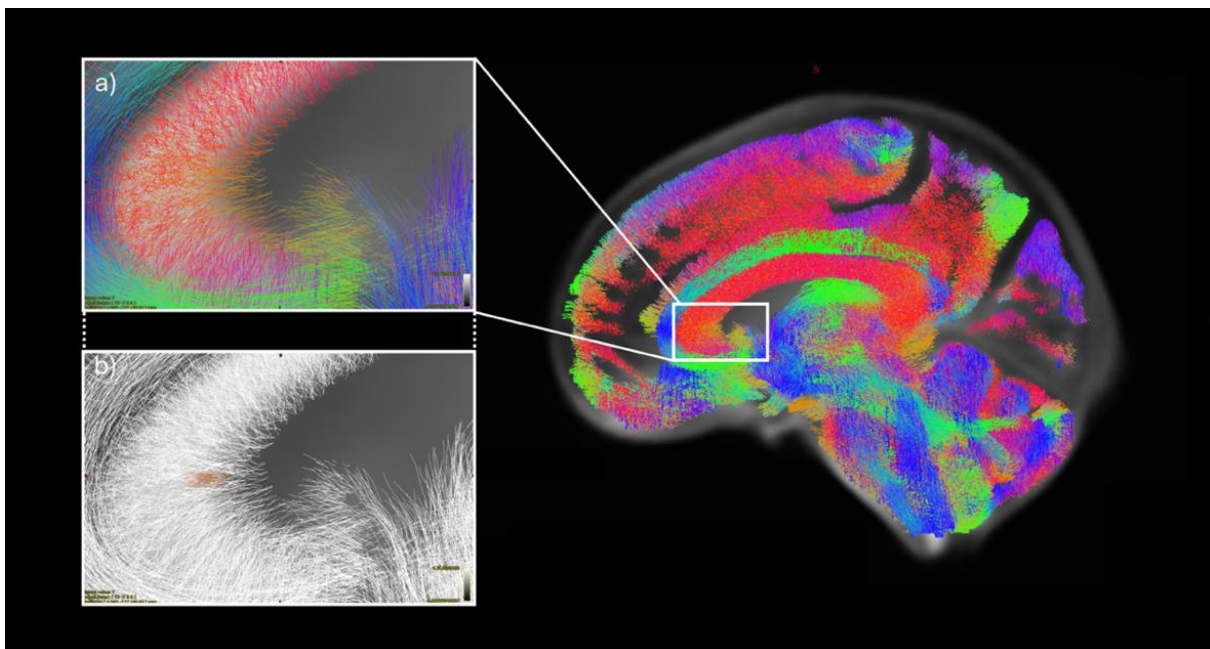


Figure 14: Whole brain tractogram, a) zoomed in on the genu of the Corpus Callosum, b) after applying a threshold for significance, visualizing only streamlines corresponding to fixels with significantly altered fiber cross-section (log (FC)).

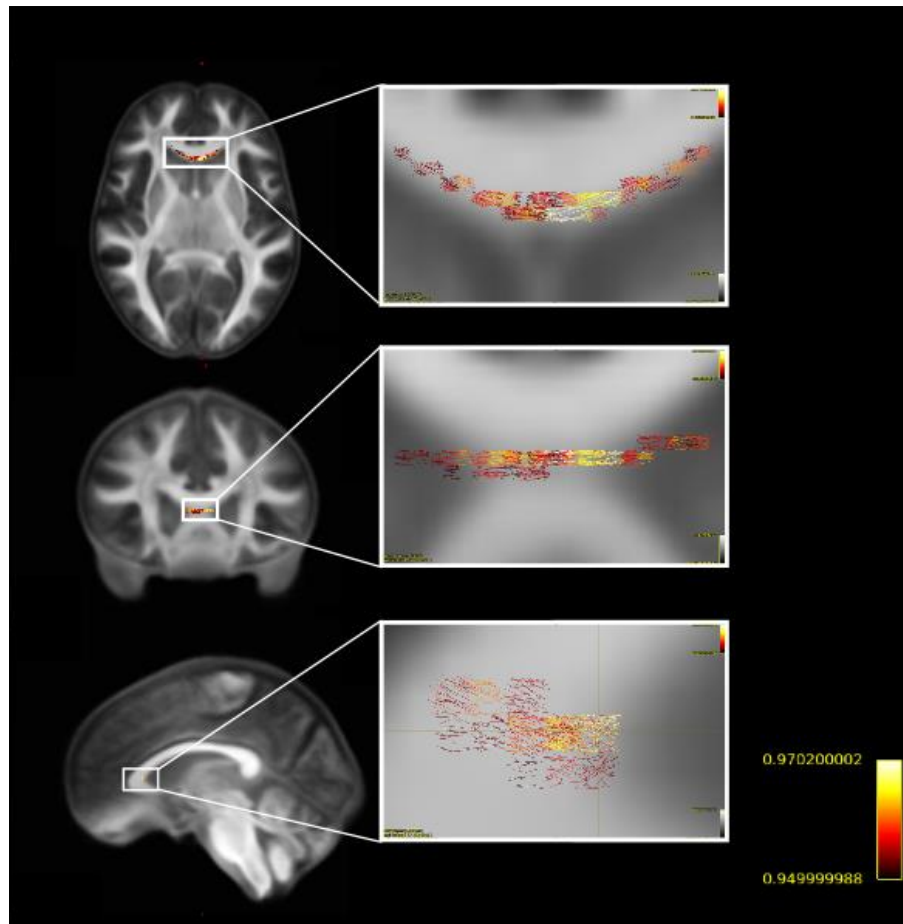


Figure 15: Visualization of streamline segments corresponding to significant fixels (FWE-corrected P -value 0.05). Streamlines are colored by significance level, presented as $1-p$.

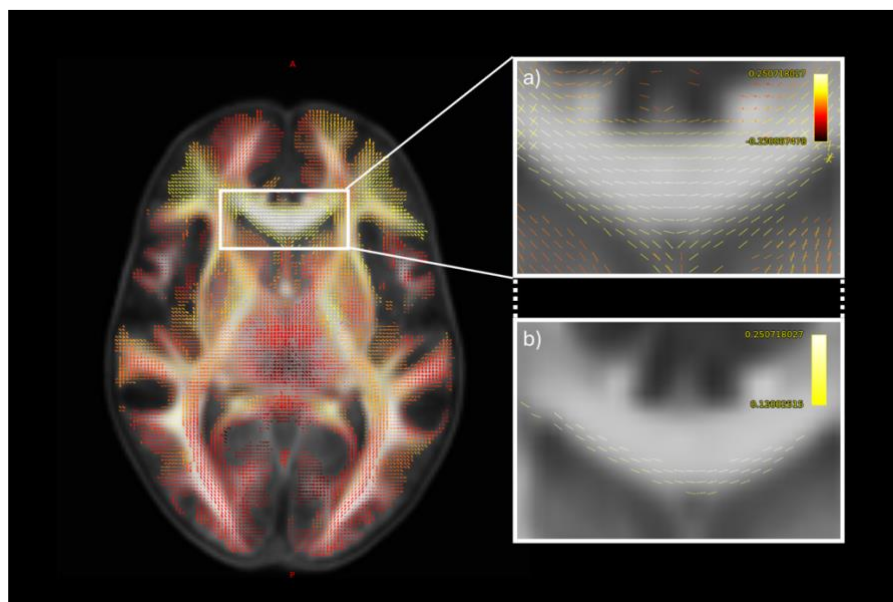


Figure 16: Whole brain fixel image colored according to absolute effect size. a) Zoomed in on the genu of the CC and b) showing only significant fixels.

No significant differences in log (FC) were found between the PRE and POST group or between the PERI and POST group. Additionally, no significant differences were found in FD or FDC for any of the groups.

3.3 FA analysis

Voxelwise analysis of FA using tract-based spatial statistics (TBSS) revealed no significant differences in fractional anisotropy across the three groups. In the anterior corpus callosum a non-significant difference in FA was observed ($p = 0.31$), as shown in Figure 17.

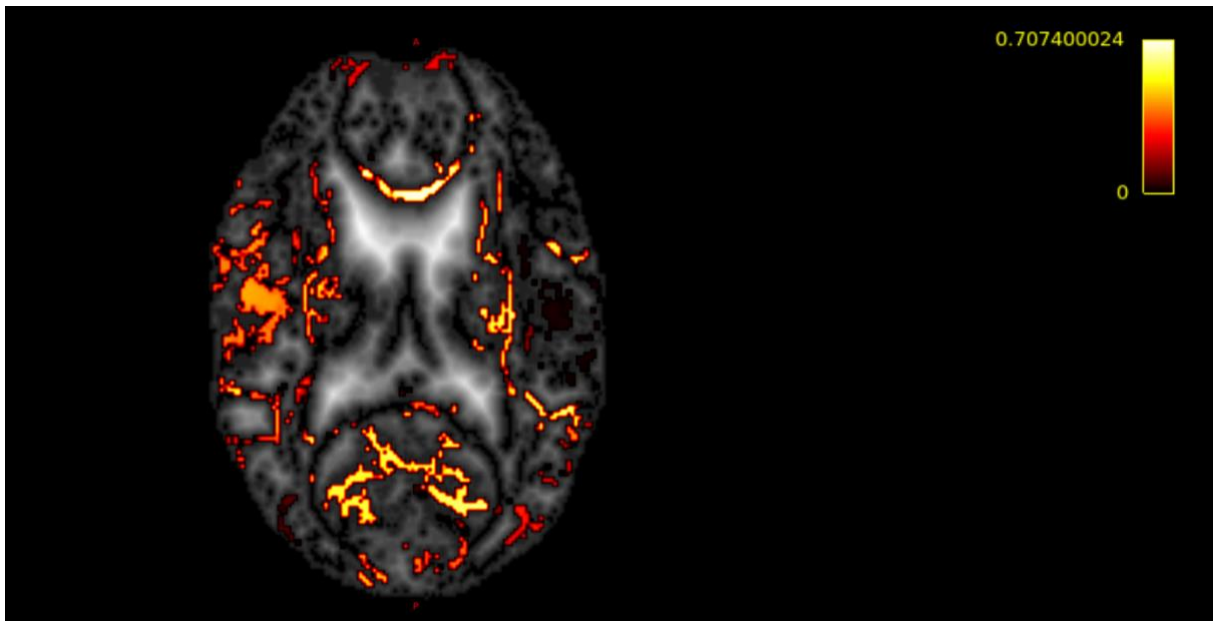


Figure 17: Whole-brain FA map with values colored according to $1-p$, with p reflecting the significance level.

4. Discussion

4.1 Interpretation of results

Upon whole-brain FBA analysis, fixels in the anterior Corpus Callosum showed significantly altered log (FC) when comparing pre- to perimenopausal women. No difference between the three groups was found for FD or FDC. Further pairwise testing showed that this difference could be attributed to an increase in log (FC) in the anterior CC of perimenopausal women compared to premenopausal women. No significant differences in log (FC) were found between pre- and post-, or peri- and postmenopausal women. Additionally, no significant differences in FA were found between the three groups.

When trying to interpret the observed increase in log (FC), one must keep in mind that an increase in FC can be caused by different biological phenomena. As FC is derived from the deformation field used during spatial registration, the metric itself simply reflects an increase in the macroscopic morphology of a fiber and cannot distinguish between different biological causes.

A first possible explanation for the increase in log (FC) in the CC of the peri-group compared to the pre-group is an increase in the number of axons within the fibers of the CC. This, however, is unlikely, because although the adult human brain is highly plastic, adaptations mainly include synaptic remodeling, local axonal sprouting and small extensions of existing axons, rather than the formation of new, long-range axonal projections. A second possible explanation is increased myelination, as it has been shown that even within axons, individual branches can show varying degrees of myelination.⁹⁴ However, an increased FC could just as well point towards disintegration of myelin sheets. In a recent work by Klosinski et al., electron microscopy (EM) was used to assess the structural integrity of white matter in reproductively incompetent mice. These EM images clearly show an expansion of the white matter compartment due to a loss of myelin integrity.⁵⁷

To assess what explanation would be most plausible, a closer look into the adaptive responses of oligodendrocytes, the cells producing myelin, can be helpful. Under normal circumstances, oligodendrocytes minimize neuronal energy consumption, support saltatory nerve conduction and provide metabolic support.⁵⁹ However, oligodendrocytes can transform into a disease-associated state when confronted with a build-up of amyloid plaques and disease-associated microglia. Importantly, this altered cell-state is not tied to a specific brain pathology. The shift to a disease-associated state has been observed to various extents in different brain diseases, after brain injury and in the normal aging brain.⁹⁵ In an attempt to aid the brain, disease-associated oligodendrocytes will upregulate genes associated with stress and inflammation and downregulate genes associated with homeostasis and myelination. If amyloid-beta pathology, oxidative stress and inflammation remain, oligodendrocyte dysfunction and apoptosis eventually occur, leading to a disruption of myelin sheaths, a known driver of neurodegeneration.⁹⁶ During menopause transition, it is hypothesized that decreased glucose metabolism leads to the use of myelin as an alternative energy substrate. Additionally, amyloid-beta pathology is known to increase, potentially shifting a proportion of oligodendrocytes into the disease-associated state, further reducing myelination.

An additional argument for interpreting the increased FC as a loss of white matter compactness arises when taking a closer look at the anatomical region affected. The genu of the CC is a so-called 'late-myelinated region'. These regions derive their name from the fact that they are myelinated in the later phases of development, resulting in a thinner myelin sheet and more unmyelinated axons. Consequently, these layers are more vulnerable to myelin breakdown and have consistently been found as the first regions affected by neurodegenerative processes.^{97 98} Although much uncertainty remains on the processes affecting myelination during menopause transition, a loss of structural compactness in the white matter for now seems to be the most plausible explanation for the increase in log (FC). However, other possible explanations should be explored as well.

Strangely, while a significant increase in log (FC) is observed when comparing pre- to perimenopause, no changes are found between the pre- and post-, or peri- and post-group. One possible explanation relates to the age distribution of the sample. As the mean age of the post-group lies very close to the mean of the peri-group, 54 years and 51 years respectively, brain changes characteristic for postmenopause may not yet fully have presented themselves.

When looking at FA, no significant differences were found between menopausal phases. This finding is incongruent with previous literature. One study by Mosconi et al. found significantly lower FA in the right external capsule of postmenopausal women, compared to perimenopausal women. This incongruence could potentially be attributed to a difference in

sample size and sample demographics. While the study by Mosconi et al. sampled 57 perimenopausal and 74 postmenopausal women with a mean age of 50 and 57 respectively, the current study investigated only 14 peri- and 22 postmenopausal women with a mean age of 51 and 54 respectively. However, as no effect size was reported in the study of Mosconi et al., it is possible that a small, biologically non-meaningful difference in FA between the peri- and post-group got amplified by the large sample size. Another explanation could be differences in the pre- and postprocessing of the DWI data. In the paper of Mosconi et al. Statistical Parametric Mapping (SPM12) and Matlab 7.8 are employed, using fully automated pipelines. However, besides co-registration, spatial normalization and smoothing, it is not mentioned what exact preprocessing steps or corrections were performed, hampering reproducibility. Additionally, the statistical approach used to assess differences in FA is not explicitly described. However, as Tract-Based Spatial Statistics (TBSS) is not mentioned, it is plausible that a different statistical method is used. If so, this would introduce an additional methodological difference with the present research.

Additionally, Mosconi et al. apply an a priori mask to the images before statistical testing. This mask ensured that only regions involved in the brain estrogen network, with known cognitive functions, were included into the statistical analysis. This mask included the fusiform gyrus, the anterior and posterior cingulate cortex, the inferior and superior parietal lobule, the inferior, medial, middle and superior frontal cortex, the inferior and medial orbitofrontal cortex, the inferior, middle, and superior temporal gyrus, the precuneus, insula, medial temporal lobe, putamen and thalamus. For analyzing WM, the brainstem was included as well. However, from the methodological section of the paper, it remains unclear whether this mask was also applied before analysis of FA and, if the mask was applied, whether the corpus callosum (CC) was included in this analysis or not.

In this work, no a priori mask was applied, as the research question was exploratory in nature. Instead, a whole-brain analysis was performed. A case can be made for both the region-of-interest (ROI) analysis as used by Mosconi et al. and the whole-brain analysis performed in this thesis. With ROI testing, sensitivity increases, meaning that the likelihood of detecting regions that are truly different is bigger compared to a whole-brain analysis. However, concern have been raised on the lack of transparency regarding the timing of ROI selection, as choosing ROIs after observing preliminary results can increase the risk of inflated or false-positive findings.⁹⁹ Additionally, overly strict masks in ROI-analyses can lead to false negatives as well, as regions where no effect is initially expected are excluded from the analysis.¹⁰⁰ Whole-brain analysis may therefore be more appropriate when the underlying biological mechanisms are still poorly understood. However, their statistical power to detect subtle effects is lower, which may increase the likelihood of false negatives. Transparency regarding both the choice of the analytical approach and the timing of ROI selection is therefore warranted.

4.2 Limitations

While the findings presented in this thesis provide valuable insights, several limitations should be acknowledged regarding study design, sample characteristics, and DWI quality control. The first limitation concerns the cross-sectional design of the present study. Because the three groups consist of different women, confounding due to group-differences unrelated to menopause status cannot be excluded. For example, educational level or cardiometabolic health, factors known to affect WM, may have differed between perimenopausal and premenopausal women, potentially introducing bias. However, strict exclusion criteria were applied, and women with medical conditions such as hormonal, metabolic or chronic disease

(e.g., severe hypertension, diabetes, dysfunctions of the thyroid) were not eligible for participation. Therefore, no major confounding is expected to be present.

Another limitation is the unequal sample sizes, with the peri-group having a smaller sample size ($n=14$), compared with the pre- ($n=28$) and post-group ($n=22$). This disbalance may have affected statistical power, as power generally increases with total sample size, but decreases when group sizes are unequal. Therefore, the peri- to pre-group comparison should have been most affected by reduced power. Interestingly, this was the only pairwise comparison where statistically significant results were obtained. Therefore, additional regions might have reached significance if the sample sizes would have been more balanced.

A third limitation was the fact that some women classified as perimenopausal at the first consultation, may actually have been postmenopausal. Perimenopause was defined as having had the last menstrual cycle between 3 and 12 months prior. However, women whose last cycle occurred within this timeframe may not have menstruated again and would retrospectively belong to the postmenopausal group. The potential misclassification of postmenopausal women to the peri-group may in part explain the lack of significant findings in this pairwise comparison. While this limitation is valid, requiring 12 months to confirm menopausal status before conducting analyses is not always feasible. However, if follow-up scans of the participants in the present study are conducted, it would be advisable to rerun the analyses using the updated menopause status. As the retrospective classification is more accurate more reliable results can be obtained.

Another limitation regarding sample characteristics is the relatively low mean age of the postmenopausal group (54 years). As a result, the sample may not be fully representative of the postmenopausal phase as a whole but rather characterize early postmenopause (Stage +1 according to the STRAW criteria). Given that hormone levels were sampled, further categorization of women into menopausal substages, based on their hormonal profiles, could have provided additional insights. However, as such classification can be time-consuming, future work may benefit from the development of tools automating the classification-process, using available hormone and cycle data.

A last limitation concerns the quality assessment of the diffusion-weighted imaging data. Although a visual inspection was performed of the quality assessment reports generated by QSIprep, no systematic evaluation of these outputs was performed. As a result, participants with data quality issue may have gone unnoticed.

4.3 Future perspectives

The findings within this paper offer preliminary insights into white matter changes across menopause transition. However, several avenues remain open for future investigation. First and foremost, some recommendation on future fixel-based research can be made. Firstly, efforts should be made to maintain balanced sample sizes across groups, to ensure sufficient statistical power. Secondly, particular attention should be paid to the composition of the postmenopausal group, ensuring that women across different postmenopausal substages are adequately represented. Thirdly, if follow-up scans are obtained, retrospective reclassification of misidentified perimenopausal women is warranted, as well as analysis of the images with the corrected menopausal staging. Additionally, future research on white matter changes throughout menopause transition may benefit from adopting a longitudinal study design. As menopause is a dynamic biological transition, mapping brain changes within individuals is essential for characterizing the trajectories of change, including their transient or persistent

nature. Additionally, it will allow for menopause-related effects to better be distinguished from aging effects. A longitudinal study design may also reduce confounding, and may help characterize the genetic, environmental, and behavioral factors influencing neuroendocrine aging in the female brain. Follow-up should therefore ideally last until older age to better investigate the hypothesized link between menopause-related brain changes, and the development of AD later in life. Furthermore, a continued effort should be made within the neuro-imaging community for the transparent reporting of all pre- and postprocessing steps, to further improve reproducibility.

Besides DWI imaging, other techniques for assessing myelin content and myelin integrity should be considered, both in human and animal research. The reason for this, is that a strong theoretical framework and complementary evidence is needed for the biological interpretation of DWI derived measures such as FC, FD, FDC and FA. In humans, Myelin Water Imaging (MWI)¹⁰¹, Magnetization Transfer Imaging (MTI)¹⁰² and multicomponent relaxometry MRI⁵⁹ have been proposed. For example, multicomponent relaxometry MRI has been able to show a reversible reduction in myelin content in athletes after running a marathon.⁵⁹ Additionally, strong correlations have been found between the MWI derived measure 'myelin water', and histological stainings of myelin in human brain tissue.¹⁰¹ Performing a similar analyses on women going through menopause would help us gain insight into changes in cerebral myelin content specifically. Other, related but non-neuroimaging research domains that need further attention include the investigation of oligodendrocytes and their role in aging and neuroendocrine aging specifically. Also, a clarification of their role in myelin homeostasis during menopause transition is needed.

Once greater insight has been gained into menopause-related brain changes, it will be equally important to translate these findings into more applied research. Additional preclinical studies and randomized clinical trials are needed to evaluate the safety and efficacy of menopausal hormone therapy. Such efforts are increasingly relevant, as a growing number of women seek evidence-based strategies to maintain both physical and brain health throughout the menopausal transition and later life.

5. Conclusion

This thesis investigated white matter changes associated with the menopausal transition using diffusion-weighted imaging. Measures of both micro- and macrostructure were examined, including fractional anisotropy (FA), fiber density (FD), fiber cross-section (FC) and the combined metric fiber density and cross-section (FDC). Notably, this is the first study to apply a fixel-based analysis to a cohort of menopausal women.

Overall, the findings indicate that menopause transition is associated with measurable alterations in white matter architecture. Compared with premenopausal women, perimenopausal women showed increased FC in the genu of the corpus callosum, a region not previously implicated in menopause-related white matter research.

Menopause has previously been associated with cerebral hypometabolism and increased amyloid-beta deposition, both of which are linked to white matter degeneration. Therefore, it is hypothesized that the observed increase in FC may reflect reduced white matter compactness leading to volumetric expansion in the genu of the corpus callosum. However, this interpretation should be approached with caution given the limitations related to sample characteristics, study design, and data quality control.

Taken together, these findings demonstrate that menopause is accompanied by measurable changes in the brain's white matter compartment. This study therefore contributes to a deeper understanding of menopause-related, neurobiological changes and female brain aging as a whole. Furthermore, the findings within this thesis may help clarify the relationship between menopause, cognitive symptoms, and women's increased risk of Alzheimer's Disease (AD). Nevertheless, further research is needed to investigate how myelin content changes across menopausal transition and to identify the biological mechanisms driving these alterations. In addition, longitudinal within-subject studies will be essential for mapping the trajectory of menopause-related brain changes over time. A better understanding of these processes may eventually lead to improved symptom management and may potentially inform measures for AD prevention.

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Statement on the use of Generative AI-tools

Throughout this thesis, the artificial intelligence tools ChatGPT, Claud, Co-pilot and Elicit were used in a limited and supportive fashion. Specifically, these tools were mainly consulted to help identify relevant scientific literature and to assist with the adjustment and debugging of the image processing codes. To a lesser extent, the tools were used to help with complex phrases where more academic wording was sought. Additionally, all AI-generated suggestions were evaluated critically and adapted where necessary before inclusion. Their use was strictly limited to supporting the writing and technical process, and did not extend to the generation of data, results, or scientific reasoning.

Poster

Decoding the Female Brain

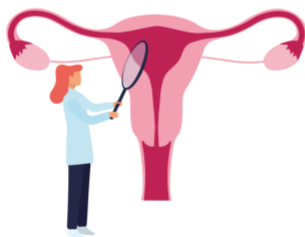
Investigating Cerebral White Matter Changes throughout Menopausal Transition

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What Cerebral White Matter changes can be observed during Menopause Transition?

During menopause transition, large hormonal shifts take place. Oestrogen and progesterone fluctuate and eventually drop to very low levels. But, not only the reproductive tract is changing: **the brain is greatly affected too.**

Women
are more at risk for
Alzheimer Disease (AD)
than men, even when
taking their greater
longevity into account.

Pathological
mechanisms of
AD start **decades**
before any
symptoms occur

Oestrogen regulates brain energy metabolism

Without oestrogen, mitochondrial function declines, pushing the brain into a **bio-energetic crisis**. Some of the changes resemble the early changes seen in AD.



Methodology

Fixel-based analysis (**FBA**) is performed on diffusion-weighted images (DWI) to derive Fiber Density (**FD**), Fiber Cross-section (**FC**) and Fiber Density and Cross-section (**FDC**).

These metrics of white matter integrity are compared between **pre-, peri-, and postmenopausal women** in a cross-sectional study design. Constrained Spherical Deconvolution (**CSD**) is used to estimate the orientation and properties of individual fibers. Data will be analysed using **MRtrix3**.

Call for collaboration!

Expertise in **analysis**
of **diffusion data**
using **MRtrix**

Larger or longitudinal
datasets of
menopausal women



Questions or remarks?
Want to collaborate?
kato.herregods@ugent.be



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Appendix

Appendix A: 06_qsiprep_IRTG.sh

```
#!/bin/bash

# -----
# Script: Run QSIprep 1.0.1 on BIDS dataset
# -----

# This script is a modification of the FMRIPrep script as written by Ann-Christin Kimmig and reviewed by Bernd
Kardatzki, University of Tübingen and the QSIprep script as written by Amelia Rauland, University of Philadelphia.
# Modified by Kato Herregods, University of Ghent for IRTG007 study, University of Tübingen. 24.10.2025
# It runs QSIprep on a given BIDS-dataset using QSIprep 1.0.1 in an Apptainer.

#To run the script, type the following command in the terminal:
#cd PATH/TO/06_qsiprep_IRTG.sh
#chmod +x 06_qsiprep_IRTG.sh
#./06_qsiprep_IRTG.sh [-v VPN_FILE] INPUT_DIR
#Example:
#./06_qsiprep_IRTG.sh -v /home/kato/01_BIDS_IRTG07_crosssec/list_subjects/vpn16.txt
/home/kato/01_BIDS_IRTG07_crosssec

#To run different subjects in parallel, open different terminals and run for different subjects by changing the
VPN_FILE argument.

# Ensure the script is executed, not sourced
[[ "${BASH_SOURCE[0]}" != "${0}" ]] && { echo "Please don't source but execute me!"; return; }

# Define Apptainer/Singularity path
APPTAINER_IMG="/nic/sw/IRTG/sif/qsiprep-1.0.1.sif"
FREESURFER_PATH="/nic/sw/FreeSurfer"
FS_LICENSE="/nic/sw/FreeSurfer/license.txt" # Path to FreeSurfer license file

# Default values (can be overridden)
: ${NPROCS:=16} #NPROCS = number of processes/parallel jobs
: ${OMP_THREADS:=4} #Number of threads = Virtual cores created through a technology calles simultaneous
multithreading (SMT)
```

```
: ${MEM_GB:=16}

# Function to display usage instructions when called from the command line
## It explains all command-line options the script accepts
function Usage(){
    echo
    echo "Usage: ${0##*/} [-n NPROCS] [-t OMP_THREADS] [-o OUTPUT_DIR] [-v VPN_FILE] INPUT_DIR"
    echo
    echo "  -n NPROCS      Number of processes (default: $NPROCS)"
    echo "  -t OMP_THREADS Number of threads per process (default: $OMP_THREADS)"
    echo "  Alternatively, NPROC, OMP_THREADS and MEM_GB can be set via the command line
before running this script using: 'export NPROCS=X' and 'export OMP_THREADS=X'"
    echo "  -o OUTPUT_DIR  Output directory (default: <INPUT_DIR>/derivatives/qsiprep)"
    echo "  -v VPN_FILE    File with participants to be processed (optional, e.g.
\"/p/irtg/IRTGXX/00_Codes_IRTGXX/vpn_lists/vpn1.txt\"), default: processes all unprocessed participants"
    echo "  -f BIDS_FILTER BIDS filter for selecting specific sessions and/or datatypes (optional). Either enter your
file path or type \"ses01\" to only process first session and \"ses02\" for second session"
    echo
    echo "INPUT_DIR is mandatory and used as base folder for output and logfiles (default:
<INPUT_DIR>/derivatives/qsiprep/logs)."
    echo "Recommended INPUT_DIR is \"p/irtg/IRTGXX/01_BIDS_IRTGXX\""
    echo "$1"
}

# Parse command-line arguments

while getopts "hn:t:o:v:" OPT; do
    case $OPT in
        # Option -h: Show usage and exit
        h ) Usage; exit 0 ;;

        # Option -n: Adapt number of processes
        n ) NPROCS="$OPTARG" ;;

        # Option -t: Adapt number of threads per process
        t ) OMP_THREADS="$OPTARG" ;;

        # Option -o: Use alternative output directory
        o ) OUTPUT_DIR="$OPTARG" ;;

        # Option -v: add vpn_list to only process specific VPs
```

```

v ) VPN_FILE="$OPTARG" ;;

# Catch invalid options or missing arguments
? ) echo "${0##*/}: unexpected option \"-$OPTARG\" or missing argument"; exit 1 ;;

esac
done

# Shift arguments to move past the options
shift $((OPTIND - 1))

# Ensure INPUT_DIR is provided
if [ $# -lt 1 ]; then
    Usage "Need exactly one folder name as the INPUT_DIR argument!"
    exit 1
else
    # Set INPUT_DIR to the absolute path of the given argument
    INPUT_DIR="$(realpath $1)"
    echo "Processing input directory: $INPUT_DIR"
fi

# Check if INPUT_DIR exists
[[ -d $INPUT_DIR ]] || { echo "${0##*/}: $INPUT_DIR not found"; exit 1; }

# Define paths
: "${OUTPUT_DIR:="$INPUT_DIR/derivatives/qsiprep"}"
WORK_DIR="$INPUT_DIR/derivatives/work/qsiprep_work"
LOG_DIR="$OUTPUT_DIR/logs"

# Create necessary directories
echo mkdir -p "$OUTPUT_DIR" "$WORK_DIR" "$LOG_DIR"
mkdir -p "$OUTPUT_DIR" "$WORK_DIR" "$LOG_DIR"

# Check whether the environment variable TEMPLATEFLOW_HOME is set. If it is not set, it will assign a
default fallback value.
if [ -z "$TEMPLATEFLOW_HOME" ]; then
    export TEMPLATEFLOW_HOME="$HOME/.cache/templateflow" #Only executed if TEMPLATE_FLOW is
empty. Export makes the variable visible to qsirecon
    echo "TEMPLATEFLOW_HOME was not set. Using default: \"$TEMPLATEFLOW_HOME\""

```

```

fi

# Teach qsiprep container to not use its own (almost empty) template repository
if [ -z $APTAINERENV_TEMPLATEFLOW_HOME ]; then
    export APTAINERENV_TEMPLATEFLOW_HOME="$TEMPLATEFLOW_HOME"
    echo "APTAINERENV_TEMPLATEFLOW_HOME was not set. Setting to:
\"$APTAINERENV_TEMPLATEFLOW_HOME\""
fi

# Check if vpn.txt exists and read subject list
PARTICIPANT_LABELS=()

if [[ -f "$VPN_FILE" ]]; then
    # Check for Windows-style line endings before converting
    if grep -q $'\r' "$VPN_FILE"; then
        if command -v dos2unix &> /dev/null; then
            dos2unix "$VPN_FILE"
        else
            echo "Warning: dos2unix not found. Attempting manual conversion."
            sed -i 's/\r$//' "$VPN_FILE"
        fi
    fi

    # Read file into array and remove "sub-" prefix
    mapfile -t PARTICIPANT_LABELS < <(sed 's/^sub-/' "$VPN_FILE")
    echo "Using participants from $VPN_FILE: ${PARTICIPANT_LABELS[@]}"
else
    # Get the list of subjects from INPUT_DIR if no VPN file is provided
    mapfile -t PARTICIPANT_LABELS < <(find "$INPUT_DIR" -maxdepth 1 -type d -name "sub-*" | sed 's|^./sub-||' |
    sort -n)

    # Check if subjects were found
    if [ ${#PARTICIPANT_LABELS[@]} -eq 0 ]; then
        echo "Error: No subjects found in $INPUT_DIR. Check if the dataset is correctly structured."
        exit 1
    fi

    echo "Subjects found: ${PARTICIPANT_LABELS[@]}"
fi

```

```
# Convert array to space-separated string
PARTICIPANT_LABELS_STR="${PARTICIPANT_LABELS[@]}"

# Start loop for QSIprep
echo "Starting QSIprep..."

#####

#           Run QSIprep in apptainer           #
#####

# Loop over each subject/participant label (you can define PARTICIPANT_LABELS_STR with your list)
for subject in $PARTICIPANT_LABELS_STR; do
    # Display the participant label being processed
    echo "Processing participant: $subject"

    # Run the Apptainer command for each subject with the dynamic output spaces
    qsiprep_failed=0
    {
        apptainer run \
            --cleanenv \
            -B "$TEMPLATEFLOW_HOME:$APPTAINERENV_TEMPLATEFLOW_HOME" \
            -B "$WORK_DIR:/work_dir" \
            -B "$INPUT_DIR:/input_dir" \
            -B "$OUTPUT_DIR:/output_dir" \
            -B "$FREESURFER_PATH:$FREESURFER_PATH" \
            "$APPTAINER_IMG" \
            "/input_dir" \
            "/output_dir" \
        --notrack \
            participant \
            --anat-modality T1w \
            --work-dir "/work_dir" \
            ${PARTICIPANT_LABELS_STR:+--participant-label $subject} \
            --fs-license-file "$FS_LICENSE" \
            --skip-bids-validation \
```

```

--output-resolution 1.5 \
--separate-all-dwis \
-v -v \
--stop-on-first-crash \
--nprocs "$NPROCS" \
--nthreads ${OMP_THREADS} \
--omp-nthreads "$OMP_THREADS" \
1> >(tee -a "$LOG_DIR/sub-${subject}_${date +"%Y-%m-%d-%H-%M"}_out.log") \
2> >(tee >(grep -i -e "warning" -e "error" >> "$LOG_DIR/sub-${subject}_${date +"%Y-%m-%d-%H-%M"}_err.log") >&2);
} || qsiprep_failed=1

done

# If qsiprep failed we need to know about it
if [ ${qsiprep_failed} -gt 0 ]; then
    echo QSIPREPFail
else
    echo QSIPREPSUCCESS
fi

```

Appendix B: 07_FBA_analysis.sh

```

#!/bin/bash

set -euo pipefail

# Written by Andrew Jahn, University of Michigan, 02.25.2019
# Modified by Rik88 for MYP2 study @ UGent, Belgium, 11.20.2022
# Modified by Kato Herregods for IRTG, Tübingen, Germany

#To run the script follow these steps:
#cd /PATH/TO/08_FBA_analysis.sh
#chmod +x 08_FBA_analysis.sh
# ./08_FBA_analysis.sh

```

```
##### STEP 1 #####
#           Define PATHS           #
#####

INPUT_DIR="/home/kato/01_BIDS_IRTG07_crosssec"
SUBJECT_LIST="/home/kato/01_BIDS_IRTG07_crosssec/list_subjects/vpn_all_included_subjects.txt"
TEMPLATE_DIR="$INPUT_DIR/derivatives/mrtrix3/template"

mkdir -p "$TEMPLATE_DIR"

##### STEP 2 #####
#           Convert data to .mif format           #
#####

#Loop over subjects
while read -r subject; do
    echo "Converting .nii to .mif format for sub-${subject} ..."

    #Determine session
    if [ -d "$INPUT_DIR/derivatives/qsiprep/sub-${subject}/ses-01" ]; then
        SESSION="ses-01"
    elif [ -d "$INPUT_DIR/derivatives/qsiprep/sub-${subject}/ses-02" ]; then
        SESSION="ses-02"
    fi

    #Define output directory
    OUTPUT_DIR="$INPUT_DIR/derivatives/mrtrix3/sub-${subject}/${SESSION}"
    mkdir -p "$OUTPUT_DIR"

    #Convert data to .mif format
    mrconvert -grad \
        "$INPUT_DIR/derivatives/qsiprep/sub-${subject}/${SESSION}/dwi/sub-${subject}_${SESSION}_acq-
DGD103_dir-AP_space-ACPC_desc-preproc_dwi.b" \
        "$INPUT_DIR/derivatives/qsiprep/sub-${subject}/${SESSION}/dwi/sub-${subject}_${SESSION}_acq-
DGD103_dir-AP_space-ACPC_desc-preproc_dwi.nii.gz" \
```

```

"$OUTPUT_DIR/sub-`${subject}_acq-DGD103_preproc_dwi.mif" \
-force

done < "$SUBJECT_LIST"

##### STEP 3 #####
#   Computing the tissue specific response functions           #
#####

#Computing the average tissue response function using the response functions generated by QSIrecon with the
mrtrix_multishell_msmt_ACT-hsvs workflow

#Loop over subjects
while read -r subject; do
    echo "Computing response functions for `${subject} (DGD103)..."

    #Determine session
    if [ -d "$INPUT_DIR/derivatives/qsiprep/sub-`${subject}/ses-01" ]; then
        SESSION="ses-01"
    elif [ -d "$INPUT_DIR/derivatives/qsiprep/sub-`${subject}/ses-02" ]; then
        SESSION="ses-02"
    fi

    ##Define output directory
    OUTPUT_DIR="$INPUT_DIR/derivatives/mrtrix3/sub-`${subject}/${SESSION}"

    #Computing the respons function
    dwi2response dhollander "$OUTPUT_DIR/sub-`${subject}_acq-DGD103_preproc_dwi.mif" \
        "$OUTPUT_DIR/response_wm.txt" \
        "$OUTPUT_DIR/response_gm.txt" \
        "$OUTPUT_DIR/response_csf.txt"

done < "$SUBJECT_LIST"

##### STEP 4 #####

```

```
# Computing the average, tissue specific response function      #
#####

#Generating the average tissue response function for the three tissue types
INPUT_FILES_WM=( $INPUT_DIR/derivatives/mrtrix3/sub-*/ses-*/response_wm.txt )
INPUT_FILES_GM=( $INPUT_DIR/derivatives/mrtrix3/sub-*/ses-*/response_gm.txt )
INPUT_FILES_CSF=( $INPUT_DIR/derivatives/mrtrix3/sub-*/ses-*/response_csf.txt )

responsemean "${INPUT_FILES_WM[@]}" "$TEMPLATE_DIR/group_average_response_wm.txt"
responsemean "${INPUT_FILES_GM[@]}" "$TEMPLATE_DIR/group_average_response_gm.txt"
responsemean "${INPUT_FILES_CSF[@]}" "$TEMPLATE_DIR/group_average_response_csf.txt"

##### STEP 5 #####
# Upsampling the dw images                                     #
#####

#Loop over subjects
while read -r subject; do
    echo "Upsampling dwi of subject sub-${subject} ..."

    #Determine session
    if [ -d "$INPUT_DIR/derivatives/qsiprep/sub-${subject}/ses-01" ]; then
        SESSION="ses-01"
    elif [ -d "$INPUT_DIR/derivatives/qsiprep/sub-${subject}/ses-02" ]; then
        SESSION="ses-02"
    fi

    #Define output directory
    OUTPUT_DIR="$INPUT_DIR/derivatives/mrtrix3/sub-${subject}/${SESSION}"

    #Upsampling of dw images
    mrgrid "$OUTPUT_DIR/sub-${subject}_acq-DGD103_preproc_dwi.mif" regrid -vox 1.25
    "$OUTPUT_DIR/sub-${subject}_acq-DGD103_preproc_upsampled.mif"

    #Upsampling of mean B0 images. This upsampled image will later be used for creating a binary brain mask for
    each participant (step 5)
```

```

mrgrid "$INPUT_DIR/derivatives/qsiprep/sub-`${subject}`/$SESSION/dwi/sub-`${subject}`_${SESSION}_acq-
DGD103_dir-AP_space-ACPC_dwired.nii.gz" regrid -vox 1.25 "$OUTPUT_DIR/sub-`${subject}`_${SESSION}_acq-
DGD103_dir-AP_space-ACPC_dwired_upsampled.nii"
done < "$SUBJECT_LIST"

##### STEP 6 #####
#       Compute binary brain mask image for each subject       #
#####

#This step diverts from those described in the MRtrix readthedocs. The reason for this is the poor performance of
'dwi2mask' in creating an accurate binary brain mask. Therefore, mri_synthstrip is used, an alternative skull
stripping function, able to create binary brain masks.
#Loop over subjects
while read -r subject; do
    echo "Computing the brain mask image using synthstrip for sub-`${subject}` ..."

    #Determine session
    if [ -d "$INPUT_DIR/derivatives/qsiprep/sub-`${subject}`/ses-01" ]; then
        SESSION="ses-01"
    elif [ -d "$INPUT_DIR/derivatives/qsiprep/sub-`${subject}`/ses-02" ]; then
        SESSION="ses-02"
    fi

    #Define output directory
    OUTPUT_DIR="$INPUT_DIR/derivatives/mrtrix3/sub-`${subject}`/$SESSION"

    #Computing the brain mask images from the upsampled mean BO image (=dwired)
    mri_synthstrip -i "$OUTPUT_DIR/sub-`${subject}`_${SESSION}_acq-DGD103_dir-AP_space-
ACPC_dwired_upsampled.nii" -o "$OUTPUT_DIR/sub-`${subject}`_${SESSION}_acq-DGD103_dir-AP_space-
ACPC_dwired_upsampled_stripped.nii" -m "$OUTPUT_DIR/sub-`${subject}`_acq-DGD103_mask.nii"

    #Converting the created masks from .nii to .mif files to avoid incompatibility issues later on (e.g. in step creating
the population template)
    mrconvert "$OUTPUT_DIR/sub-`${subject}`_acq-DGD103_mask.nii" "$OUTPUT_DIR/sub-`${subject}`_acq-
DGD103_mask.mif" -datatype bit

done < "$SUBJECT_LIST"

```

#This step diverts from those described in the MRtrix readthedocs. The reason for this is the poor performance of 'dwi2mask' in creating an accurate binary brain mask. Therefore, mri_synthstrip is used, an alternative skull stripping function, able to create binary brain masks.

```
##### STEP 7 #####
#      Fibre Orientation Distribution estimation      #
#####

#Loop over subjects
while read -r subject; do
    echo "Estimation of FOD for wm, gm, csf in sub-${subject} ..."

    #Determine session
    if [ -d "$INPUT_DIR/derivatives/qsiprep/sub-${subject}/ses-01" ]; then
        SESSION="ses-01"
    elif [ -d "$INPUT_DIR/derivatives/qsiprep/sub-${subject}/ses-02" ]; then
        SESSION="ses-02"
    fi

    #Define output directory
    OUTPUT_DIR="$INPUT_DIR/derivatives/mrtrix3/sub-${subject}/${SESSION}"

    #Estimation of fiber orientation distribution for wm, gm and csf.
    dwi2fod msmt_csd \
        $OUTPUT_DIR/sub-${subject}_acq-DGD103_preproc_upsampled.mif \
        $TEMPLATE_DIR/group_average_response_wm.txt $OUTPUT_DIR/sub-${subject}_wm_fod.mif \
        $TEMPLATE_DIR/group_average_response_gm.txt $OUTPUT_DIR/sub-${subject}_gm_fod.mif \
        $TEMPLATE_DIR/group_average_response_csf.txt $OUTPUT_DIR/sub-${subject}_csf_fod.mif \
        -mask $OUTPUT_DIR/sub-${subject}_acq-DGD103_mask.mif

done < "$SUBJECT_LIST"

##### STEP 8 #####
#      Joint bias field correction and intensity normalisation      #
#####
```

```
#Loop over subjects
while read -r subject; do
    echo "Bias field correction and intensity normalisation in sub-`${subject}` ..."

    #Determine session
    if [ -d "$INPUT_DIR/derivatives/qsiprep/sub-`${subject}`/ses-01" ]; then
        SESSION="ses-01"
    elif [ -d "$INPUT_DIR/derivatives/qsiprep/sub-`${subject}`/ses-02" ]; then
        SESSION="ses-02"
    fi

    #Define output directory
    OUTPUT_DIR="$INPUT_DIR/derivatives/mrtrix3/sub-`${subject}`/$SESSION"

    #Joint bias field correction and intensity normalisation
    mtnormalise \
        $OUTPUT_DIR/sub-`${subject}`_wm_fod.mif $OUTPUT_DIR/sub-`${subject}`_wm_norm.mif \
        $OUTPUT_DIR/sub-`${subject}`_gm_fod.mif $OUTPUT_DIR/sub-`${subject}`_gm_norm.mif \
        $OUTPUT_DIR/sub-`${subject}`_csf_fod.mif $OUTPUT_DIR/sub-`${subject}`_csf_norm.mif \
        -mask $OUTPUT_DIR/sub-`${subject}`_acq-DGD103_mask.mif

done < "$SUBJECT_LIST"

##### STEP 9 #####
#      Generate a study-specific unbiased FOD template      #
#####

#Putting all FOD images in a single folder and putting a set of corresponding mask images (with the same prefix
as the FOD images) in another folder (using masks speeds up registration significantly)
mkdir -p "$TEMPLATE_DIR/fod_input"
mkdir -p "$TEMPLATE_DIR/mask_input"

#Symbolic link of all FOD images (and masks) into a single input folder. This way the entire population is used to
build the template.
#PRE.mif behaves the same like the original file wmfod_norm.mif. If you change wmfod_norm.mif, PRE.mif also
changes bcs it is the same file. + almost no extra disc space
```

```

echo "Creating symbolic link of all FOD images and masks into a single input folder ..."

#Looping over subjects
while read -r subject; do

    #Determine session
    if [ -d "$INPUT_DIR/derivatives/qsiprep/sub-`${subject}`/ses-01" ]; then
        SESSION="ses-01"
    elif [ -d "$INPUT_DIR/derivatives/qsiprep/sub-`${subject}`/ses-02" ]; then
        SESSION="ses-02"
    fi

    #Define output directory
    OUTPUT_DIR="$INPUT_DIR/derivatives/mrtrix3/sub-`${subject}`/$SESSION"

    #Create symbolic links
    ln -sr $OUTPUT_DIR/sub-`${subject}`_wm_norm.mif $TEMPLATE_DIR/fod_input/sub-`${subject}`_PRE.mif
    ln -sr $OUTPUT_DIR/sub-`${subject}`_acq-DGD103_mask.mif $TEMPLATE_DIR/mask_input/sub-
`${subject}`_PRE.mif

done < "$SUBJECT_LIST"

#Build the unbiased FOD template
population_template $TEMPLATE_DIR/fod_input -mask_dir $TEMPLATE_DIR/mask_input
$TEMPLATE_DIR/wmfod_template.mif -voxel_size 1.25

##### STEP 10 #####
#   Register all subject FOD images to the FOD template   #
#####

#Looping over subjects
while read -r subject; do

    echo "Registration to template for sub-`${subject}` ..."

    #Determine session

```

```

if [ -d "$INPUT_DIR/derivatives/qsiprep/sub-`${subject}`/ses-01" ]; then
    SESSION="ses-01"
elif [ -d "$INPUT_DIR/derivatives/qsiprep/sub-`${subject}`/ses-02" ]; then
    SESSION="ses-02"
fi

#Define output directory
OUTPUT_DIR="$INPUT_DIR/derivatives/mrtrix3/sub-`${subject}`/$SESSION"

#Register all subject FOD images to the FOD template
mrregister $OUTPUT_DIR/sub-`${subject}`_wm_norm.mif \
-mask1 $OUTPUT_DIR/sub-`${subject}`_acq-DGD103_mask.mif \
$TEMPLATE_DIR/wmfod_template.mif \
-nl_warp $OUTPUT_DIR/sub-`${subject}`_subject2template_warp.mif \
$OUTPUT_DIR/sub-`${subject}`_template2subject_warp.mif

done < "$SUBJECT_LIST"

##### STEP 11 #####
#           Compute the template mask           #
#####

#Looping over subjects
while read -r subject; do
    echo "Registration to template for sub-`${subject}` ..."

    #Determine session
    if [ -d "$INPUT_DIR/derivatives/qsiprep/sub-`${subject}`/ses-01" ]; then
        SESSION="ses-01"
    elif [ -d "$INPUT_DIR/derivatives/qsiprep/sub-`${subject}`/ses-02" ]; then
        SESSION="ses-02"
    fi

    #Define output directory
    OUTPUT_DIR="$INPUT_DIR/derivatives/mrtrix3/sub-`${subject}`/$SESSION"

    #Compute the template mask, which is the intersection of all subject's masks in template space

```

```

mrtransform $OUTPUT_DIR/sub- $\{subject\}$ _acq-DGD103_mask.mif -warp $OUTPUT_DIR/sub-
 $\{subject\}$ _subject2template_warp.mif -interp nearest -datatype bit $OUTPUT_DIR/sub-
 $\{subject\}$ _dwi_mask_in_template_space.mif

done < "$SUBJECT_LIST"

#Compute the template mask as the intersection of all warped masks
INPUT_MASKS=( $INPUT_DIR/derivatives/mrtrix3/sub-*/ses-*/sub-*_dwi_mask_in_template_space.mif )

echo "Path to all warped masks to create template mask:  $\{INPUT\_MASKS[@]\}$ "

mrmath " $\{INPUT\_MASKS[@]\}$ " min $TEMPLATE_DIR/template_mask.mif -datatype bit

#MRtrix3 strongly advises visualising the resulting template mask to make sure all regions of the brain that are
intended to be analysed are included. If not, this is either caused by a faulty individual subject mask, problems in
the template building process or problems with registration of the individual subjects. The root cause of the
problem should be found and solved. More information can be found on the mrtrix3 readthedocs page under
MSMT-CSD FBA and the Mrtrix3 forum.

##### STEP 12 #####
#   Compute a white matter template analysis fixel mask   #
#####

echo "Creating white matter template fixel mask..."

fod2fixel -mask $TEMPLATE_DIR/template_mask.mif -fmls_peak_value 0.06
$TEMPLATE_DIR/wmfod_template.mif $TEMPLATE_DIR/fixel_mask

#MRtrix3 strongly advises visualising the output fixel mask using mrview. This can be done by opening the
index.mif found in ../template/fixel_mask via the fixel plot tool. If fixels are missing, especially in regions where
fibers cross, the mask should be regenerated with a lower value for -fmls_peak_value. More information can be
found on the mrtrix3 readthedocs page under MSMT-CSD FBA and the Mrtrix3 forum.

##### STEP 13 #####
#   Warp FOD images to template space   #

```

```
#####

#Looping over subjects
while read -r subject; do
    echo "Warping FOD image to template space for sub-`${subject}` ..."

    #Determine session
    if [ -d "$INPUT_DIR/derivatives/qsiprep/sub-`${subject}`/ses-01" ]; then
        SESSION="ses-01"
    elif [ -d "$INPUT_DIR/derivatives/qsiprep/sub-`${subject}`/ses-02" ]; then
        SESSION="ses-02"
    fi

    #Define output directory
    OUTPUT_DIR="$INPUT_DIR/derivatives/mrtrix3/sub-`${subject}`/$SESSION"

    #Warping FOD images to template space
    mrtransform $OUTPUT_DIR/sub-`${subject}`_wm_norm.mif -warp $OUTPUT_DIR/sub-
`${subject}`_subject2template_warp.mif -reorient_fod no $OUTPUT_DIR/sub-
`${subject}`_fod_in_template_space_NOT_REORIENTED.mif

done < "$SUBJECT_LIST"

##### STEP 14 #####
#      Segment FOD images to estimate fixels and AFD      #
#####

#Looping over subjects
while read -r subject; do
    echo "Warping FOD image to template space for sub-`${subject}` ..."

    #Determine session
    if [ -d "$INPUT_DIR/derivatives/qsiprep/sub-`${subject}`/ses-01" ]; then
        SESSION="ses-01"
    elif [ -d "$INPUT_DIR/derivatives/qsiprep/sub-`${subject}`/ses-02" ]; then
        SESSION="ses-02"
    fi
```

```

#Define output directory
OUTPUT_DIR="$INPUT_DIR/derivatives/mrtrix3/sub-${subject}/${SESSION}"

#Segmentation of each FOD lobe to identify the number and orientation of fixels in each voxel.
#The output also contains the apparent fibre density (AFD) value per fixel (estimated as the FOD lobe integral):
    fod2fixel -mask $TEMPLATE_DIR/template_mask.mif $OUTPUT_DIR/sub-
${subject}_fod_in_template_space_NOT_REORIENTED.mif
$OUTPUT_DIR/fixel_in_template_space_NOT_REORIENTED -afd fd.mif

done < "$SUBJECT_LIST"

##### STEP 15 #####
#           Reorient fixels           #
#####

#Looping over subjects
while read -r subject; do
    echo "Reorienting fixels for sub-${subject} ..."

    #Determine session
    if [ -d "$INPUT_DIR/derivatives/qsiprep/sub-${subject}/ses-01" ]; then
        SESSION="ses-01"
    elif [ -d "$INPUT_DIR/derivatives/qsiprep/sub-${subject}/ses-02" ]; then
        SESSION="ses-02"
    fi

    #Define output directory
    OUTPUT_DIR="$INPUT_DIR/derivatives/mrtrix3/sub-${subject}/${SESSION}"

    #Reorient the fixels
    fixelreorient $OUTPUT_DIR/fixel_in_template_space_NOT_REORIENTED $OUTPUT_DIR/sub-
${subject}_subject2template_warp.mif $OUTPUT_DIR/fixel_in_template_space

done < "$SUBJECT_LIST"

##### STEP 16 #####

```

```
#      Assign subject fixels to template fixels      #
#####

#Looping over subjects
while read -r subject; do
    echo "Assigning subject fixels to template fixels sub-`${subject}` ..."

    #Determine session
    if [ -d "$INPUT_DIR/derivatives/qsiprep/sub-`${subject}`/ses-01" ]; then
        SESSION="ses-01"
    elif [ -d "$INPUT_DIR/derivatives/qsiprep/sub-`${subject}`/ses-02" ]; then
        SESSION="ses-02"
    fi

    #Define output directory
    OUTPUT_DIR="$INPUT_DIR/derivatives/mrtrix3/sub-`${subject}`/$SESSION"

    #Create new directory
    mkdir -p "$TEMPLATE_DIR/fd"

    #Assign subject fixels to template fixels
    fixelcorrespondence $OUTPUT_DIR/fixel_in_template_space/fd.mif $TEMPLATE_DIR/fixel_mask
$TEMPLATE_DIR/fd sub-`${subject}`_fd.mif

done < "$SUBJECT_LIST"

##### STEP 17 #####
#      Compute the fibre cross-section (FC) metric and log(FC)      #
#####

#Looping over subjects
while read -r subject; do
    echo "Computing FC and log(FC) sub-`${subject}` ..."

    #Determine session
    if [ -d "$INPUT_DIR/derivatives/qsiprep/sub-`${subject}`/ses-01" ]; then
        SESSION="ses-01"
```

```

    elif [ -d "$INPUT_DIR/derivatives/qsiprep/sub-`${subject}`/ses-02" ]; then
        SESSION="ses-02"
    fi

    #Define output directory
    OUTPUT_DIR="$INPUT_DIR/derivatives/mrtrix3/sub-`${subject}`/$SESSION"

    #Create new directories
    mkdir -p "$TEMPLATE_DIR/fc"
    mkdir -p "$TEMPLATE_DIR/log_fc"

    #Compute FC
    warp2metric $OUTPUT_DIR/sub-`${subject}`_subject2template_warp.mif -fc $TEMPLATE_DIR/fixel_mask
    $TEMPLATE_DIR/fc sub-`${subject}`_fc.mif

    #Copy index.mif and directions.mif from fc directory to log_fc directory so a full fixel directory is created, needed
    for later processing step (fixelfilter)
    cp $TEMPLATE_DIR/fc/index.mif $TEMPLATE_DIR/fc/directions.mif $TEMPLATE_DIR/log_fc

    #Compute log(FC)
    mrcalc $TEMPLATE_DIR/fc/sub-`${subject}`_fc.mif -log $TEMPLATE_DIR/log_fc/sub-`${subject}`_logfc.mif

done < "$SUBJECT_LIST"

##### STEP 18 #####
# Compute a combined measure of fibre density and cross-section (FDC)#
#####

#Looping over subjects
while read -r subject; do
    echo "Assigning subject fixels to template fixels sub-`${subject}` ..."

    #Create directory
    mkdir -p "$TEMPLATE_DIR/fdc"

    #Copy index.mif and directions.mif from fc directory to fdc directory so a full fixel directory is created, needed
    for later processing step (fixelfilter)

```

```

cp $TEMPLATE_DIR/fc/index.mif $TEMPLATE_DIR/fc/directions.mif $TEMPLATE_DIR/fdc

#Compute FDC
    mrcalc \
    $TEMPLATE_DIR/fd/sub-`${subject}_fd.mif \
    $TEMPLATE_DIR/fc/sub-`${subject}_fc.mif \
    -mult $TEMPLATE_DIR/fdc/sub-`${subject}_fdc.mif

done < "$SUBJECT_LIST"

##### STEP 19 #####
#   Perform whole-brain fibre tractography on the FOD template   #
#####

#Following steps are executed from the template directory
cd "$TEMPLATE_DIR"

tckgen -angle 22.5 -maxlen 250 -minlen 10 -power 1.0 \
    wmfd_template.mif -seed_image template_mask.mif -mask template_mask.mif \
    -select 20000000 -cutoff 0.06 tracks_20_million.tck

##### STEP 20 #####
#   Reduce biases in tractogram densities   #
#####

tcksift tracks_20_million.tck wmfd_template.mif \
    tracks_2_million_sift.tck -term_number 2000000

##### STEP 21 #####
#   Generate fixel-fixel connectivity matrix   #
#####

fixelconnectivity fixel_mask/ tracks_2_million_sift.tck matrix/

```

```
##### STEP 22 #####
#      Smooth fixel data using fixel-fixel connectivity      #
#####

fixelfilter $TEMPLATE_DIR/fd smooth $TEMPLATE_DIR/fd_smooth -matrix $TEMPLATE_DIR/matrix
fixelfilter $TEMPLATE_DIR/log_fc smooth $TEMPLATE_DIR/log_fc_smooth -matrix $TEMPLATE_DIR/matrix
fixelfilter $TEMPLATE_DIR/fdc smooth $TEMPLATE_DIR/fdc_smooth -matrix $TEMPLATE_DIR/matrix

#####

echo "===== FIXEL-BASED ANALYSIS COMPLETED SUCCESSFULLY ====="

echo " RUN 09_FBA_stats_f_test.sh FOR STATISTICAL ANALYSIS "
```

Appendix C: 08_FBA_stats_f_test.sh

```
#!/bin/bash

set -euo pipefail

# Written by Andrew Jahn, University of Michigan, 02.25.2019
# Modified by Kato Herregods for IRTG, Tübingen, Germany
# In this script, f-tests are performed for three different measures: fiber density (FD), the logarithm of fiber cross-
section# (log(FC)) and the combination of the two (FDC). For these analyses it is necessary to create three .txt
files. Details on these .txt files can be found on the mrtrix3 readthedocs page, under fixel-based analysis. They
should be located inside the $TEMPLATE_DIR.
#For the analysis of FDC and log(FC), the design matrix includes the covariates age and intracranial volume. For
the analysis of FD, the design matrix only includes the covariate age, as recommended by the developers of FBA.

#To execute the script, run these commands in the terminal:
#cd PATH/TO/08_FBA_stats_f_test.sh
#chmod +x 08_FBA_stats_f_test.sh
# ./08_FBA_stats_f_test.sh

##### STEP 1 #####
#      Define PATHS      #
#####
```

```

INPUT_DIR="/home/kato/01_BIDS_IRTG07_crosssec"
SUBJECT_LIST="/home/kato/01_BIDS_IRTG07_crosssec/list_subjects/vpn_all_included_subjects_MHTincluded.t
xt"
TEMPLATE_DIR="$INPUT_DIR/derivatives/mrtrix3/template"

mkdir -p "$TEMPLATE_DIR"
mkdir -p "$TEMPLATE_DIR/f_stat"
cd $TEMPLATE_DIR

##### STEP 2 #####
#           F-test for fiber density           #
#####

#Pairwise comparisson of fiber density in PRE vs POST.
DESIGN="$TEMPLATE_DIR/f_stat/design_matrix_FD_covar_age.txt"
CONTRAST="$TEMPLATE_DIR/f_stat/contrast_matrix_FD_covar_age.txt"
CONNECTIVITY="$TEMPLATE_DIR/matrix"
F_INDEX="$TEMPLATE_DIR/f_stat/f_contrast_index.txt"

#Run fixelcfestats. Is there a significant difference in fiber density between any of the groups
fixelcfestats $TEMPLATE_DIR/fd_smooth $TEMPLATE_DIR/f_stat/fd_files.txt $DESIGN $CONTRAST
$CONNECTIVITY $TEMPLATE_DIR/f_stat/fd -fests $F_INDEX -nthreads ${OMP_NUM_THREADS} -nshuffles
5000

echo "===== F-TEST OF FD COMPLETED SUCCESSFULLY ====="

##### STEP 3 #####
#           F-test for fiber cross-section           #
#####

#Pairwise comparisson of fiber density in PRE vs POST.
DESIGN="$TEMPLATE_DIR/f_stat/design_matrix_FC_FDC_covar_age_TIV.txt"
CONTRAST="$TEMPLATE_DIR/f_stat/contrast_matrix_FC_FDC_covar_age_TIV.txt"

```

```
CONNECTIVITY="$TEMPLATE_DIR/matrix"
F_INDEX="$TEMPLATE_DIR/f_stat/f_contrast_index.txt"

#Run fixlcfestats. Is there a significant difference in fiber density between any of the groups
fixlcfestats $TEMPLATE_DIR/log_fc_smooth $TEMPLATE_DIR/f_stat/log_fc_files.txt $DESIGN $CONTRAST
$CONNECTIVITY $TEMPLATE_DIR/f_stat/log_fc -ftests $F_INDEX -nthreads ${OMP_NUM_THREADS} -
nshuffles 5000

echo "===== F-TEST OF LOG(FC) COMPLETED SUCCESSFULLY ====="

##### STEP 3 #####
#           F-test for FDC           #
#####

#Pairwise comparisson of fiber density in PRE vs POST.
DESIGN="$TEMPLATE_DIR/f_stat/design_matrix_FC_FDC_covar_age_TIV.txt"
CONTRAST="$TEMPLATE_DIR/f_stat/contrast_matrix_FC_FDC_covar_age_TIV.txt"
CONNECTIVITY="$TEMPLATE_DIR/matrix"
F_INDEX="$TEMPLATE_DIR/f_stat/f_contrast_index.txt"

#Run fixlcfestats. Is there a significant difference in fiber density between any of the groups
fixlcfestats $TEMPLATE_DIR/fdc_smooth $TEMPLATE_DIR/f_stat/fdc_files.txt $DESIGN $CONTRAST
$CONNECTIVITY $TEMPLATE_DIR/f_stat/fdc -ftests $F_INDEX -nthreads ${OMP_NUM_THREADS} -nshuffles
5000

echo "===== F-TEST OF FDC COMPLETED SUCCESSFULLY ====="
```

Appendix D: 08_FBA_stats_pairwise_fc.sh

```
#!/bin/bash

set -euo pipefail
```

```
export OMP_NUM_THREADS=2
export MKL_NUM_THREADS=2
export OPENBLAS_NUM_THREADS=2

# Written by Andrew Jahn, University of Michigan, 02.25.2019
# Modified by Kato Herregods for IRTG, Tübingen, Germany
# In this script, pairwise statistical analyses are performed for three different measures: the logarithm of fiber
cross-section# (log(FC)), fiber density (FD) and the combination of the two (FDC). For these pairwise analyses it
is necessary to generate### three .txt files including the subjects that you want to compare.
# In this script, PRE_vs_PERI_subject_list.txt, PERI_vs_POST_subject_list.txt and PRE_vs_POST_subject_list.txt
are the files created for this purpose.
# They should be located inside the $TEMPLATE_DIR.

#To run the script follow these steps:
#cd PATH/TO/08_FBA_stats_log_fc.sh
#chmod +x 08_FBA_stats_log_fc.sh
# ./08_FBA_stats_log_fc.sh

##### STEP 1 #####
#           Define PATHS           #
#####

INPUT_DIR="/home/kato/01_BIDS_IRTG07_crosssec"
SUBJECT_LIST="/home/kato/01_BIDS_IRTG07_crosssec/list_subjects/vpn_all_included_subjects.txt"
TEMPLATE_DIR="$INPUT_DIR/derivatives/mrtrix3/template"

mkdir -p "$TEMPLATE_DIR"
cd $TEMPLATE_DIR

##### STEP 2 #####
#           Fiber cross section in PRE vs POST           #
#####

#Pairwise comparison of fiber density in PRE vs POST.
DESIGN="$TEMPLATE_DIR/t_stat/1.PREvsPOST/design_matrix_PREvsPOST.txt"
CONTRAST="$TEMPLATE_DIR/t_stat/1.PREvsPOST/contrast_matrix.txt"
```

```
CONNECTIVITY="$TEMPLATE_DIR/matrix"

# Run fixelcfestats. In what fixels is log(FC) significantly smaller in the POST vs PRE group?
#fixelcfestats $TEMPLATE_DIR/log_fc_smooth
$TEMPLATE_DIR/t_stat/1.PREvsPOST/PREvsPOST_logfcfiles.txt $DESIGN $CONTRAST $CONNECTIVITY
$TEMPLATE_DIR/t_stat/stats_PREvsPOST_logfc -nthreads ${OMP_NUM_THREADS} -nshuffles 5000

#echo "==== COMPARISON OF FIBRE CROSS-SECTION in PRE vs POST COMPLETED SUCCESSFULLY
===="

##### STEP 3 #####
#           Fiber cross-section in PRE vs PERI           #
#####

#Pairwise comparisson of fiber density in PRE vs POST.
DESIGN="$TEMPLATE_DIR/t_stat/2.PREvsPERI/design_matrix_PREvsPERI.txt"
CONTRAST="$TEMPLATE_DIR/t_stat/2.PREvsPERI/contrast_matrix.txt"
CONNECTIVITY="$TEMPLATE_DIR/matrix"

# Run fixelcfestats. In what fixels is log(fc) significantly smaller in the POST vs PRE group?
#fixelcfestats $TEMPLATE_DIR/log_fc_smooth $TEMPLATE_DIR/t_stat/2.PREvsPERI/PREvsPERI_logfcfiles.txt
$DESIGN $CONTRAST $CONNECTIVITY $TEMPLATE_DIR/t_stat/stats_PREvsPERI_logfc -nthreads
${OMP_NUM_THREADS} -nshuffles 5000

#echo "==== COMPARISON OF FIBRE CROSS-SECTION in PRE vs PERI COMPLETED SUCCESSFULLY
===="

##### STEP 4 #####
#           Fiber cross-section in PERI vs POST           #
#####

#Pairwise comparisson of fiber density in PERI vs POST.
DESIGN="$TEMPLATE_DIR/t_stat/3.PERIvsPOST/design_matrix_PERIvsPOST.txt"
CONTRAST="$TEMPLATE_DIR/t_stat/3.PERIvsPOST/contrast_matrix.txt"
CONNECTIVITY="$TEMPLATE_DIR/matrix"

# Run fixelcfestats. In what fixels is log(fc) significantly smaller in the POST vs PERI group?
```

```
fixelcfstats $TEMPLATE_DIR/log_fc_smooth
$TEMPLATE_DIR/t_stat/3.PERIvsPOST/PERIvsPOST_logfcfiles.txt $DESIGN $CONTRAST $CONNECTIVITY
$TEMPLATE_DIR/t_stat/stats_PERIvsPOST_logfc -nthreads ${OMP_NUM_THREADS} -nshuffles 5000

echo "===== COMPARISON OF FIBRE CROSS-SECTION in PERI vs POST COMPLETED SUCCESSFULLY
====="
```

Appendix E: FBA_matrices.Rmd

```
library(readxl)

data <- read_excel("/Users/katoherregods/Documents/MASTERPROEF/Metadata/Tübingen/IRTG07_Final_Subject_List_DWI_dropouts_removed_MHT_included.xlsx")

#Mean-centering the covariates age and total intracranial volume
data$AGE_centered <- scale(data$AGE, scale = FALSE)

data$GROUP <- factor(data$GROUP)
data

## # A tibble: 66 × 9
##   ID          GROUP SESSION  MHT  AGE log_TIV    TIV Notes AGE_centered[,1]
##   <chr>         <fct>   <dbl> <dbl> <dbl>   <dbl>   <dbl> <chr>
##   <dbl>
## 1 IRTG07_007001 3         1      1    55    14.3 1.69e6 <NA>
## 7.18
## 2 IRTG07_007002 1         1      1    41    14.1 1.34e6 <NA>
## -6.82
## 3 IRTG07_007004 1         1      1    45    14.3 1.61e6 <NA>
## -2.82
## 4 IRTG07_007005 3         1      1    49    14.1 1.40e6 <NA>
## 1.18
## 5 IRTG07_007007 2         1      1    47    14.1 1.37e6 <NA>
## -0.818
## 6 IRTG07_007008 1         1      1    40    14.1 1.36e6 <NA>
## -7.82
## 7 IRTG07_007009 3         1      1    53    14.1 1.32e6 <NA>
## 5.18
## 8 IRTG07_007011 1         1      1    41    14.1 1.40e6 <NA>
## -6.82
## 9 IRTG07_007012 1         1      1    45    14.2 1.51e6 <NA>
## -2.82
## 10 IRTG07_007013 1         1      1    38    14.2 1.51e6 <NA>
## -9.82
## # i 56 more rows

#DESIGN MATRIX F-TEST FC and FDC
```

```
#Creating the design matrix for FC and FDC
design_matrix_FC_FDC <- model.matrix(~ GROUP + AGE_centered + log_TIV -1, data = data)
design_matrix_FC_FDC
```

##	GROUP1	GROUP2	GROUP3	AGE_centered	log_TIV
## 1	0	0	1	7.1818182	14.33995
## 2	1	0	0	-6.8181818	14.10597
## 3	1	0	0	-2.8181818	14.28982
## 4	0	0	1	1.1818182	14.14897
## 5	0	1	0	-0.8181818	14.13141
## 6	1	0	0	-7.8181818	14.12073
## 7	0	0	1	5.1818182	14.09512
## 8	1	0	0	-6.8181818	14.14906
## 9	1	0	0	-2.8181818	14.22625
## 10	1	0	0	-9.8181818	14.22995
## 11	1	0	0	-8.8181818	14.25508
## 12	0	0	1	6.1818182	14.12847
## 13	1	0	0	-9.8181818	14.26262
## 14	0	1	0	5.1818182	14.21141
## 15	1	0	0	-4.8181818	14.21583
## 16	0	0	1	1.1818182	14.14803
## 17	0	1	0	2.1818182	14.26987
## 18	0	0	1	6.1818182	14.23226
## 19	1	0	0	-3.8181818	14.28057
## 20	0	0	1	9.1818182	14.19796
## 21	0	1	0	2.1818182	14.33620
## 22	0	1	0	6.1818182	14.14132
## 23	0	0	1	7.1818182	14.33444
## 24	0	1	0	2.1818182	14.26049
## 25	1	0	0	-0.8181818	14.24468
## 26	0	0	1	11.1818182	14.12774
## 27	1	0	0	-5.8181818	14.11033
## 28	1	0	0	-8.8181818	14.35413
## 29	0	0	1	5.1818182	14.14553
## 30	0	1	0	0.1818182	14.30148
## 31	0	0	1	8.1818182	14.16885
## 32	0	1	0	1.1818182	14.28185
## 33	0	0	1	6.1818182	14.24530
## 34	1	0	0	-11.8181818	14.25006
## 35	1	0	0	-10.8181818	14.38500
## 36	1	0	0	-5.8181818	14.39924
## 37	0	1	0	-6.8181818	14.19816
## 38	0	0	1	11.1818182	14.30225
## 39	1	0	0	-12.8181818	14.14096
## 40	0	1	0	4.1818182	14.19177
## 41	0	1	0	7.1818182	14.22592
## 42	1	0	0	-9.8181818	14.30079
## 43	0	0	1	2.1818182	14.11516
## 44	0	1	0	11.1818182	14.24616
## 45	1	0	0	2.1818182	14.16944

```
## 46      0      0      1      4.1818182 14.10767
## 47      0      0      1     -0.8181818 14.29562
## 48      0      0      1      3.1818182 14.41516
## 49      0      0      1      8.1818182 14.27476
## 50      1      0      0     -3.8181818 14.23915
## 51      1      0      0      1.1818182 14.13846
## 52      0      0      1      7.1818182 14.27308
## 53      1      0      0     -8.8181818 14.26022
## 54      1      0      0     -6.8181818 14.16866
## 55      0      0      1      7.1818182 14.20821
## 56      1      0      0    -11.8181818 14.14055
## 57      1      0      0     -1.8181818 14.14159
## 58      1      0      0      3.1818182 14.24883
## 59      0      0      1      8.1818182 14.20132
## 60      0      0      1      8.1818182 14.23620
## 61      1      0      0     -1.8181818 14.24944
## 62      1      0      0     -0.8181818 14.20473
## 63      1      0      0     -6.8181818 14.08989
## 64      1      0      0     -7.8181818 14.16753
## 65      0      1      0      2.1818182 14.16415
## 66      0      1      0      5.1818182 14.16766
## attr(,"assign")
## [1] 1 1 1 2 3
## attr(,"contrasts")
## attr(,"contrasts")$GROUP
## [1] "contr.treatment"

#Export the design matrix to a .txt file (for Later use in mrtrix). Row num
bering, column headers and quotes around values are removed
write.table(design_matrix_FC_FDC,
            file = "/Users/katoherregods/Documents/MASTERPROEF/Statistics/
correction_age_TIV/design_matrix_FC_FDC.txt",
            row.names = FALSE,
            col.names = FALSE,
            quote = FALSE)

#CONTRAST MATRIX F-TEST FC and FDC

#Create a contrast matrix for FC en FDC
contrast_matrix_FC_FDC <- matrix(c(1,0,0,1,-1,-1,0,0,0,0), nrow = 2)
contrast_matrix_FC_FDC

##      [,1] [,2] [,3] [,4] [,5]
## [1,]    1    0   -1    0    0
## [2,]    0    1   -1    0    0

#Export the contrast matrix to a .txt file
write.table(contrast_matrix_FC_FDC,
            file = "/Users/katoherregods/Documents/MASTERPROEF/Statistics/
correction_age_TIV/contrast_matrix_FC_FDC.txt",
            row.names = FALSE,
```

```
col.names = FALSE,  
quote = FALSE)
```

#DESIGN MATRIX F-TEST FD

#Creating the design matrix. Only age included, TIV NOT

```
design_matrix_FD <- model.matrix(~ GROUP + AGE_centered -1, data = data)  
design_matrix_FD
```

##	GROUP1	GROUP2	GROUP3	AGE_centered
## 1	0	0	1	7.1818182
## 2	1	0	0	-6.8181818
## 3	1	0	0	-2.8181818
## 4	0	0	1	1.1818182
## 5	0	1	0	-0.8181818
## 6	1	0	0	-7.8181818
## 7	0	0	1	5.1818182
## 8	1	0	0	-6.8181818
## 9	1	0	0	-2.8181818
## 10	1	0	0	-9.8181818
## 11	1	0	0	-8.8181818
## 12	0	0	1	6.1818182
## 13	1	0	0	-9.8181818
## 14	0	1	0	5.1818182
## 15	1	0	0	-4.8181818
## 16	0	0	1	1.1818182
## 17	0	1	0	2.1818182
## 18	0	0	1	6.1818182
## 19	1	0	0	-3.8181818
## 20	0	0	1	9.1818182
## 21	0	1	0	2.1818182
## 22	0	1	0	6.1818182
## 23	0	0	1	7.1818182
## 24	0	1	0	2.1818182
## 25	1	0	0	-0.8181818
## 26	0	0	1	11.1818182
## 27	1	0	0	-5.8181818
## 28	1	0	0	-8.8181818
## 29	0	0	1	5.1818182
## 30	0	1	0	0.1818182
## 31	0	0	1	8.1818182
## 32	0	1	0	1.1818182
## 33	0	0	1	6.1818182
## 34	1	0	0	-11.8181818
## 35	1	0	0	-10.8181818
## 36	1	0	0	-5.8181818
## 37	0	1	0	-6.8181818
## 38	0	0	1	11.1818182
## 39	1	0	0	-12.8181818
## 40	0	1	0	4.1818182
## 41	0	1	0	7.1818182

```
## 42      1      0      0 -9.8181818
## 43      0      0      1  2.1818182
## 44      0      1      0 11.1818182
## 45      1      0      0  2.1818182
## 46      0      0      1  4.1818182
## 47      0      0      1 -0.8181818
## 48      0      0      1  3.1818182
## 49      0      0      1  8.1818182
## 50      1      0      0 -3.8181818
## 51      1      0      0  1.1818182
## 52      0      0      1  7.1818182
## 53      1      0      0 -8.8181818
## 54      1      0      0 -6.8181818
## 55      0      0      1  7.1818182
## 56      1      0      0 -11.8181818
## 57      1      0      0 -1.8181818
## 58      1      0      0  3.1818182
## 59      0      0      1  8.1818182
## 60      0      0      1  8.1818182
## 61      1      0      0 -1.8181818
## 62      1      0      0 -0.8181818
## 63      1      0      0 -6.8181818
## 64      1      0      0 -7.8181818
## 65      0      1      0  2.1818182
## 66      0      1      0  5.1818182
## attr("assign")
## [1] 1 1 1 2
## attr("contrasts")
## attr("contrasts")$GROUP
## [1] "contr.treatment"

#Export the design matrix to a .txt file (for later use in mrtrix). Row num
bering, column headers and quotes around values are removed
write.table(design_matrix_FD,
            file = "/Users/katoherregods/Documents/MASTERPROEF/Statistics/
correction_age_TIV/design_matrix_FD.txt",
            row.names = FALSE,
            col.names = FALSE,
            quote = FALSE)
```

#CONTRAST MATRIX FOR F-TEST FD

```
#Create a contrast matrix for FD
contrast_matrix_FD <- matrix(c(1,0,0,1,-1,-1,0,0), nrow = 2)
contrast_matrix_FD

##      [,1] [,2] [,3] [,4]
## [1,]    1    0   -1    0
## [2,]    0    1   -1    0

#Export the contrast matrix to a .txt file
write.table(contrast_matrix_FD,
```

```
file = "/Users/katoherregods/Documents/MASTERPROEF/Statistics/
correction_age_TIV/contrast_matrix_FD.txt",
row.names = FALSE,
col.names = FALSE,
quote = FALSE)
```

#DESIGN MATRIX FOR PAIRWISE COMPARISON LOG (FC) PREvsPOST

```
# Keep only participants from group 1 and group 3
data_subset <- subset(data, GROUP %in% c(1, 3))

# Make sure GROUP is treated as a factor with only levels 1 and 3
data_subset$GROUP <- factor(data_subset$GROUP, levels = c(1, 3))

# Create design matrix for comparing only group 1 vs 3
design_matrix_PREvsPOST <- model.matrix(
  ~ GROUP + AGE_centered + log_TIV ,
  data = data_subset
)

# Save design matrix to txt file
write.table(
  design_matrix_PREvsPOST,
  file = "design_matrix_PREvsPOST.txt",
  quote = FALSE,
  sep = "\t",
  row.names = FALSE,
  col.names = TRUE
)

# View design matrix
design_matrix_PREvsPOST
```

```
##      (Intercept) GROUP3 AGE_centered log_TIV
## 1             1      1    7.1818182 14.33995
## 2             1      0   -6.8181818 14.10597
## 3             1      0   -2.8181818 14.28982
## 4             1      1    1.1818182 14.14897
## 5             1      0   -7.8181818 14.12073
## 6             1      1    5.1818182 14.09512
## 7             1      0   -6.8181818 14.14906
## 8             1      0   -2.8181818 14.22625
## 9             1      0   -9.8181818 14.22995
## 10            1      0   -8.8181818 14.25508
## 11            1      1    6.1818182 14.12847
## 12            1      0   -9.8181818 14.26262
## 13            1      0   -4.8181818 14.21583
## 14            1      1    1.1818182 14.14803
## 15            1      1    6.1818182 14.23226
## 16            1      0   -3.8181818 14.28057
## 17            1      1    9.1818182 14.19796
```

```
## 18      1      1      7.1818182 14.33444
## 19      1      0     -0.8181818 14.24468
## 20      1      1     11.1818182 14.12774
## 21      1      0     -5.8181818 14.11033
## 22      1      0     -8.8181818 14.35413
## 23      1      1      5.1818182 14.14553
## 24      1      1      8.1818182 14.16885
## 25      1      1      6.1818182 14.24530
## 26      1      0    -11.8181818 14.25006
## 27      1      0    -10.8181818 14.38500
## 28      1      0     -5.8181818 14.39924
## 29      1      1     11.1818182 14.30225
## 30      1      0    -12.8181818 14.14096
## 31      1      0     -9.8181818 14.30079
## 32      1      1      2.1818182 14.11516
## 33      1      0      2.1818182 14.16944
## 34      1      1      4.1818182 14.10767
## 35      1      1     -0.8181818 14.29562
## 36      1      1      3.1818182 14.41516
## 37      1      1      8.1818182 14.27476
## 38      1      0     -3.8181818 14.23915
## 39      1      0      1.1818182 14.13846
## 40      1      1      7.1818182 14.27308
## 41      1      0     -8.8181818 14.26022
## 42      1      0     -6.8181818 14.16866
## 43      1      1      7.1818182 14.20821
## 44      1      0    -11.8181818 14.14055
## 45      1      0     -1.8181818 14.14159
## 46      1      0      3.1818182 14.24883
## 47      1      1      8.1818182 14.20132
## 48      1      1      8.1818182 14.23620
## 49      1      0     -1.8181818 14.24944
## 50      1      0     -0.8181818 14.20473
## 51      1      0     -6.8181818 14.08989
## 52      1      0     -7.8181818 14.16753
## attr(,"assign")
## [1] 0 1 2 3
## attr(,"contrasts")
## attr(,"contrasts")$GROUP
## [1] "contr.treatment"

#Export the design matrix to a .txt file
write.table(design_matrix_PREvsPOST,
            file = "/Users/katoherregods/Documents/MASTERPROEF/Statistics/
correction_age_TIV/design_matrix_PREvsPOST.txt",
            row.names = FALSE,
            col.names = FALSE,
            quote = FALSE)
```

#DESIGN MATRIX FOR PAIRWISE COMPARISON LOG (FC) PREvsPERI

```
# Keep only participants from group 1 and group 2
data_subset_2 <- subset(data, GROUP %in% c(1, 2))

# Make sure GROUP is treated as a factor with only levels 1 and 2
data_subset_2$GROUP <- factor(data_subset_2$GROUP, levels = c(1, 2))

# Create design matrix for comparing only group 1 vs 2
design_matrix_PREvsPERI <- model.matrix(
  ~ GROUP + AGE_centered + log_TIV ,
  data = data_subset_2
)

# Save design matrix to txt file
write.table(
  design_matrix_PREvsPERI,
  file = "design_matrix_PREvsPERI.txt",
  quote = FALSE,
  sep = "\t",
  row.names = FALSE,
  col.names = TRUE
)

# View design matrix
design_matrix_PREvsPERI
```

##	(Intercept)	GROUP2	AGE_centered	log_TIV
## 1	1	0	-6.8181818	14.10597
## 2	1	0	-2.8181818	14.28982
## 3	1	1	-0.8181818	14.13141
## 4	1	0	-7.8181818	14.12073
## 5	1	0	-6.8181818	14.14906
## 6	1	0	-2.8181818	14.22625
## 7	1	0	-9.8181818	14.22995
## 8	1	0	-8.8181818	14.25508
## 9	1	0	-9.8181818	14.26262
## 10	1	1	5.1818182	14.21141
## 11	1	0	-4.8181818	14.21583
## 12	1	1	2.1818182	14.26987
## 13	1	0	-3.8181818	14.28057
## 14	1	1	2.1818182	14.33620
## 15	1	1	6.1818182	14.14132
## 16	1	1	2.1818182	14.26049
## 17	1	0	-0.8181818	14.24468
## 18	1	0	-5.8181818	14.11033
## 19	1	0	-8.8181818	14.35413
## 20	1	1	0.1818182	14.30148
## 21	1	1	1.1818182	14.28185
## 22	1	0	-11.8181818	14.25006
## 23	1	0	-10.8181818	14.38500
## 24	1	0	-5.8181818	14.39924
## 25	1	1	-6.8181818	14.19816

```
## 26      1      0 -12.8181818 14.14096
## 27      1      1   4.1818182 14.19177
## 28      1      1   7.1818182 14.22592
## 29      1      0  -9.8181818 14.30079
## 30      1      1  11.1818182 14.24616
## 31      1      0   2.1818182 14.16944
## 32      1      0  -3.8181818 14.23915
## 33      1      0   1.1818182 14.13846
## 34      1      0  -8.8181818 14.26022
## 35      1      0  -6.8181818 14.16866
## 36      1      0 -11.8181818 14.14055
## 37      1      0  -1.8181818 14.14159
## 38      1      0   3.1818182 14.24883
## 39      1      0  -1.8181818 14.24944
## 40      1      0  -0.8181818 14.20473
## 41      1      0  -6.8181818 14.08989
## 42      1      0  -7.8181818 14.16753
## 43      1      1   2.1818182 14.16415
## 44      1      1   5.1818182 14.16766
## attr("assign")
## [1] 0 1 2 3
## attr("contrasts")
## attr("contrasts")$GROUP
## [1] "contr.treatment"

#Export the design matrix to a .txt file
write.table(design_matrix_PREvsPERI,
            file = "/Users/katoherregods/Documents/MASTERPROEF/Statistics/
correction_age_TIV/design_matrix_PREvsPERI.txt",
            row.names = FALSE,
            col.names = FALSE,
            quote = FALSE)
```

#DESIGN MATRIX FOR PAIRWISE COMPARISON LOG (FC) PERIvsPOST

```
# Keep only participants from group 2 and group 3
data_subset_3 <- subset(data, GROUP %in% c(2, 3))

# Make sure GROUP is treated as a factor with only levels 2 and 3
data_subset_3$GROUP <- factor(data_subset_3$GROUP, levels = c(2, 3))

# Create design matrix for comparing only group 2 vs 3
design_matrix_PERIvsPOST <- model.matrix(
  ~ GROUP + AGE_centered + log_TIV ,
  data = data_subset_3
)

# Save design matrix to txt file
write.table(
  design_matrix_PERIvsPOST,
  file = "design_matrix_PERIvsPOST.txt",
```

```
quote = FALSE,
sep = "\t",
row.names = FALSE,
col.names = TRUE
)

# View design matrix
design_matrix_PERIVsPOST

##      (Intercept) GROUP3 AGE_centered log_TIV
## 1             1      1    7.1818182 14.33995
## 2             1      1    1.1818182 14.14897
## 3             1      0   -0.8181818 14.13141
## 4             1      1    5.1818182 14.09512
## 5             1      1    6.1818182 14.12847
## 6             1      0    5.1818182 14.21141
## 7             1      1    1.1818182 14.14803
## 8             1      0    2.1818182 14.26987
## 9             1      1    6.1818182 14.23226
## 10            1      1    9.1818182 14.19796
## 11            1      0    2.1818182 14.33620
## 12            1      0    6.1818182 14.14132
## 13            1      1    7.1818182 14.33444
## 14            1      0    2.1818182 14.26049
## 15            1      1   11.1818182 14.12774
## 16            1      1    5.1818182 14.14553
## 17            1      0    0.1818182 14.30148
## 18            1      1    8.1818182 14.16885
## 19            1      0    1.1818182 14.28185
## 20            1      1    6.1818182 14.24530
## 21            1      0   -6.8181818 14.19816
## 22            1      1   11.1818182 14.30225
## 23            1      0    4.1818182 14.19177
## 24            1      0    7.1818182 14.22592
## 25            1      1    2.1818182 14.11516
## 26            1      0   11.1818182 14.24616
## 27            1      1    4.1818182 14.10767
## 28            1      1   -0.8181818 14.29562
## 29            1      1    3.1818182 14.41516
## 30            1      1    8.1818182 14.27476
## 31            1      1    7.1818182 14.27308
## 32            1      1    7.1818182 14.20821
## 33            1      1    8.1818182 14.20132
## 34            1      1    8.1818182 14.23620
## 35            1      0    2.1818182 14.16415
## 36            1      0    5.1818182 14.16766
## attr(,"assign")
## [1] 0 1 2 3
## attr(,"contrasts")
## attr(,"contrasts")$GROUP
## [1] "contr.treatment"
```

```
#Export the design matrix to a .txt file
write.table(design_matrix_PERIVsPOST,
            file = "/Users/katoherregods/Documents/MASTERPROEF/Statistics/
correction_age_TIV/design_matrix_PERIVsPOST.txt",
            row.names = FALSE,
            col.names = FALSE,
            quote = FALSE)
```

Appendix F: 09_freesurfer_reconall.sh

```
#!/bin/bash

# Written by Kato Herregods for IRTG, Tübingen, Germany 16.05.2026

set -euo pipefail

export OMP_NUM_THREADS=2
export MKL_NUM_THREADS=2
export OPENBLAS_NUM_THREADS=2

#To run the script follow these steps:
#cd /PATH/TO/09_freesurfer_reconall.sh
#chmod +x 09_freesurfer_reconall.sh
# ./09_freesurfer_reconall.sh

##### STEP 1 #####
#           Define PATHS           #
#####

INPUT_DIR="/home/kato/01_BIDS_IRTG07_crosssec"
SUBJECT_LIST="/home/kato/01_BIDS_IRTG07_crosssec/list_subjects/vpn_63_88.txt"

##### STEP 2 #####
#           Run recon-all on the T1mprage           #
#####

while read -r subject; do
```

```

echo "Performing freesurfer's recon-all for sub- $\{subject\}$  ..."

#Determine session
if [ -d "$INPUT_DIR/sub- $\{subject\}$ /ses-01" ]; then
    SESSION="ses-01"
elif [ -d "$INPUT_DIR/sub- $\{subject\}$ /ses-02" ]; then
    SESSION="ses-02"
fi

#By default, the output of recon-all is placed in the directory in which FreeSurfer was installed, called
SUBJECTS_DIR. For convenience, the output is redirected to the derivatives folder.
export SUBJECTS_DIR="$INPUT_DIR/derivatives/freesurfer"
mkdir -p "$SUBJECTS_DIR"

recon-all -s "sub- $\{subject\}$ " -i "$INPUT_DIR/sub- $\{subject\}$ /$SESSION/anat/sub-
 $\{subject\}$ _ $\{SESSION\}$ _T1w.nii.gz" -all

done < "$SUBJECT_LIST"

```

Appendix G: 09_freesurfer_TIV.sh

```

#Run this line in the terminal from the freesurfer directory

asegstats2table --subjects sub-007001 sub-007002 sub-007004 sub-007005 sub-007007 sub-007008 sub-
007009 sub-007011 sub-007012 sub-007013 sub-007014 sub-007017 sub-007018 sub-007020 sub-007021 sub-
007022 sub-007023 sub-007025 sub-007026 sub-007027 sub-007028 sub-007029 sub-007030 sub-007031 sub-
007032 sub-007033 sub-007035 sub-007037 sub-007038 sub-007040 sub-007042 sub-007044 sub-007045 sub-
007048 sub-007049 sub-007050 sub-007051 sub-007052 sub-007053 sub-007054 sub-007055 sub-007056 sub-
007058 sub-007059 sub-007060 sub-007061 sub-007062 sub-007063 sub-007067 sub-007069 sub-007070 sub-
007072 sub-007073 sub-007074 sub-007075 sub-007076 sub-007077 sub-007079 sub-007080 sub-007081 sub-
007082 sub-007083 sub-007085 sub-007086 sub-007087 sub-007088 --meas volume --tablefile aseg_stats.txt

```

Appendix H: 10_FA_analysis.sh

```

#!/bin/bash

set -euo pipefail

```

```
# Written by Andrew Jahn, University of Michigan, 02.25.2019
# Modified by Kato Herregods for IRTG, Tübingen, Germany

#To run the script follow these steps:
#cd PATH/TO/10_FA_analysis.sh
#chmod +x 10_FA_analysis.sh
# ./10_FA_analysis.sh

##### STEP 1 #####
#           Define PATHS           #
#####

INPUT_DIR="/home/kato/01_BIDS_IRTG07_crosssec"
SUBJECT_LIST="/home/kato/01_BIDS_IRTG07_crosssec/list_subjects/vpn_all_included_subjects_MHTincluded.txt"
TBSS_DIR="${INPUT_DIR}/derivatives/mrtrix3_DT/TBSS"

mkdir -p "${TBSS_DIR}"

##### STEP 2 #####
#           Comput FA maps           #
#####

while read -r subject; do
    echo "Computing FA for sub-${subject} ..."

    # Determine session
    if [ -d "$INPUT_DIR/derivatives/qsiprep/sub-${subject}/ses-01" ]; then
        SESSION="ses-01"
    elif [ -d "$INPUT_DIR/derivatives/qsiprep/sub-${subject}/ses-02" ]; then
        SESSION="ses-02"
    else
        echo "No session found for sub-${subject}"
        continue
    fi
```

```
# Input files
DWI="$INPUT_DIR/derivatives/mrtrix3/sub-`${subject}`/$SESSION/sub-`${subject}`_acq-
DGD103_preproc_dwi.mif"
MASK="$INPUT_DIR/derivatives/qsiprep/sub-`${subject}`/$SESSION/dwi/sub-`${subject}`_`${SESSION}`_acq-
DGD103_dir-AP_space-ACPC_desc-brain_mask.nii.gz"

# Output files
DT="$INPUT_DIR/derivatives/mrtrix3_DT/sub-`${subject}`/$SESSION/sub-`${subject}`_`${SESSION}`_dt.mif"
FA="$INPUT_DIR/derivatives/mrtrix3_DT/sub-`${subject}`/$SESSION/sub-`${subject}`_FA.mif"
FA_NII="$TBSS_DIR/sub-`${subject}`_FA.nii.gz"

mkdir -p "$INPUT_DIR/derivatives/mrtrix3_DT/sub-`${subject}`/$SESSION"

# Fit tensor
dwi2tensor \
    "$DWI" \
    "$DT" \
    -mask "$MASK" \
    -force

# Extract FA
tensor2metric \
    "$DT" \
    -fa "$FA" \
    -force

# Convert to NIfTI for TBSS
mrconvert \
    "$FA" \
    "$FA_NII" \
    -force

done < "$SUBJECT_LIST"

##### STEP 3 #####
#                               #
```

```
#####

echo "Running TBSS..."

cd "$TBSS_DIR"

tbss_1_preproc *.nii.gz
tbss_2_reg -T
tbss_3_postreg -S
tbss_4_prestats 0.2

##### STEP 3 #####
#      Run voxelwise statistics (randomise)      #
#####

cd "$INPUT_DIR"

Text2Vest "$INPUT_DIR"/design_matrix_FA.txt "$INPUT_DIR"/design_matrix_FA.mat
Text2Vest "$INPUT_DIR"/contrast_matrix_FA.txt "$INPUT_DIR"/contrast_matrix_FA.con
Text2Vest "$INPUT_DIR"/f_contrast_index_FA.txt "$INPUT_DIR"/f_contrast_index_FA.fts

echo "Running voxelwise statistics..."

cd "$TBSS_DIR/stats"

# Requires:
# design.mat
# design.con
# design.fts

randomise \
  -i all_FA_skeletonised.nii.gz \
  -o menopause_FA \
  -m mean_FA_skeleton_mask.nii.gz \
  -d "$INPUT_DIR/design_matrix_FA.mat" \
  -t "$INPUT_DIR/contrast_matrix_FA.con" \
```

```
-f "$INPUT_DIR/f_contrast_index_FA.fts" \
-n 5000 \
--T2

echo "Analysis complete."
echo "Results saved in."
echo "$TBSS_DIR/stats/"
```

Appendix I: 11_qsirecon_IRTG.sh

```
#!/bin/bash

set -euo pipefail

# Written by Kato Herregods for IRTG, Tübingen, Germany 16.05.2026

#To execute the script, run following commands in the terminal:
#cd PATH/TO/07_qsirecon_IRTG.sh
#chmod +x 07_qsirecon_IRTG.sh
# ./07_qsirecon_IRTG.sh

# Script: Run QSirecon 1.1.2 on preprocessed BIDS dataset.
# The script expects multishell diffusion data. If both single and multishell were acquired, it is recommended to
use the BIDS_FILTER argument,
# specifying to only process multishell data.
#
# The script will generate response functions and fiber orientation distribution (FOD) for white matter, gray matter
and cerebrospinal fluid using the mrtrix_multishell_msmt_ACT-hsvs workflow.
# It will also generate a MATLAB format .mat file containing connectivity matrices for the selected atlases, in this
case the Brainnetome246Ext atlas

# TEMPLATEFLOW_HOME is needed to retrieve output space maps, as internet access within clinic network is
difficult, instead it refers to locally stored maps on the server. Make sure that you initialized this path once in your
terminal, after that it will be saved for the future:

# export TEMPLATEFLOW_HOME=/nic/sw/IRTG/templateflow
# export APPTAINERENV_TEMPLATEFLOW_HOME=/templateflow
```

```
#
# Freesurfer (FS) must have have run for FS ouputs to be integrated into the recon analysis.
# If no FS outputs are found, QSIrecon will run without FS output integration.
# -----

# The script:
# - performs multi-shell, multi-tissue, CSD of preprocessed diffusion weighted images using QSIrecon 1.1.2.dev0

# Ensure the script is executed, not sourced
[[ "${BASH_SOURCE[0]}" != "${0}" ]] && { echo "Please don't source but execute me!"; return; }

# Define Apptainer/Singularity path
APTAINER_IMG="/nic/sw/IRTG/sif/qsirecon-1.1.2.dev0.sif"
FREESURFER_PATH="/nic/sw/FreeSurfer"
FS_LICENSE="/nic/sw/FreeSurfer/license.txt" # Path to FreeSurfer license file

# Default values (can be overridden)
: ${NPROCS:=16} #NPROCS = number of processes/parallel jobs
: ${OMP_THREADS:=4} #Number of threads = Virtual cores created through a technology calles simultaneous
multithreading (SMT)
: ${MEM_GB:=16n}

# Function to display usage instructions when called from the command line
# This function explains how to run the script, what arguments it expects and what each option means.
# The usage function's purpose is to help users understand how to use the script correctly.

function Usage(){
    echo
    echo "Usage: ${0##*/} [OPTIONS] INPUT_DIR OUTPUT_DIR"
    echo
    echo " -n NPROCS      Number of processes (default: $NPROCS)"
    echo " -t OMP_THREADS Number of threads per process (default: $OMP_THREADS)"
    echo "
        Alternatively, NPROC, OMP_THREADS and MEM_GB can be set via the command line
        before running this script using: 'export NPROCS=X' and 'export OMP_THREADS=X'"
}
```

```

echo " -v VPN_FILE    File with participants to be processed (optional, e.g.
\"/p/irtg/IRTGXX/00_Codes_IRTGXX/vpn_lists/vpn1.txt)\", default: processes all unprocessed participants"

echo " -f BIDS_FILTER  BIDS filter for selecting specific datatypes and/or sessions. Either enter your file path
or type \"ses01\" to only process first session and \"ses02\" for second session"

echo

echo "Recommended INPUT_DIR is \"/p/irtg/IRTGXX/01_BIDS_IRTGXX/derivatives/qsiprep\""
echo "Recommended OUTPUT_DIR is \"/p/irtg/IRTGXX/01_BIDS_IRTGXX/derivatives/qsirecon\""

echo "INPUT_DIR and OUTPUT_DIR are mandatory. OUTPUT_DIR is used as base folder for logfiles
(default: <OUTPUT_DIR>/logs)"

echo "$1"
}

# Parse command-line arguments
# getopt is a built-in Bash tool. It automatically reads and interprets flags and arguments that the user types on
the command line.

while getopt ":hn:t:v:f:" OPT; do
    case $OPT in
        # Option -h: Show usage and exit
        h ) Usage; exit 0 ;;

        # Option -n: Adapt number of processes
        n ) NPROCS="$OPTARG" ;;

        # Option -t: Adapt number of threads per process
        t ) OMP_THREADS="$OPTARG" ;;

        # Option --participant-label: add vpn_list to only process specific VPs
        v ) VPN_FILE="$OPTARG" ;;

        # Option -f: add BIDS filter to only process specific sessions and/or datatypes.
        f ) BIDS_FILTER="$OPTARG" ;;

        # Catch invalid options or missing arguments
        ? ) echo "${0##*/}: unexpected option \"-$OPTARG\" or missing argument"; exit 1 ;;

    esac
done

# Shift arguments to move past the options as OPTIND will point to the first non-optional argument.
# After this shift, $1 becomes the first required argument (INPUT_DIR) and $2 becomes the second required
argument (OUTPUT_DIR)

```

```
## The script can now safely use $1, $2 ... for non-optional arguments. The code is used to move past the options
so the rest of the script can focus on the main input arguments.
shift $((OPTIND - 1))

# Ensure INPUT_DIR is provided.
if [ $# -lt 2 ]; then

    Usage "Need INPUT_DIR and OUTPUT_DIR arguments!"
    exit 1
else
    # Set INPUT_DIR and OUTPUT_DIR to the absolute path of the given argument
    INPUT_DIR="$(realpath $1)" ## Take the first argument passed to the script (here the input folder) and convert
it into a real path using 'realpath'. If for example the user type ./data, it is now turned into /home/user/project/data.
This path is then stored in the variable INPUT_DIR
    OUTPUT_DIR="$(realpath $2)"
    echo "Processing input directory: $INPUT_DIR" ## The input directory is now printed to the terminal
    echo "Processing output directory: $OUTPUT_DIR"
fi

# Check if INPUT_DIR and OUTPUT_DIR exists
[[ -d $INPUT_DIR ]] || { echo "${0##*/}: $INPUT_DIR not found"; exit 1; }
[[ -d $OUTPUT_DIR ]] || { echo "${0##*/}: $OUTPUT_DIR not found"; exit 1; }

# Define paths based on INPUT_DIR and OUTPUT_DIR
WORK_DIR="$(realpath "$INPUT_DIR/./work/qsirecon_work")"
LOG_DIR="$OUTPUT_DIR/logs"

# Create necessary directories
echo mkdir -p "$WORK_DIR" "$LOG_DIR"
mkdir -p "$WORK_DIR" "$LOG_DIR"

# Make sure the aptainer can find the BIDS_FILTER file
BIDS_FILTER=$(realpath "$BIDS_FILTER")
BIDS_FILTER_DIR=$(dirname "$BIDS_FILTER")
BIDS_FILTER_FILE=$(basename "$BIDS_FILTER")
```

```
# Check whether the environment variable TEMPLATEFLOW_HOME is set. If it is not set, it will assign a
default fallback value.
if [ -z "$TEMPLATEFLOW_HOME" ]; then
    export TEMPLATEFLOW_HOME="/nic/sw/IRTG/templateflow" #Only executed if TEMPLATE_FLOW is empty.
Export makes the variable visible to qsirecon
    echo "TEMPLATEFLOW_HOME was not set. Using default: \"$TEMPLATEFLOW_HOME\""
fi

# Teach qsiprep container to not use its own (almost empty) template repository
if [ -z $APTAINERENV_TEMPLATEFLOW_HOME ]; then
    export APTAINERENV_TEMPLATEFLOW_HOME="/templateflow"
    echo "APTAINERENV_TEMPLATEFLOW_HOME was not set. Setting to: \"/templateflow\"
fi

# Check if vpn.txt exists and read subject list
PARTICIPANT_LABELS=()

if [[ -f "$VPN_FILE" ]]; then
    # Check for Windows-style line endings before converting
    if grep -q $'\r' "$VPN_FILE"; then
        if command -v dos2unix &> /dev/null; then
            dos2unix "$VPN_FILE"
        else
            echo "Warning: dos2unix not found. Attempting manual conversion."
            sed -i 's/\r$//' "$VPN_FILE"
        fi
    fi

    # Read file into array and remove "sub-" prefix
    mapfile -t PARTICIPANT_LABELS < <(sed 's/^sub-/' "$VPN_FILE")
    echo "Using participants from $VPN_FILE: ${PARTICIPANT_LABELS[@]}"
else
    # Get the list of subjects from INPUT_DIR if no VPN file is provided
    mapfile -t PARTICIPANT_LABELS < <(find "$INPUT_DIR" -maxdepth 1 -type d -name "sub-*" | sed
's|^.*sub-||' | sort -n)
```

```
# Check if subjects were found
if [ ${#PARTICIPANT_LABELS[@]} -eq 0 ]; then
    echo "Error: No subjects found in $INPUT_DIR. Check if the dataset is correctly structured."
    exit 1
fi

echo "Subjects found: ${PARTICIPANT_LABELS[@]}"
fi

# Convert array to space-separated string
PARTICIPANT_LABELS_STR="${PARTICIPANT_LABELS[@]}"

# Check if Freesurfer output exists and conditionally define FS_ARG as argument to pass to the flag: '--fs-
subjects-dir'.
FS_DIR="$(realpath "$INPUT_DIR/..fmrip/sourcedata/freesurfer")"

if [[ -d "$FS_DIR" ]]; then
    FS_ARG="--fs-subjects-dir $FS_DIR"
    echo "FS_DIR found. QSIrecon will run with FreeSurfer integration"
else
    FS_ARG=""
    echo "Warning: FS_DIR does not exist. QSIrecon will run without FreeSurfer integration."
fi

# Start loop for QSIrecon
echo "Starting QSIrecon..."

echo "FS_DIR = $FS_DIR"

echo "BIDS_FILTER = $BIDS_FILTER"

#####
#           Run QSIrecon in aptainer           #
#####

# Loop over each subject/participant label (you can define PARTICIPANT_LABELS_STR with your list)
```

```

for subject in $PARTICIPANT_LABELS_STR; do
    # Display the participant label being processed
    echo "Processing participant: $subject"

qsirecon_failed=0
{
    aptainer run \
        --containall \
        --writable-tmpfs \
        -B "$TEMPLATEFLOW_HOME:$APPTAINERENV_TEMPLATEFLOW_HOME" \
        -B "$WORK_DIR:/work_dir" \
        -B "$INPUT_DIR:/input_dir" \
        -B "$OUTPUT_DIR:/output_dir" \
        -B "$FREESURFER_PATH:$FREESURFER_PATH" \
        -B "$FS_DIR:$FS_DIR" \
        -B "$BIDS_FILTER_DIR:/filters" \
        $APPTAINER_IMG \
        "/input_dir" \
        "/output_dir" \
        participant \
        ${PARTICIPANT_LABELS_STR:+--participant-label $subject} \
        --input-type qsiprep \
        --fs-license-file "$FS_LICENSE" \
        $FS_ARG \
        --work-dir "/work_dir" \
        --recon-spec mrtrix_multishell_msmr_ACT-hsvs \
        -v -v \
        --stop-on-first-crash \
        --atlases Brainnetome246Ext \
        --bids-filter-file "/filters/$BIDS_FILTER_FILE" \
        --nprocs "$NPROCS" \
        --omp-nthreads "$OMP_THREADS" \
        1> >(tee -a "$LOG_DIR/sub-${subject}_${date +"%Y-%m-%d-%H-%M"}_out.log") \
        2> >(tee >(grep -i -e "warning" -e "error" >> "$LOG_DIR/sub-${subject}_${date +"%Y-%m-%d-%H-%M"}_err.log") >&2);
    } || qsirecon_failed=1

```

```
done

# If qsirecon failed we need to know about it - upload the log to outputs
if [ ${qsirecon_failed} -gt 0 ]; then
    echo QSIPRECONFAIL
else
    echo QSIRECONSUCCESS
fi
```