Brain function and glucocorticoids in obesity and type 2 diabetes including effects of lifestyle interventions

Andreas Stomby
A small step for science, a giant leap for me
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Abstract

**Background** Obesity and associated metabolic dysregulation are linked to impaired cognitive function and alterations in brain structure, which increases the risk of age-related dementia. Increased glucocorticoid (GC) exposure may be a potential mediator of these negative effects on the brain.

**Methods and results** In paper 1, we tested the relationship between cortisol levels, brain morphology and cognitive function in 200 women and men. Salivary cortisol levels were negatively related to cortical surface areas in prefrontal brain regions in both sexes. In participants with type 2 diabetes, high salivary cortisol levels were associated with lower memory performance. In paper 2, we tested in 70 overweight women the effects on tissue-specific GC metabolism of a Paleolithic diet or a diet following the Nordic nutrition recommendations. The 24-month interventions led to decreased expression of the GC-activating enzyme 11βHSD1 in adipose tissue, interpreted as a normalization of an obesity-related disturbance in GC metabolism. Furthermore, GC metabolism by 5α-reductase increased substantially after 2 years, an unexpected and novel result. The outcomes did not differ by diet. In paper 3, 20 women included in paper 2 were examined with functional magnetic resonance imaging (fMRI) while performing a memory task at baseline and after 6 months. Memory performance improved and functional brain responses increased in the hippocampus. Once again, the results were similar in both diet groups. In paper 4, 24 overweight participants with type 2 diabetes were examined with fMRI, using the same memory test as in paper 3, at baseline and after 12 weeks of intervention with a Paleolithic diet with or without exercise training. Functional brain response increased in the hippocampus, but memory was not improved. The addition of physical exercise did not alter the results.

**Conclusion** Cortisol levels are linked to prefrontal brain structure and, at least in type 2 diabetes, lower memory performance. Furthermore, the dysregulated GC metabolism in obesity can be reversed by long-term diet-induced weight loss. Finally, dietary interventions with associated metabolic improvements alter functional brain responses during memory testing, including increased activation of the hippocampus. Whether these changes are linked to alterations in GC exposure and mediate improved cognition requires further study.

**Keywords** Obesity, type 2 diabetes, glucocorticoid, cortisol, 11beta hydroxysteroid dehydrogenase type 1, episodic memory, functional magnetic resonance imaging, paleolithic diet, exercise
Original papers


## Abbreviations

<table>
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<tr>
<td>11βHSD</td>
<td>11beta hydroxysteroid dehydrogenase</td>
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<tr>
<td>5α-R1</td>
<td>5α-reductase type 1</td>
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<tr>
<td>5α-R2</td>
<td>5α-reductase type 2</td>
</tr>
<tr>
<td>ACTH</td>
<td>Adrenocorticotropic hormone</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
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<td>AUC</td>
<td>Area under the curve</td>
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<tr>
<td>BDNF</td>
<td>Brain-derived neurotrophic factor</td>
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<td>BMI</td>
<td>Body mass index (kg/m²)</td>
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<td>BOLD</td>
<td>Blood oxygen level-dependent signal</td>
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<td>CRH</td>
<td>Corticotropin-releasing hormone</td>
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<td>CVD</td>
<td>Cardiovascular disease</td>
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<td>fMRI</td>
<td>Functional magnetic resonance imaging</td>
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<td>GC</td>
<td>Glucocorticoid</td>
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<td>GR</td>
<td>Glucocorticoid receptor</td>
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<td>HPAA</td>
<td>Hypothalamic-pituitary-adrenal axis</td>
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<td>MNI</td>
<td>Montreal Neurological Institute</td>
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<td>MR</td>
<td>Mineralocorticoid receptor</td>
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<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<td>NNR</td>
<td>Nordic Nutrition Recommendations</td>
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<td>OG</td>
<td>Observational group</td>
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<tr>
<td>PD</td>
<td>Paleolithic diet</td>
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<tr>
<td>PD-EX</td>
<td>Paleolithic diet combined with high-intensity exercise</td>
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<tr>
<td>PUFA</td>
<td>Polyunsaturated fatty acids</td>
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<td>ROI</td>
<td>Region of interest</td>
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<td>SFA</td>
<td>Saturated fatty acids</td>
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<td>T2DM</td>
<td>Type 2 diabetes mellitus</td>
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<tr>
<td>THE</td>
<td>Tetrahydrocortisone</td>
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<td>THF</td>
<td>Tetrahydrocortisol</td>
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Populärvetenskaplig sammanfattning


Stresshormonet kortisol produceras i binjurarna och är nödvändigt för att kunna reagera på och tolerera yttre påfrestningar i form av psykisk eller fysisk stress. I det långa loppen leder dock förhöjda kortisolnivåer till omfattande negativa effekter på kroppen såsom bukfetma, nedsatt känslighet för hormonet insulin (förstadium till typ 2-diabetes) och högt blodtryck. Dessutom kan förhöjda kortisolnivåer orsaka försämrad minne samt förändrad hjärnstruktur som är mycket viktiga för minnesfunktion – de främre delarna av hjärnan (prefrontalcortex) samt en del mitt i hjärnan kallad hippocampus.

Fetma och framför allt typ 2-diabetes är kopplat till en ökad kortisolproduktion. Dessutom ökar aktivering av inaktivt kortison till aktivt kortisol i fettväven vid fetma och typ 2-diabetes. Dessa effekter leder till en ökad exponering för stresshormonet kortisol och har föreslagits kunna vara en orsak till att fetma orsakar livsstilssjukdomar och att dessa i sin tur ökar risken att drabbas av demens.

Målet med denna avhandling var att undersöka om förändrade nivåer av stresshormonet kortisol kan vara en länk mellan livsstilssjukdomar, försämrad minnesfunktion och förändringar i hjärnans struktur. Dessutom undersöktes hur omsättningen av kortisol påverkas av en traditionell kost enligt de nordiska näringsrekommendationerna eller en modifierad stenålderskost. Till sist har vi undersökt om hjärnans aktiveringsmönster i samband med minnestestning förändrades av att följa dessa dieter.

Studie 1 inkluderade 200 kvinnor och män som ett representativt urval från befolkningen. Vi fann att högre kortisolnivåer i salivprover var relaterat till mindre yta i flera delar av prefrontalcortex. Dessutom var högre kortisolnivåer associerade med sämre arbetsminne hos män, men inte hos kvinnor, samt sämre långtidsminne hos personer med typ 2 diabetes.

Studie 2 inkluderade 70 överviktiga kvinnor som genomgått klimakteriet. Dessa kvinnor åt en modifierad stenålderskost eller en traditionell kost enligt de nordiska näringsrekommendationerna i två år. Båda dieterna orsakade en kraftig viktminskning som kunde kopplas till minskade nivåer av enzymet 11βHSD1 vilket aktiverar inaktivt kortison till aktivt kortisol i
fettväv. Denna minskning kan leda till förbättrad balans i ämnesomsättningen eftersom fettväven exponeras för lägre nivåer av kortisol.

I studie 3 undersöktes tjugo individer från studie 2 med funktionell magnetkameraavbildning av hjärnan och ett minnestest innan studien och efter 6 månader. Efter att ha följt dieterna ökade hjärnaktiveringen i flertalet hjärnregioner, däribland hippocampus, vid minnesinkodning. Minnesfunktionen förbättrades; denna förbättring kan dock ha orsakats av att deltagarna utförde samma test två gånger.


Sammanfattningsvis stärker denna avhandling hypotesen att stresshormonet kortisol kan vara en viktig länk mellan fetma, typ 2-diabetes och förändrad hjärnfunktion. Dessutom talar resultaten för att omsättningen av kortisol och hjärnans funktion kan förbättras av livsstilsförändringar. Om dessa förändringar kan ha gynnsamma effekter på minnesfunktion över tid och därmed minska risken att drabbas av demenssjukdom bör studieras vidare.
Background

Obesity and type 2 diabetes

During the last century, the major risk factors for disease and death have shifted from poverty, undernutrition, and infections to obesity, diabetes, and physical inactivity (1). A remarkable improvement in living conditions has thus opened the way to a new collection of diseases to prevent and treat to avoid a deterioration of health.

These diseases and risk factors are not confined to the developed parts of the world. During the last four decades, people in both high- and low-income countries have gained weight, and the largest increases in body mass index (BMI) have occurred primarily in low-income countries. The result is a doubled prevalence of obesity from 1980–2008, increasing from 5 to 10% in men and from 8 to 14% in women (2). In northern Sweden, the general trends are in line with global data; from 1986 to 2004, the prevalence of obesity doubled to 21% among men and women (3).

Obesity substantially increases the risk of developing type 2 diabetes mellitus (T2DM) (4). Insulin resistance is the primary feature of T2DM, which subsequently leads to a failure of the pancreas to produce sufficient insulin and thus to rising blood glucose levels. The estimated number of individuals with diabetes globally ranges from about 300 to 350 million, or 6.4 to 7% (5, 6). About 80 to 90% of those affected are estimated to have T2DM. The prevalence has increased substantially since 1980, mainly because of aging and population growth but also because of an increased age-specific prevalence of diabetes. T2DM is a major health concern today and will probably become even more important given that as many as 440 million people globally (7.7%) may have diabetes in 2030 (5, 6). In northern Sweden, the prevalence of self-reported diabetes has been stable at around 5–10% in older women and 10–15% in older men. The increased obesity prevalence in that region has thus not led to the expected higher prevalence of diabetes. This apparent conundrum may be explained by improvement in other risk factors for diabetes such as increased physical activity, improved dietary composition, and a lower rate of smoking, and provides evidence that the T2DM epidemic can possibly be limited by lifestyle modification (7). However, blood glucose levels have increased slightly, which may indicate that we face an eventual increase in T2DM in northern Sweden, as well (8).

Obesity and T2DM, combined with hypertension and hyperlipidemia, are the most important risk factors for cardiovascular disease (CVD) (9), the leading cause of death globally (10). In addition, obesity increases the risk of cancer (11) and musculoskeletal disease (12) whereas T2DM is the leading cause of blindness, lower limb amputations, and end-stage kidney disease
(10, 13). Of note, both obesity and T2DM increase the risk of dementia, which is important because additive effects with aging may increase the future prevalence of dementia substantially (14). The rationale is thus obvious for studying potential mechanisms linking obesity to metabolic dysregulation and neurodegenerative diseases and for identifying potential means of treatment.

**The brain in obesity and T2DM**

Negative effects on brain function in obesity and metabolic dysregulation have been increasingly acknowledged during the last decade. Accordingly, obesity and T2DM as well as lifestyle modifications with increased physical activity and healthy diets were recently proposed as representing important modifiable factors that can prevent a future rise in dementia (15).

The tight association between obesity and metabolic dysregulation such as hypertension, dyslipidemia, and insulin resistance, which ultimately leads to T2DM, has to be taken into consideration when interpreting epidemiological data on cognitive function in obesity because the metabolic dysregulations alone may have negative effects on the brain (16). Nevertheless, although these vascular risk factors mediate some of the relationship, obesity, and especially central obesity, during midlife is independently related to lowered cognitive performance with aging. The most consistently affected cognitive domains are processing speed, verbal memory, executive function, and cognitive flexibility (17-22). This relationship is a bit inconsistent, however, and in some cohorts, midlife obesity has not been linked to cognition later in life (21, 22). Of note, in individuals >65 years of age, a high BMI may be beneficial with respect to cognitive function. This inconsistency may be explained by weight loss in the early phase of dementia as well as altered body composition with aging (21, 22).

T2DM can be considered the most severe metabolic dysregulation associated with obesity and has major negative impacts throughout the body, including the brain. Several cognitive domains such as processing speed, attention, verbal memory, cognitive flexibility, and verbal fluency are impaired in T2DM during midlife and aging (21, 23-25). The magnitude of these impairments is about -0.3 to -0.5 standard deviations and thus relatively small compared to cut-off levels used in a clinical diagnosis of minor cognitive impairment, typically -1.65 standard deviations (26). In addition, these impairments seem to appear early in the progression of T2DM, and the rate of cognitive decline in T2DM is only slightly increased compared to healthy individuals (27-29). To what extent these impairments can be prevented or even reversed and whether doing so decreases the dementia risk are reasonable questions.
Because obesity, T2DM, and cognitive impairments share common risk factors, such as low socioeconomic status (30), it cannot be excluded that the direction of the relationship among obesity, T2DM, and impaired cognition is reversed, i.e., that individuals with a lower cognitive performance are at increased risk of developing obesity and T2DM (31). In line with this, obesity, the metabolic syndrome, and T2DM have been shown to be already associated with altered brain function and structure in childhood and adolescence (31-33).

**Dementia risk in obesity and metabolic dysfunction**

Individuals with a high cognitive reserve, such as that related to a high education or cognitively demanding work, have a lowered risk of dementia. The reason may be the ability to cope with larger impairments in cognition before reaching a dementia threshold (34). Furthermore, the concept of brain maintenance suggests that individuals unaffected by age-related brain changes and pathologies are less likely to develop dementia (35). In line with these concepts, the cognitive impairments and brain changes seen in obesity and metabolic dysfunction should associate with an increased prevalence of dementia, which seems to be the case.

Indeed, midlife obesity, especially when associated with waist circumference, increases by about 50 to 100% the risk of developing dementia with aging (36-45). This association remains significant after correction for multiple cardiovascular risk factors. In addition, among older individuals at around 70 years of age, the association is reversed, i.e., a low BMI is related to increased dementia risk; however, this risk may relate to altered body composition because a large waist circumference remains associated with increased dementia risk in the elderly independent of BMI (44). Notably, a recent study including data from 2 million patients suggested that the risk of dementia may actually be lower in those with a higher BMI in midlife (46). Thus, the last word has definitely not been said regarding the putative negative effects of obesity on brain health.

When obesity is accompanied by T2DM, the risk of dementia increases even further, and this association is probably true regardless of whether or not T2DM is present during midlife or in later years (25, 36, 38, 47, 48). Accordingly, obesity and T2DM have been listed as two of the most important targets to decrease the burden of dementias in the future (26).

**Brain imaging in obesity and metabolic dysfunction**

Obesity in midlife is associated with lower total brain volume and total gray matter volume (49, 50). However, both negative and positive correlations between gray matter volume and BMI have been found for different parts of
the frontal lobe, temporal lobe, and cerebellum (50). Midlife obesity may also increase the rate of brain volume loss and cortical thinning of the temporal and prefrontal cortex with aging (51, 52). The same pattern seems to be present in elderly individuals with and without Alzheimer’s disease, with the most consistent findings of a negative association with the volume of the prefrontal cortex and medial temporal lobes (53-57).

With the increasing metabolic dysfunction that T2DM represents, alterations in brain morphology are even more pronounced (see (58) for review). Thus, reductions in total brain volume, increased global, cortical and subcortical atrophy, increased prevalence of white matter hyperintensities, and altered white matter microstructure have been reported (59-65). This atrophy seems more pronounced in the hippocampus (66-69), although not in all studies (62). Specific effects on hippocampal volume are of particular interest because hippocampal atrophy precedes the development of Alzheimer’s disease (70).

The majority of functional brain imaging in obesity has been done with an emphasis on the regulation of appetite, focusing on the dopaminergic reward system and appetite control in relation to either food intake or presentation of food pictures (71). A recent study has, however, suggested that compared to normal-weight women, severely obese women have increased glucose metabolism in the posterior cingulate gyrus and anterior cerebellum, as measured with positron emission tomography. After gastric bypass and major weight loss, glucose metabolism decreased in these brain areas. Concomitantly, executive function improved, but the effect of repeated testing was not controlled for (72).

Functional magnetic resonance imaging (fMRI) in T2DM has revealed altered connectivity among several brain regions included in the default mode network (73-77). These alterations have mainly consisted of decreased connectivity between the hippocampus and medial and lateral prefrontal cortex, temporal cortex, and lingual gyrus (74, 77). However, some results have been contradictory, suggesting instead increased connectivity in the medial frontal cortex and lingual gyrus (75, 76). Of interest, intranasal insulin administration has reversed some of these alterations in connectivity in T2DM (78). Nevertheless, studies are lacking that have tested the effects of lifestyle interventions on functional brain responses in obesity and T2DM.

**Altered brain structure and function form a basis for cognitive impairments**

As stated above, the most consistent findings from brain imaging in obesity and T2DM are reduced volume of the prefrontal cortex and hippocampus, reduced functional connectivity between parts of these brain regions, and altered white matter microstructure. Executive function is one of the most
consistently affected cognitive domains in obesity and T2DM. Executive function is often considered to modulate other cognitive processes to achieve flexible and goal-directed behavior. Consequently, several cognitive domains rely on maintained executive performance. Of interest, the prefrontal cortex is a key region for executive function and may thus link alterations in brain morphology to cognitive impairments in obesity and T2DM. Accordingly, reduced orbitofrontal volume has been negatively related to BMI and positively related to executive function in obese females (53). However, others have found reduced volume of the left dorsolateral prefrontal cortex and lower executive performance (measured with trail-making test B) in obese individuals, without the expected association between these abnormalities (57).

In T2DM, the relationship between alterations in cognitive function and findings from brain imaging is more consistent. Cortical and subcortical brain atrophy and white matter lesions relate to executive performance and information processing speed (79). In addition, microstructural abnormalities in white matter, measured with diffusion tensor imaging in T2DM, correlate with slower processing speed and lower memory performance (64, 65). The hippocampus has been a major focus for imaging studies in T2DM, and hippocampal atrophy has been suggested to form the basis for memory impairments in metabolic disease. However, the evidence for a mediating effect of decreased hippocampal volume on memory performance in T2DM is inconsistent (67, 80, 81).

**Putative links among obesity, metabolic dysfunction, and brain health**

Many potential mechanisms are possible to explain how obesity and associated metabolic dysregulations could exert these negative effects on brain structure and function. The most obvious may be the increased risk of macrovascular lesions, causing lacunar infarcts and major strokes, which substantially increases the risk of vascular dementia (82). Microvascular disease in T2DM may also be important because the degree of retinopathy predicts cognitive impairment (83). Of note, however, brain imaging has not confirmed an increased number of microvascular lesions in T2DM (84).

Substances produced in fat may also contribute to brain dysfunction. Adipose tissue produces hundreds of polypeptides with autocrine, paracrine, and endocrine functions, called adipokines (85). These adipokines can have direct effects on the brain, providing a possible link among obesity, cognitive impairment, and dementia (86-88). In elderly people, high leptin levels are associated with a lower incidence of dementia and cognitive decline (89), which may be attributed to increased long-term potentiation and synaptic plasticity in the hippocampus (90). However, this protective effect may not
be present in obese elderly persons, perhaps because of leptin resistance (91, 92).

Adipose tissue also secretes pro-inflammatory cytokines, and obesity consequently is considered a state of low-grade chronic inflammation (93). This inflammatory process, estimated by increased levels of circulating C-reactive protein, interleukins 1 and 6, and tumor necrosis factor alpha, increases the rate of general cognitive decline and the risk of Alzheimer’s disease (94, 95). Reduced hippocampal volume has been suggested as a possible mediator of these inflammation-related impairments (96). Furthermore, inflammation in obese people may damage the blood–brain barrier, which in itself increases the risk of dementia (97, 98).

Insulin does not regulate glucose metabolism in the brain (99) but still acts as an important neuropeptide, and insulin receptors are present in both cortical and subcortical brain regions. One main function of insulin is suppression of appetite via hypothalamic signaling (100). However, other structures such as the hippocampus have a high density of insulin receptors, and insulin may act as a neuroprotective agent improving synaptic plasticity (101). On the other hand, elevated fasting insulin levels caused by insulin resistance have been associated with impairment of long-term memory and global cognitive function (102, 103) and an increased risk of Alzheimer’s disease (104). In addition, Alzheimer’s disease is characterized by insulin resistance (105). Insulin-degrading enzyme degrades both insulin and Aβ protein. This dual activity is probably important because accumulation of Aβ amyloid is believed to be central to the development of Alzheimer’s disease, and increased insulin levels may compete with Aβ as a target of insulin-degrading enzyme, leading to elevated Aβ levels (106). It is, however, notable that increased fasting plasma levels of insulin in obesity and insulin resistance are not reflected by increased insulin levels in the brain (107, 108). Furthermore, both acute and long-term intranasal insulin administration improves episodic memory function in cognitively healthy as well as impaired individuals (109). Also, intranasal insulin administration can strengthen functional connectivity between the hippocampus and the medial prefrontal cortex in T2DM (78). The circumstances under which insulin may be detrimental or beneficial to brain health clearly need to be further elucidated.

Glycemic control may also affect brain function. In both type 1 diabetes mellitus (110) and T2DM (111), a high HbA1c level has been related to slower psychomotor speed and reduced working memory, episodic memory, and general cognitive ability. Moreover, even in elderly individuals without T2DM, a high HbA1c has been associated with impaired memory function, perhaps mediated by an altered hippocampal microstructure (81). Although this link would indicate that tight glycemic control is beneficial, other studies have suggested an increased risk of dementia in T2DM individuals with
recurrent hypoglycemia (112). Dementia may also increase the risk of hypoglycemic episodes; thus, it cannot be excluded that the impaired cognition causes hypoglycemia rather than the other way around (113). Nevertheless, the potential benefit of lowering glucose levels must be weighed against the increased risk of hypoglycemia.

A key link among obesity, metabolic dysfunction, and brain damage may also be increased tissue-specific glucocorticoid (GC) exposure. This possibility is further elucidated below.

**Effects of lifestyle modification on cognitive performance and brain health**

Several weight loss studies have assessed cognitive performance, but the quality of the studies with respect to randomization, control for effects of repeated testing, and the inclusion of a weight-stable control group is weak in general. This weakness was evident from the results of a meta-analysis in which weight loss had an overall small but significant positive effect on memory performance and attention/executive function even though very few of the included studies found significant effects on their own (114).

A high level of physical activity may protect against age-related cognitive decline and dementia (115, 116). This relationship holds true both in studies with self-reported physical activity and those with objectively measured physical activity (116). Moderately intense physical activity may even promote cognitive function in participants with minor cognitive impairments (117, 118). Exercise seems to have broad effects on several cognitive domains, but the most prominent effect may be on executive function dependent on the prefrontal cortex (119). This possibility is interesting because a high level of physical activity may protect against prefrontal brain atrophy in particular (120). Moreover, other aspects of physical fitness, such as coordination and balance, have been related to better executive control and working memory performance (121). Whether the positive association between physical activity and cognitive function is affected by BMI and metabolic dysfunction is currently unclear.

These observational data are supported by a number of randomized controlled interventions with moderately intense aerobic exercise in sedentary individuals at 60–80 years of age and without T2DM. Six months of aerobic exercise led to increased gray matter volume in the prefrontal cortex, specifically in the anterior cingulate gyrus, inferior frontal gyrus, and the superior temporal gyrus (122, 123). These structural effects may be reflected by improved verbal memory, executive function, and increased brain responses in the prefrontal and temporal cortices during tests of executive function (124). Furthermore, 12 months of resistance exercise has also been suggested to improve executive function and increase brain
responses in the prefrontal and temporal cortices (125). Others have found mainly decreased brain responses in the prefrontal and temporal cortices during the same tasks of executive function after an aerobic exercise intervention (126). One year of aerobic exercise may also increase hippocampus volume, improve spatial memory, and increase brain-derived neurotrophic factor (BDNF) levels (127). Of importance, these studies did not specifically include overweight or obese participants. Nevertheless, in at least two studies, participants had a mean BMI > 25 kg/m² (126, 128).

Studies involving participants with T2DM have been less encouraging. The Look-Ahead Movement and Mind study included 976 overweight and obese subjects with T2DM. Eight years of intensive lifestyle intervention did not have beneficial effects on global cognition, verbal fluency, executive function, verbal memory, attention, or processing speed compared to diabetes support and education. Of note, the cognitive functions were measured only at the 8-year follow-up and not at baseline (129). The ACCORD MIND study included 2977 participants with T2DM at high risk for CVD, randomized to either intensive glucose-lowering therapy (HbA1c < 42 mmol/mol) or standard therapy (HbA1c 53–63 mmol/mol) for 40 months. Although total brain volume decreased less in the intensive treatment group compared to standard treatment, there were no improvements in processing speed, verbal memory, or executive function (130). Results of these two large randomized controlled trials suggest that neither weight loss nor improvement of hyperglycemia with lifestyle alterations or by intensive drug treatment can reverse the cognitive deficits associated with T2DM. One reason, at least in the ACCORD MIND study, may be an increased prevalence of hypoglycemic episodes (112). In addition, however, the timing of the intervention may be highly important because the brain impairments may appear early in the progression of T2DM.

Nevertheless, a recently published large randomized controlled trial, FINGER, included 1260 participants at increased risk for Alzheimer’s disease related to the presence of overweight and metabolic dysfunction. Participants were randomized to either a multi-domain approach including nutritional advice, physical activity, social activities, and management of vascular risk factors and the other group to standard health advice. After 2 years, there were beneficial effects on memory, executive function, and psychomotor speed in the intervention group (131). In line with this result, performing moderately intense exercise regularly has been recommended to reduce the risk of developing dementia (132).

In conclusion, in obesity without T2DM, aerobic and resistance exercise may improve spatial and episodic memory function as well as executive function, effects that may be linked to increased gray matter volume in the prefrontal cortex and the hippocampus as well as to altered functional brain responses primarily in the prefrontal cortex (122, 125-128). On the other
hand, the two largest interventions for improving weight and metabolic dysregulation in T2DM have failed to show that doing so is beneficial with respect to cognition. However, the effects on functional brain responses of other lifestyle modifications such as specific diets, as well as lifestyle modification in T2DM, remain to be studied.

**GC metabolism in obesity and T2DM**

*The hypothalamic–pituitary–adrenal axis*

The hypothalamic–pituitary–adrenal axis (HPAA) regulates the production of the active GC cortisol in humans. The periventricular nucleus in the hypothalamus secretes corticotropin-releasing hormone (CRH), which stimulates the release of adrenocorticotropic hormone (ACTH) from the pituitary. ACTH in turn stimulates cortisol synthesis and release from the zona fasciculata in the adrenal cortex. The HPAA is activated in response to threats to homeostasis, i.e., the equilibrium of physiological processes. These threats can be either physical or psychological, such as fasting, exercise training, trauma, or stress (Figure 1 (133)).

![Figure 1. Stress is the main driver of the hypothalamic-pituitary-adrenal axis (HPAA). Higher brain regions such as the medial prefrontal cortex and hippocampus can integrate cognitive functions e.g. memory and executive functions with the endocrine stress response through inhibition of the HPAA. Cortisol regulates the HPAA through negative feedback on the](image-url)
hypothalamus and the pituitary. Cortisol and cortisone are interconverted by 11βHSD1 and 11βHSD2 locally in tissues. Obesity and T2DM causes homeostatic stress and elevated drive in the HPAA. Furthermore, the conversion of inactive cortisone to active cortisol is increased in adipose tissue. Consequently, obesity and T2DM increases cortisol exposure with negative effects on metabolic regulation and brain function. CRH = corticotropin-releasing hormone. ACTH = adrenocorticotropic hormone. Plus = increased in obesity and/or T2DM. Cross = brain areas damaged by elevated cortisol levels.

**Cortisol signaling and effects**

Cortisol binds to the GC receptor (GR) in the cytoplasm, is translocated into the cell nucleus where it binds to GC response elements on the DNA, and either stimulates or suppresses gene transcription. These genomic effects, dependent on protein synthesis, are rather slow. However, GR signaling can have rapid non-genomic effects (134). Cortisol can also bind to and activate mineralocorticoid receptors (MRs). To prevent cortisol from interfering with aldosterone signaling on MRs, 11beta hydroxysteroid dehydrogenase type 2 (11βHSD2) converts active cortisol into inactive cortisone in tissues where MRs are present, such as the kidney. However, cortisol signaling via MRs may be an important pathway for cortisol effects in the brain, especially the hippocampus (135).

Cortisol has widespread short- and long-term effects throughout the body. As the name ‘glucocorticoid’ implies, it was first considered to be a glucoregulatory hormone, increasing glucose levels during fasting. It does, however, have several other effects that can be considered beneficial in the acute stress response, such as a reduced inflammatory response, elevated blood pressure, and increased arousal. These effects are beneficial in the short term, but when cortisol levels are chronically elevated (as in Cushing’s syndrome), it has major negative impacts throughout the body, causing insulin resistance, hypertension, visceral adiposity, chronic inflammation, osteoporosis, and brain damage (136-138).

**The HPAA in obesity and T2DM**

The remarkable resemblance between Cushing’s syndrome, caused by chronically elevated cortisol levels, and the metabolic syndrome has led researchers to hypothesize that altered cortisol levels may be a key mechanism for development of obesity and associated metabolic dysregulation. Accordingly, both obesity and T2DM are reflected by an increased excretion of GC metabolites in urine (139, 140); however, while circulating cortisol levels are probably increased in T2DM (141-144), obesity
rather is associated with normal or even decreased circulating cortisol levels, at least in the morning fasting state (145). Nevertheless, results of studies using stable isotope tracers have suggested that the total production of GCs is increased in both conditions (146). In addition to these alterations, reduced feedback inhibition of the HPAA in response to dexamethasone has been suggested in obesity (147) and T2DM (148), but this concept has been challenged by other studies (140, 149, 150). Increased circulating cortisol levels after stimulation with ACTH and CRH may also suggest a hypersensitive HPAA in obesity (151) and T2DM (144). Thus, present research indicates an altered HPAA function in both obesity and T2DM, but is this altered function relevant for these diseases?

The findings regarding HPAA regulation and cortisol levels in obesity are clearly conflicting. Because circulating cortisol levels are lowered in obesity, looking at tissue-specific GC metabolism may be of greater relevance in this condition. Nevertheless, higher plasma cortisol levels in blood samples taken before lunch are associated with increased diastolic blood pressure in men and increased triglyceride levels in women (152). In T2DM, elevated cortisol levels have been associated with poorer glycemic control (153, 154), hypertension (153), increased cholesterol levels (154), and an increased number of diabetic complications (155). In addition, high morning plasma cortisol levels may also be associated with ischemic heart disease (154). Furthermore, impaired feedback inhibition of the HPAA has been linked to impaired declarative memory performance in T2DM as well as Alzheimer’s disease (156, 157). These studies indeed indicate that altered cortisol levels and HPAA dysfunction may play a role in T2DM morbidity.

**GCs as a link among obesity, T2DM, and cognitive impairment**

GRs and MRs are highly expressed in hippocampal regions, i.e., the dentate gyrus and cornu ammonis area 1, as well as the prefrontal cortex in humans (158, 159). These receptors are thought to play an important role in the feedback inhibition of cortisol on the HPAA. Increased cortisol secretion after acute stress is indeed associated with deactivations in the hippocampus and prefrontal cortex, as measured with positron emission tomography and fMRI (160). Furthermore, the hippocampus is central to episodic, declarative, and spatial memory function, and the prefrontal cortex mediates executive function, resulting in goal-directed behaviors. Thus, the hippocampus and prefrontal cortex can integrate the cognitive resources and autonomic HPAA response needed to deal with a stressor (161). Of importance, the relationship between cortisol and these brain regions may be bi-directional (162). In the 1980s, Sapolsky et al. proposed the GC cascade hypothesis based on experimental research in rodents and primates. Severe psychosocial stress and GC administration in the central nervous system
were associated with hippocampal atrophy in primates, which was thought to increase cortisol secretion, causing exacerbated brain damage (163). This hypothesis has been brought into question by the fact that more recent studies have not replicated these results using more physiological stress paradigms and GC doses (164). Nevertheless, several studies have found shortening of apical dendrites, decreased dendritic arborization, and decreased numbers of dendritic spines on pyramidal neurons in the hippocampus and the medial prefrontal cortex in response to increased GC levels and stress (165, 166).

Evidence for a link among the HPAA, cortisol, and the brain in humans relies heavily on brain imaging studies. Lupien et al. were first to suggest this link in healthy humans with findings that elderly men with high cortisol levels that increased over a 5-year period had smaller hippocampi and impaired episodic memory compared to men with low–moderate cortisol levels that remained stable (167). Since that study, an association between different indices of HPAA activity and hippocampal volume has been replicated (168), but it also has been challenged (157, 169, 170). These inconsistent results may trace to several factors, such as different cohorts, ages, comorbidities, and indices of HPAA activity and, as must be remembered, the relationship between cortisol levels and hippocampal volume is probably rather weak. When focused on the prefrontal cortex, results have been a bit more consistent. High daily saliva cortisol levels have been related to a reduced cortical thickness in most prefrontal regions (170) and reduced feedback inhibition after dexamethasone to a lower volume of the left cingulate gyrus (171). Some results, however, have challenged this relationship, but it must be noted that these studies analyzed the prefrontal cortex as a single region, possibly missing effects on specific subregions within the prefrontal cortex (169, 172).

As observed above, most data point to higher circulating cortisol levels in T2DM. Therefore, alterations in cortisol levels have been hypothesized to provide a link among T2DM, cognitive impairments, brain damage, and an increased risk of dementia (173). This proposed association has been supported by studies suggesting hippocampal atrophy as a consequence of a dysregulated HPAA in T2DM (67, 174) and that this atrophy may be linked to impaired declarative memory functions (144). Furthermore, individuals suffering from both T2DM and depression, which is associated with altered HPAA regulation, have an increased rate of cognitive decline as well as increased dementia risk compared to those suffering from T2DM alone (48, 175).
Tissue-specific GC metabolism in obesity and T2DM

Inactive cortisone and active cortisol can be interconverted locally in tissues by 11-beta hydroxysteroid dehydrogenase type 1 (11βHSD1, cortisone → cortisol) and type 2 (11βHSD2, cortisol → cortisone). These enzymes provide means of regulating GC signaling in tissues independent of circulating cortisol levels. Intriguingly, even though circulating cortisol levels are only slightly altered in obesity, 11βHSD1 activity is increased in subcutaneous and visceral adipose tissue (140, 176, 177), leading to increased local cortisol production (178). This increased expression and activity have been linked to abdominal obesity, insulin resistance, and inflammation (140, 179).

11βHSD1 is highly expressed in liver, as well. Results of initial studies in this area, using the elevation of plasma cortisol levels after oral cortisone administration as an estimate of hepatic 11βHSD1 activity, suggested that obesity was associated with decreased 11βHSD1 activity in the liver (140, 177, 180). Of note, however, several recent studies using more advanced techniques with labeled cortisol tracers have produced findings suggesting that hepatic 11βHSD1 is actually sustained in obesity and T2DM (146, 181, 182). These contrasting results could arise from an increased clearance rate of cortisol by hepatic 5α-reductase in obesity and T2DM, leading to reduced enrichment of plasma cortisol after oral cortisone, even though the hepatic 11βHSD1 activity is unaltered (183, 184).

The putative importance of these alterations in tissue-specific GC metabolism is highlighted in transgenic mice overexpressing adipose 11βHSD1. Even though circulating corticosterone levels remain normal, these mice develop abdominal obesity, hypertension, dyslipidemia, and insulin resistance (185). Furthermore, 11βHSD1-knockout mice are resistant to diet-induced weight gain and metabolic dysregulation (186). Taken together with studies in humans, these experimental studies clearly highlight 11βHSD1 as a potential drug target for treatment of obesity and T2DM.

Three randomized controlled trials have evaluated the effects of 11βHSD1 inhibitors in T2DM (187-189). In general, these inhibitors lowered HbA1c levels and blood pressure. Because the effect sizes were limited, however, further development of these drugs has stalled. Hughes et al. recently highlighted one possible explanation for the unexpectedly small effects, suggesting that 11βHSD1 can convert cortisone → cortisol as well as cortisol → cortisone in vivo (190). This concept implies that 11βHSD1 inhibitors may have to selectively inhibit the activation of inactive cortisone → cortisol to have major effects on metabolic regulation in T2DM.
Tissue-specific GC metabolism and cognitive function

11βHSD1 is expressed throughout the human brain and seems to act predominantly as a reductase, converting cortisone \( \rightarrow \) cortisol (191). 11βHSD1-deficient mice are resistant to age-related cognitive impairments (192), and inhibition of 11βHSD1 lowers intra-hippocampal corticosterone levels, an effect linked to improved spatial memory in rodents (193, 194). In humans, an elevated ratio of cortisol to cortisone metabolites in 24-hour urine samples, reflecting increased systemic 11βHSD1 activity (Figure 2), has been associated with a more rapid decline in processing speed and increased brain atrophy during a 6-year period in elderly men (195). Of note, in the same cohort, the ratio of cortisol:cortisone metabolites correlated with hippocampal atrophy (195). Taken together, these findings suggest that 11βHSD1 in the brain may provide a target for treatments of cognitive impairment. Sandeep et al. provided further support for this idea, reporting that treatment with the non-selective 11βHSD inhibitor carbenoxolone improved verbal fluency in healthy elderly men as well as verbal memory in elderly men with T2DM (196). However, a recent study that examined the effect of a selective 11βHSD1 inhibitor on cognitive performance in Alzheimer’s disease found no improvements of any symptoms after 12 weeks of treatment (197). Furthermore, another study recently concluded that 11βHSD1 in the brain does not contribute to systemic cortisol production; that result does not, however, preclude that cortisol produced by 11βHSD1 can have important local effects in the brain (198). Perhaps targeting minor cognitive impairments or cognitive impairments associated with T2DM could improve the efficacy of these drugs.

![Cortisol and cortisone conversion](image)

**Figure 2.** Cortisol and cortisone are interconverted by 11βHSD1/2. Cortisol is metabolized by 5α- and 5β-reductase and cortisone by 5β-reductase in the liver. 11βHSD = 11beta hydroxysteroid dehydrogenase, THF = tetrahydrocortisol, THE = tetrahydrocortisone.
Diet and exercise in obesity and T2DM

Diet is a cornerstone of the prevention and treatment of obesity and T2DM. The debate is intense regarding present diet recommendations, with a main focus on macronutrient composition. The most recent diet recommendations in both obesity and T2DM and for the general population emphasize that a decreased intake of carbohydrates and increased intake of protein are probably, at least in T2DM, beneficial with respect to glycemic control (199, 200). Indeed, the World Health Organization recently recommended that sugar intake be reduced to < 10 % of total energy intake (201).

A negative energy balance is central to losing weight (202), a concept supported by the fact that a combination of diet and exercise causes a greater long-term (>12 months) weight loss than diet alone, probably on the basis of increased energy expenditure combined with decreased energy intake (203). Regarding specific macronutrients and weight loss, a recent meta-analysis confirmed that in the short-term (< 12 months), several diets with different macronutrient compositions can achieve a substantial and equal weight loss (204). On the other hand, a high protein intake in ad libitum diets may counteract weight regain during 12 months after a substantial weight loss (205, 206).

The optimal dietary intake of saturated (SFA), trans-unsaturated, monounsaturated, and polyunsaturated fatty acids (PUFA) is debatable. Until recently, most evidence pointed towards a lowered risk of CVD if dietary SFAs were replaced with PUFAs (207). However, results of a recent meta-analysis indicated no protective effects of a higher PUFA intake (208). Of note, that analysis was criticized for interpreting an intervention with a high intake of trans-fatty acids as a high intake of PUFAs, leading to this contradictory finding (209). The current dietary guidelines recommending a low intake of SFAs as well as trans-unsaturated fatty acids and a high intake of PUFAs are thus probably still correct with respect to current evidence and may decrease the risk of both diabetes and CVD (200, 207, 208, 210).

The Paleolithic diet

The Paleolithic diet (PD) originates from studies in rural populations believed to be unaffected by westernized culture and eating habits. Of interest are studies by Lindeberg et al. on the Kitava Island. This population evidently experiences no ischemic heart disease or stroke (211) and has a lower cardiovascular risk than a Swedish reference population (212). The dietary pattern in this population may, at least in part, reflect what the human species evolved and adapted to. It excludes the intake of grains, refined sugar and fats, sodium chloride, alcoholic beverages, and other foodstuffs that constitute a major part of the western diet (211-213). Instead,
it emphasizes a high intake of green leafy vegetables, fruits, berries, nuts, fish, and lean meat (213). Adhering to these recommendations would lead to an increased intake of PUFAs and protein and a lower glycemic load when compared to present diet recommendations (214).

A few studies have compared different types of PD with other more common diets. Recently, a 2-year intervention showed that a modified PD was superior the Nordic Nutrition Recommendations (NNR) with respect to weight loss and reduction of waist circumference and fat mass after 6 months. Although these effects were sustained after 2 years, they were not significantly different between the diets. The PD also improved triglyceride levels more than did the NNR diet after 6 months, a difference that was sustained after 24 months (215). Furthermore, a PD may improve insulin sensitivity more than a Mediterranean diet in T2DM (216).

Diet composition and brain function

The PD has not been studied in relation to cognitive performance, brain function, and dementia. An abundance of observational studies, however, have sought to identify links between specific nutrients and brain health.

Several studies have explored putative links between intake and/or plasma levels of fatty acids, cognitive impairment, and dementia. Some have suggested that low circulating levels of the PUFAs eicosapentaenoic acid and docosahexaenoic acid are related to impaired memory and executive function and an increased risk of dementia and brain atrophy (52, 217-219). Although this indication has been opposed by another study suggesting that SFAs, but not omega-3 PUFAs, are related to a decreased risk of Alzheimer’s disease (220), when adjusting SFA intake for intake of unsaturated fatty acids, the risk for Alzheimer’s disease was increased instead (221). Randomized controlled trials evaluating the effects of omega-3 supplementation on cognition and risk of dementia have also reported mixed results. A recent meta-analysis concluded that omega-3 supplementation may be beneficial with respect to memory performance, processing speed, and attention in non-demented elderly persons with impaired cognition, but not in cognitively healthy or demented elderly people (222). However, reports also exist describing improved executive function, improved white matter integrity, and increased gray matter volume in several brain regions, including the hippocampus, after 24 weeks of omega-3 supplementation in healthy overweight adults (223). Because one key feature of the PD is an increased intake of PUFAs, this increase may be beneficial to brain health.

Another possible mediator of positive effects on the brain by a PD may be polyphenols. Hundreds of different polyphenols are present in edible plants such as berries, fruits, nuts, grains, coffee, and red grapes. Many of these polyphenols have anti-oxidant effects and therefore have been highlighted as
substances that can prevent cancer, CVD, and neurodegenerative diseases (224). This suggestion has been based on experimental research in rodents receiving extremely high doses of polyphenols compared to levels found in natural foodstuffs (224, 225), with unclear clinical relevance. Epidemiological studies have also suggested that intake of some polyphenols such as flavonols may be associated with improved verbal memory and language 13 years later, but also with impaired executive function (226). A recent study tested the effect of the polyphenol resveratrol on memory performance and brain activity in elderly individuals (227). Twenty-six weeks of treatment with 200 mg resveratrol daily led to small but significant improvements in delayed memory recall and increased functional connectivity of the hippocampus (227). These effects may, however, be hard to achieve through the consumption of natural foodstuffs. For example, the concentration of resveratrol in red wine, which is considered to have a high amount, is only 20 mg/L (224). Thus, although the PD emphasizes a high intake of foods rich in polyphenols, the potential for these considerably small amounts to improve cognitive function compared to exogenous supplementation is unclear.

Folic acid is another nutrient that seems to be important with respect to cognitive function and dementia in aging. High homocysteine levels, indicating folate deficiency, have been associated with decreased hippocampal volume in non-demented individuals (228), and individuals with a high intake of folate had a lower risk of developing Alzheimer’s disease over 10 years in that study. Of note, a negative relationship has also been proposed, with an increased rate of cognitive decline in older patients with a high folate intake (229). Several randomized controlled trials have evaluated the effect of folate supplementation on cognitive function. The results are inconsistent, and improvements, impairments, and no effects have been reported (230). However, the most comprehensive study showed that 3 years of folate supplementation led to improved memory and processing speed in elderly individuals with high homocysteine levels (230).

**Tissue-specific GC metabolism, diets, and weight loss**

Weight loss can influence tissue-specific GC metabolism. Calorie restriction leads to weight loss, causing decreased excretion of GC metabolites, predominantly because of a lowered excretion of 5α-reduced metabolites. However, weight loss does not seem to affect hepatic 11βHSD1, as evidenced by an unaltered ratio between cortisol and cortisone metabolites in urine as well as an unaltered conversion of orally taken cortisone to plasma cortisol (231-233). The expression of 11βHSD1 in adipose tissue seems to decrease in response to substantial (234, 235), but not minor, weight loss (233, 236).
In addition to effects of weight loss per se, dietary macronutrients can have specific effects on GC metabolism. A high-fat, low-carbohydrate diet has been suggested to increase systemic 11βHSD1 activity and lower GC inactivation by hepatic reductases when compared to a medium-fat, medium-carbohydrate diet (237). The results of these studies imply that GCs may play an important part in mediating the metabolic effects of dietary modifications.
Aims

The overall aim of this thesis was to investigate the effects of lifestyle interventions on tissue-specific GC metabolism and memory-related functional brain responses in obesity and T2DM. Furthermore, I wanted to elucidate whether altered cortisol levels could suggest a mechanism linking obesity and metabolic dysfunction to altered brain function.

Specific aims were as follows:

- To investigate the relationship between saliva cortisol levels, brain morphology, and cognitive function and to elucidate putative effects of gender, obesity and T2DM on this relationship
- To test the long-term effects of a diet according to NNR or a modified PD on tissue-specific GC metabolism
- To investigate whether 6 months of a modified PD or diet according to NNR would improve episodic memory function and alter functional brain responses measured with fMRI in obese postmenopausal women
- To test the effects on episodic memory performance and functional brain responses of a modified PD combined with structured high-intensity exercise or general advice on physical activity for 12 weeks in men and women with T2DM
Subjects and methods

The studies included in this thesis were conducted in accordance with the Declaration of Helsinki. The studies were approved by the regional Ethical Board of Umeå University, Umeå, Sweden, and all participants gave written informed consent before inclusion in the studies.

Participants and study designs

Study 1

Study 1 included participants from the Betula study on memory health and aging (238). The Betula study started in 1988 and included a randomly selected population-based sample (S1) of 1000 men and women ranging from 35 to 80 years old, categorized into 10 age groups. Five years later, in 1993, another two population-based samples (S2 and S3) of 1000 men and women were added to the study. These cohorts have been invited to a health examination and to perform an extensive cognitive test battery every fifth year since then (238). At test occasion five (T5), conducted 2008–2010, 291 individuals from S1 and S3 were randomly invited (stratified for age and gender) to take part in magnetic resonance imaging (MRI) examinations of the brain. Furthermore, four samples of saliva from each participant were collected at T5 (Figure 3). Ninety-one participants did not provide all saliva samples, used oral GC drugs, or were diagnosed with dementia and therefore were excluded from this study, leaving 200 participants in the final cross-sectional analysis with data from T5. The participants in study 1 were selected to reflect the general population and included 100 women and 100 men aged 55–80 years. Of this sample, 60% were overweight or obese, 7% had T2DM, and 67% had hypertension, numbers that seem to reflect the prevalence of obesity-related metabolic dysfunction in the general population of northern Sweden (3).

Health examination  
Cognitive tests  
MRI examination

Saliva sampling

12 days (3–91)  
297 days (88–524)

Figure 3. Timeline of study 1. Numbers are mean (range).
**Study 2**

Study 2 included healthy obese postmenopausal women (Figure 4). A total of 210 women responded to advertisements in local newspapers and around the University hospital area, and 70 respondents were included in the study. The included women were postmenopausal and had a BMI ≥ 27 kg/m². Exclusion criteria were smoking; a restricted diet or allergy to key components of the PD; diabetes; cardiac disease; renal disease; resting blood pressure ≥ 150/90 mmHg; osteoporosis; hyper- or hypothyroidism; or treatment with statins, beta-blockers, estrogens, GCs, or drugs for psychiatric diseases. The subsample in study 2 excluded 21 of the original 70 randomized participants because they lacked three samples of 24-hour urine, cortisone conversion tests, or biopsies of subcutaneous adipose tissue. The excluded participants were not significantly different with respect to anthropometry and insulin sensitivity compared to the 49 included in the final analysis.

**Study 3**

Study 3 included a subgroup of participants from study 2. Twenty postmenopausal women who were included in study 2 during the same time period were offered the opportunity take part in fMRI examinations. Nine of these women were randomized to the modified PD and eleven to the NNR diet. These 20 women were not significantly different from the other participants in study 2 with respect to age, BMI, or insulin sensitivity. The fMRI examinations were performed at baseline and after 6 months (Figure 4).
Study 4

Study 4 included sedentary men and women with T2DM of less than 10 years in duration. The men were over age 18 years, the women were postmenopausal, and all were younger than 75 years. All were treated with lifestyle modification or metformin and had HbA1c >47 mmol/mol at inclusion. Individuals with a history of CVD or stroke, confirmed by a cardiopulmonary exercise test, were excluded. Those with a history of psychiatric, gastrointestinal, liver, kidney, or lung diseases as well as malignancies were also excluded. Uncontrolled hypertension >160/100 mmHg, microalbuminuria, and treatment with anti-thrombotic drugs or beta-blockers were also exclusion criteria. A total of 280 individuals responded to advertisements in newspapers and at primary health care centers in Umeå. Of these, 32 fulfilled the inclusion criteria and were randomized by minimization for unbalanced treatment allocation to either a modified PD with regular advice regarding physical activity or to a modified PD combined with supervised exercise (PD-EX) for 12 weeks. Two dropped out of the PD-EX group and one from the PD group, leaving 14 and 15 participants in each group, respectively. Unexpectedly, even though all participants were asked about and did not reveal any contraindications for MRI, such as claustrophobia or the presence of magnetic metals inside their body, five participants could not perform the fMRI examinations because of these contraindications. Therefore, only 12 participants from each group...
were included in the fMRI subgroup in study 4 (Figure 5). In addition to these two intervention groups, a control group (observation group, OG) was included in study 4 (Figure 5). The OG was added after the study start, and the participants were thus not randomized to this group. Instead, participants were recruited by separate advertisements or from among volunteers who declined to be included in the intervention groups because of time constraints or who were excluded because of treatment with beta-blockers or anti-thrombotic drugs. The other inclusion and exclusion criteria were similar.

**Figure 5.** Design of study 4. PD-EX = modified Paleolithic diet with structured high-intensity exercise. PD = modified Paleolithic diet. OG = observational group. fMRI = functional magnetic resonance imaging. T2DM = type 2 diabetes mellitus.

**The interventions**

**Diets**

All diets were *ad libitum*, i.e., without limitations on total calorie intake. In studies 2 and 3, the interventions consisted of either a diet according to NNR or a modified PD. The NNR represent the present dietary guidelines in Nordic countries. When studies 2 and 3 were conducted, the 4th edition of
these recommendations was most up-to-date and formed the basis for the dietary counseling in the NNR group (214). In general, this diet emphasizes a high intake of foods rich in fiber and of low-fat dairy products. In contrast, the PD group was recommended to exclude dairy products, refined sugar and fat, salt, and grains from the diet. Instead, the intake of fruit, berries, green leafy vegetables, nuts, lean meat, fish, and to some extent root vegetables was advised to be increased (213). All participants met in a group with a dietician for dietary counseling eight times during the first 6 months (studies 2 and 3) and an additional four times in the following 18 months (study 2). Dietary intake was assessed by self-reported food records during three weekdays and one weekend day at baseline and 6 and 24 months.

In study 4, both groups ate a modified PD, similar to that in study 3. In this study, there were two group sessions of dietary counseling during the first 2 weeks and once a month thereafter. Dietary intake was validated with weighted food records for 4 days at baseline and 12 weeks.

**Exercise**

In study 4, participants in both groups met with a physician during baseline measurements for individual motivational advice on physical activity. They were advised to perform at least 30 minutes of moderately intense daily physical activity. The PD group was not prohibited from performing more physical activity of longer duration or higher intensity on their own. The PD-EX group was included in an exercise program at the Sports Medicine unit, Umeå University. In this program, a trained exercise leader helped the participants perform combined aerobic and resistance exercise training for one hour three times per week during the 12-week intervention. The activities included walking on cross trainers, cycling on ergometer bicycles, and performing resistance exercises.

**Clinical measurements**

**Anthropometric measurements**

All clinical measurements in studies 2, 3, and 4 were performed at the Clinical Research Center, Umeå University Hospital, before randomization. Participants were weighed in light clothing on a calibrated scale, and length was measured with a calibrated height-measuring gauge. BMI was calculated as the weight/squared length (kg/m²). Waist circumference was measured with a tape halfway between the iliac crest and lower rib margin during gentle exhalation. Dual energy x-ray absorptiometry was used to measure body composition in studies 2, 3, and 4.
**Blood chemistry**

In studies 2, 3, and 4, fasting serum insulin, cholesterol, triglycerides, and high-density lipoprotein were analyzed at the Department for Clinical Chemistry, Umeå University Hospital. In studies 2 and 3, fasting plasma glucose also was measured at the Department for Clinical Chemistry, but in study 4, it was measured by Hemocue. Low-density lipoprotein was calculated as (serum cholesterol – serum high-density lipoprotein – serum triglycerides)/2.2. The homeostasis model for assessment of insulin resistance was calculated as (fasting glucose × fasting insulin)/22.5.

In study 2, fasting plasma cortisol levels and cortisol levels after orally taken cortisone in the cortisone conversion test were analyzed at the Department for Clinical Chemistry. In studies 3 and 4, the plasma free fatty acid levels were measured with an enzymatic calorimetric method (NEFA-HR2, Wako Chemicals). In study 4, BDNF levels were measured with an ELISA (BDNF Quintikine ELISA kit, R&D systems).

**Physical activity and fitness**

Physical activity levels were measured with the combined accelerometer and heart-rate monitor Actiheart® in studies 2, 3, and 4. In study 4, a standardized cardiopulmonary exercise test was performed before and after the intervention at the Department for Clinical Physiology, Umeå University Hospital, to determine aerobic capacity and exclude cardiovascular disease.

**Measurements of GC metabolism**

**Cortisol levels in saliva**

Study 1 included the measurement of cortisol levels in saliva, which have been widely used in psychobiological research, predominantly as a marker of stress (239). Sampling of saliva is convenient because it can be performed by participants in an unstressed home environment and does not require blood sampling, which may be a stressful event in itself. Plasma cortisol is highly bound to cortisol-binding globulin and albumin, and only a small fraction is free and biologically active. Another advantage of cortisol in saliva is that it correlates rather well with this unbound fraction of plasma cortisol (in most studies r ≥ 0.9) and is thus a good measure of cortisol exposure (239). The participants collected saliva in plastic tubes for one day at 0700, 1100, 1600, and 2300 h at home. The plastic tubes were kept in the refrigerator until being handed over to the research staff and subsequently stored at -20 °C for about 4–6 years. Cortisol levels in saliva are highly stable, both at room temperature for a couple of hours and at -20 °C for one year (240); whether
this stability is maintained over longer storage times is unclear. The cortisol levels were analyzed with a chemiluminescence immunoassay (IBL, Hamburg, Germany), and the intra- and inter-assay coefficients of variation were < 8%. The area under the curve (AUC) with respect to ground (241) for cortisol levels in the four saliva samples and the late-night cortisol level in the saliva sample taken at 2300 h were used in the analyses.

**GC metabolites in urine**

Cortisol is metabolized by 5α- and 5β-reductase in the liver and cortisone by 5β-reductase and subsequently conjugated and excreted in the urine. The majority of cortisone is excreted as 5β-tetrahydrocortisone (THE), and cortisol is excreted as 5α-tetrahydrocortisol (THF) and 5β-THF. A small fraction is excreted as free cortisone and cortisol as well as other metabolites such as cortols and cortolones (242). The ratios of these metabolites depend on the activity of the different metabolizing enzymes, mainly 5α-reductase and 5β-reductase, as well as the interconversion of cortisone and cortisol by 11βHSD1 and 11βHSD2 in the liver. Therefore, these ratios can be used to indirectly estimate the activity of these metabolic pathways. The ratio of (5α-THF + 5β-THF) to THE has been suggested to reflect hepatic 11βHSD1 activity (11βHSD2 is expressed at low levels in the liver that are probably negligible) whereas 5α-THF:5β-THF and 5α-THF:THE reflect the balance between 5α- and 5β-reductase (Figure 2 (243)). Because these indices provide indirect estimates of the activity in these metabolic pathways, interpretation must be made with caution.

**Cortisone conversion test**

Cortisone conversion testing can be considered a test of first-pass metabolism of cortisone to cortisol by 11βHSD1 in the liver. To suppress endogenous cortisol production, 1 mg of dexamethasone was taken the evening before the test. The following morning, 25 mg of cortisone acetate was taken orally, and cortisol levels were analyzed in venous blood samples in the following 4 hours.

**11βHSD1 expression in subcutaneous adipose tissue**

In this study, needle biopsies of subcutaneous adipose tissue were taken from the periumbilical area under local anesthesia. The biopsies were frozen and kept at -80 °C until analysis of 11βHSD1 mRNA levels. 11βHSD1 enzyme activity or protein levels were not measured in this study, but previous studies have shown that these measures are correlated (244).
Cognitive tests

An extensive battery of cognitive tests was used in the Betula study (238). Based on previous research, we selected four cognitive domains to include in study 1. Episodic memory performance depends heavily on the hippocampus (245), which may be damaged by high cortisol levels (168). In addition, dysregulation of the HPAA has been linked to impaired episodic memory function in T2DM (144). Episodic memory was tested in five tasks of immediate and delayed recall of words. Working memory includes the ability to maintain and update small amounts of information temporarily. This function depends on the prefrontal cortex (246), and previous research has linked increased cortisol levels to impaired working memory and executive function (169), as well as to atrophy of the prefrontal cortex (170). Working memory function was tested in a 2-back task. Visuospatial ability requires a high level of attention and executive function, and previous research has linked the cingulate gyrus to visuospatial tasks (247). Because cortisol has been negatively related to cingulate gyrus volume and executive function, we included this cognitive domain (171, 248). A block-design task was used to test visuospatial ability. Semantic memory is closely related to episodic memory but not as sensitive to aging (35). It depends on prefrontal brain regions, and elevated cortisol levels have been negatively related to semantic memory and language performance (249, 250). Semantic memory and language were tested using four fluency tasks in which words of specific themes were to be generated during one minute. Studies 3 and 4 included an episodic memory task performed within the MRI scanner (see below).

Brain imaging

MRI

The human body contains an abundance of hydrogen, predominantly in the form of water molecules. The hydrogen nuclei consist of one positively charged proton. When a strong magnetic field is applied around the body, these protons align, either parallel or anti-parallel to the magnetic field, like compass needles. Slightly more protons will be parallel than anti-parallel because the former requires a lower amount of energy. When a radiofrequency is applied, some of the parallel protons will shift to the higher anti-parallel energy state, and a perpendicular magnetization will occur. As soon as the radiofrequency disappears, these protons return to the lower parallel energy state, and the perpendicular magnetization will decay or relax, creating two contrasts, T1 and T2. T1 is based on the protons becoming parallel with the magnetic field, and T2 is based on the decay of the perpendicular magnetization. The T1 measure is rather slow and takes
hundreds to thousands of milliseconds whereas T2 is fast and appears within milliseconds. Because tissues contain different amounts of water and thus of hydrogen, T1 and T2 will differ between tissues, creating contrasts and enabling identification of specific tissues (251). Depending on the tissue under study, different pulse sequences can be used. The pulse sequence can be either T1-weighted or T2-weighted; the choice depends primarily on the repetition time (time between radiofrequency pulses) and echo time (time from radiofrequency pulse to registration of the magnetic resonance signal). All MRI examinations were performed on a 3 T MRI scanner (Discovery MR750, General Electrics). In study 1, a T1-weighted pulse sequence with a repetition time of 8.2 ms and echo time of 3.2 ms was used because it provides a high resolution and contrast between brain tissues. In studies 3 and 4, an echo planar imaging protocol (T2*-weighted (252)) was used during the fMRI scans. This is a very fast imaging protocol that can scan an entire brain volume in two seconds and is also sensitive to alterations in blood flow.

**fMRI**

fMRI measures the blood oxygen level–dependent (BOLD) signal. When neurons within a brain region are activated, there is a rapid initial increase in deoxygenated hemoglobin, i.e., metabolically active neurons consume oxygen. After 2–6 s, the blood flow in that brain region is increased because arterioles dilate and the level of oxygenated hemoglobin increases proportionally more than before the neural activity. This increase in oxygenated hemoglobin will increase the MRI signal from the activated brain region and is thus an indirect measure of neural activity. It takes another 10 s before the BOLD signal returns to the baseline level. Several studies have found strong correlations between BOLD signal and peri-synaptic neural activity, but the method has also been criticized because others have not found the BOLD signal to correlate with measures of neural activity. These controversies may depend on the specific brain region studied (253) and are important to keep in mind when interpreting fMRI data. Nevertheless, fMRI has become the most widely used functional neuroimaging method because the spatial resolution is high enough to pinpoint effects in different brain regions, the signal is fast enough to test dynamic responses to cognitive tests, and MRI is available in most hospitals.

**fMRI task**

In studies 3 and 4, the participants performed an episodic memory task inside the fMRI scanner. We chose to focus on episodic memory because it depends heavily on hippocampal and prefrontal cortex function (254, 255),
brain regions that have been suggested to be sensitive to the effects of obesity and metabolic dysregulation, as well as to nutrients and physical activity (127, 223). Critically, the face–name task induces robust hippocampal BOLD signals during both memory encoding and retrieval, allowing for evaluation of this specific brain region (256).

The face–name task had a blocked design and consisted of three blocks: memory encoding, memory retrieval, and a baseline block. A blocked design means that the different experimental conditions are presented in blocks, creating a robust BOLD response and a high signal-to-noise ratio, which facilitates analysis of the fMRI data. The drawback of a blocked design is that the BOLD signal from a single event, such as correctly remembered faces, cannot be separated from the other events in the same block, such as forgotten faces. For this distinction, an event-related design would have to be used.

During memory encoding, four faces and names were presented to the participant in each block; in total, there were 24 faces divided into six blocks. The same faces were subsequently presented with three adjacent letters, and the retrieval task was to indicate which one of these letters fit with the face. The answer was given by pressing one of three buttons. The retrieval task was also divided into six blocks. In between the blocks of memory encoding and retrieval, the baseline task was presented; a white cross appeared on the screen, and when this changed to a ring, the participants pressed a button. In total, the face–name task lasted 8 minutes. For data on stimulus times and other specifics, see papers 3 and 4.

**fMRI data analysis**

The fMRI data were processed in several steps before analysis. This preprocessing included the following standardized procedures:

- **Slice-timing**, which corrects the time shift from the first to the last slice in one brain volume by interpolating the BOLD response at an exact time point from adjacent slices.
- **Motion correction** for small movements that can cause alterations of the BOLD signal, especially in the outer parts of the brain; this process cannot, however, correct for sudden movements >10 mm.
- **Normalization**, to enable group-averaged analyses because the individual brains will be morphologically different; in our studies, the brain template from Montreal Neurological Institute (MNI) was used.
- **Smoothing**, in which a filter, commonly a full-width, half-maximum filter, is used to increase the signal-to-noise ratio and the normality
of the BOLD signal, enabling parametric statistical analysis of the data but with the drawback of a loss of spatial resolution.

After preprocessing, the general linear model was used to calculate the β-weights for the three experimental conditions of encoding, retrieval, and baseline. These β-weights were then contrasted as \( \beta_{\text{encoding}} - \beta_{\text{baseline}} \) and \( \beta_{\text{retrieval}} - \beta_{\text{baseline}} \). The contrasts were used to remove as many brain responses as possible that were not specifically related to encoding and retrieval tasks. In both studies 3 and 4, a voxel-wise global whole brain analysis and a region of interest (ROI) analysis of the hippocampus were performed. This analysis was done because we had an a priori hypothesis that the hippocampus would show increased BOLD responses after the interventions and that this response would be linked to improved memory performance. We were able to select four ROIs within the anterior and posterior hippocampus bilaterally that have been highlighted as important for episodic memory function in a previous population-based cohort study, using the same face–name paradigm (254).

In the voxel-wise global brain analysis, a one-way analysis of variance (ANOVA) with group-averaged data was used in study 3 to compare the BOLD responses after the intervention with BOLD responses before the intervention. In study 4, a paired sample t-test was used to make this comparison. In both studies, we pooled data from both intervention groups (PD + NNR in study 3, and PD + PD-EX in study 4) into one group. There were two reasons for this approach. First, there were limited to no group differences in anthropometry, biochemistry, and physical activity levels in both studies, and we hypothesized that changes in BOLD responses would be induced by these effects rather than by differences in reported nutrient intake. Second, groups with <12 participants have been estimated to support 80% power to detect significant effects on BOLD responses under a liberal threshold of \( p < 0.05 \). However, a doubled group size may support 80% power to detect significant effects on BOLD responses with a \( p < 0.05 \) corrected for multiple comparisons, which is of major importance when interpreting fMRI data (257).

To visualize the results and compare BOLD responses before and after the interventions, the peak percent BOLD signal change was extracted for each brain region with a significantly altered BOLD response in the voxel-wise analysis. The following formulas were used to calculate the %BOLD signal change \( \left( \frac{\beta_{\text{encoding}} - \beta_{\text{baseline}}}{\beta_{\text{constant}}} \times 100 \right) \) and \( \left( \frac{\beta_{\text{retrieval}} - \beta_{\text{baseline}}}{\beta_{\text{constant}}} \times 100 \right) \). In these formulas, \( \beta_{\text{constant}} \) represents the mean BOLD signal during the face–name paradigm; therefore, these formulas will represent the percent change in BOLD signal during memory encoding and retrieval compared to the mean BOLD signal throughout the memory
paradigm. This change can be positive, with a higher BOLD signal during memory encoding or retrieval, or negative, with a lower BOLD signal during memory encoding or retrieval, called a deactivation. Study 4 included the OG; thus, in that study, the %BOLD signal changes from the significant brain regions in the intervention groups were compared with the %BOLD signal changes in the OG.

In study 3, several brain regions had a significantly altered BOLD response when using a p < 0.05 corrected for multiple comparisons. However, in study 4, the effects were weaker in general, and the brain regions with the strongest effects reached only a liberal threshold of p < 0.005 uncorrected. Therefore, we applied evaluation criteria for interpreting the findings from these brain regions in study 4 to lower the risk of reporting false-positive findings. These criteria included: the %BOLD signal change should be equal in all three groups at baseline; the %BOLD signal change after the interventions should be greater than the %BOLD signal change in the OG at baseline; and the %BOLD signal change should be positive at baseline and/or after 12 weeks, i.e., clusters displaying deactivations at both measurements were excluded from further evaluations.

To explore potential group differences, we separated the data for each intervention group and performed the voxel-wise global brain analysis once more for studies 3 and 4. The %BOLD signal change in each brain region with a significantly altered BOLD response was then compared between the groups.

For the ROI analysis of the hippocampus, we selected four clusters consisting of a sphere 5 mm diameter in the anterior and posterior hippocampus (exact MNI coordinates given in papers 3 and 4). The %BOLD signal change was extracted from these coordinates. In study 3, a one-way ANOVA was used to compare the %BOLD signal change in these ROIs before and after the intervention. In study 4, a repeated-measures ANOVA was used to test the time effect and group × time interaction in %BOLD signal change.

**Statistical methods**

In study 1, linear regression models were used to study the relationship between saliva cortisol levels, cognitive performance, cortical thickness, and cortical surface area in subregions in the prefrontal cortex and volume of the hippocampus. Linear models were used because no non-linear relationships could be identified either by visual inspection of scatter plots or curve-fit analyses. All analyses included age and gender as covariates as well as the interaction term [cortisol measure × gender]. In this thesis, the interaction between cortisol measures and BMI and T2DM was also tested because previous studies have suggested that obesity and T2DM could be related to cortisol levels, brain morphology, and cognitive function. Because of the
large number of statistical tests performed, the false discovery rate at a $p < 0.05$, corrected for 24 tests, was calculated to reduce the risk of type 1 statistical errors.

In study 2, a repeated-measures ANOVA was used to test group effects, time effects, and group $\times$ time interactions. A two-tailed $p$ value $< 0.05$ was considered significant.

In study 3, a one-way ANOVA was used to test the alterations in anthropometry and biochemistry at 6 months compared to baseline.

In study 4, Mann–Whitney U tests were used for group comparisons for the change in clinical measures during the intervention. Repeated-measures ANOVA was used to test time effects and group $\times$ time interactions in %BOLD signal changes. Pearson correlations were used for the correlation analyses in studies 2, 3, and 4.
Results and Discussion

Detailed descriptions of the results are presented in the respective papers.

Paper 1

Our hypothesis in paper 1 was that higher salivary cortisol levels would be related to a thinner prefrontal cortex, lower hippocampal volume, and impaired cognitive functions dependent on these brain areas. Furthermore, we explored putative gender differences in these relationships because experimental research has suggested that estradiol may protect female rats from the adverse effects of corticosterone (active GC in rodents) on the prefrontal cortex (258). In addition, we tested whether overweight, obesity, and T2DM interact with cortisol levels in the relationship with brain morphology and cognitive function.

Cortisol levels and brain morphology

Among the 200 women and men, AUC for cortisol levels in saliva was associated with smaller cortical surface area in the left rostral and caudal anterior cingulate gyrus, bilateral rostral middle frontal gyrus, right lateral orbitofrontal cortex, bilateral medial orbitofrontal cortex, and right superior frontal gyrus (Table 3, paper 1). After correction for multiple comparisons using the false discovery rate at p < 0.05, the medial orbitofrontal cortices and right superior frontal gyrus did not remain significant. Furthermore, AUC for cortisol levels was related to increased cortical thickness in the left rostral anterior cingulate gyrus, left medial orbitofrontal cortex, and bilateral caudal middle frontal gyrus. However, none of these associations remained significant after correction for the false discovery rate at p < 0.05. AUC for cortisol levels was not related to volume of the whole hippocampus or hippocampal subregions. Cortisol level at 2300 h was not related to brain morphology at all.

The interaction between gender and AUC for cortisol levels was significant for the cortical surface area of the left rostral middle frontal gyrus. The post-hoc analysis revealed that this negative association was present in women (b = -0.284, p = 0.003), but not men (b = 0.069, p = 0.437). Moreover, there was a significant interaction on the cortical thickness of the bilateral caudal middle frontal gyrus. In the post-hoc analyses, the positive association was evident in women (left, b = 0.215, p = 0.03; right, b = 0.255, p = 0.009) but not men (left, b = -0.124, p = 0.193; right, b = -0.054, p = 0.564).

There was a significant interaction between BMI and AUC for cortisol levels on the cortical thickness of the right inferior frontal gyrus pars orbitalis (BMI × AUC cortisol, p = 0.018). In a post-hoc analysis with normal
weight, overweight, and obese participants separated, the association was significant among the overweight participants alone (normal weight, b = -0.139, p = 0.22; overweight, b = 0.208, p = 0.036; obese, b = 0.263, p = 0.162). There was a significant interaction between T2DM and AUC for cortisol levels on the cortical surface area in the right inferior frontal gyrus pars opercularis (T2DM × AUC cortisol, p = 0.033), but when those with and without T2DM were analyzed separately, AUC for cortisol levels was not significantly associated with cortical surface area in either group (healthy, b = -0.052, p = 0.456; T2DM, b = 0.394, p = 0.061).

Cortisol levels and cognitive performance
The overall relationship between both AUC for cortisol levels and cortisol level at 2300 h and performance on the cognitive tests was non-significant. However, there was a significant interaction between gender and AUC for cortisol levels on 2-back performance. The post-hoc analysis revealed that in men, higher AUC for cortisol levels was related to lower 2-back performance (b = -0.244, p = 0.044), but this relationship was not evident in women (b = 0.135, p = 0.182).

Furthermore, there was a significant interaction between AUC for cortisol levels and T2DM on episodic memory performance (p = 0.022). In the post-hoc analysis, AUC for salivary cortisol levels was negatively associated with episodic memory performance in those with T2DM (Figure 6, β = -0.535, p = 0.029). BMI did not interact with any cortisol measure on the cognitive test results.

Figure 6. The association between episodic memory performance and area under the curve (AUC) cortisol level in saliva in 14 participants with type 2 diabetes.

Discussion
Total daily cortisol exposure estimated by AUC for saliva cortisol levels measured at four timepoints during one day was related to reduced cortical
surface area in several prefrontal brain regions. The strongest association was present in the left caudal anterior cingulate cortex. This finding extends previous results in males of an association between reduced volume of the anterior cingulate cortex and abnormal negative feedback in the HPAA (171).

Unexpectedly, we found AUC for cortisol levels to be negatively related to cortical surface area rather than cortical thickness. This outcome is a novel finding and in contrast to findings in a recent study in 388 middle-aged men of a negative relationship between total daily cortisol exposure and cortical thickness but not surface area (170). In our study, AUC for cortisol levels was positively related to cortical thickness in the caudal middle frontal gyrus bilaterally and the left medial orbitofrontal cortex, extending to the left rostral anterior cingulate cortex; however, these results did not remain significant after correction for multiple comparisons. Cortical thickness and surface area are considered two separate entities with different genetic backgrounds (259). According to the radial unit hypothesis, surface area is determined by the number of cortical columns and thickness by the number of cells within each column (260). Cortical surface area is the main determinant of inter-individual differences in cortical volume (261) and was recently suggested to be more strongly correlated with general cognitive ability than with cortical thickness (262). On the other hand, cortical thickness has been suggested to be more sensitive to the effects of Alzheimer’s disease and aging (263, 264). In conclusion, these studies may suggest that differences in cortical surface area may have a larger impact on cognition than does cortical thickness, but that the latter is more sensitive to environmental factors.

There are gender differences in the neuronal effects of chronic stress and increased corticosterone levels in rodents. Specifically, in male rodents, chronic stress causes decreased length and branching of apical dendrites in pyramidal neurons in layer III in the medial prefrontal cortex. In contrast, the dendritic length increases in response to chronic stress in female rodents, an effect that disappears after ovariectomy (258, 265). We found that the association between AUC for cortisol levels and cortical surface area was negative in both women and men in all prefrontal brain regions except the left rostral middle frontal gyrus, in which the negative association was present only among women. For measures of cortical thickness, the positive association with AUC for cortisol levels in the caudal middle frontal gyrus also was present only among women but did not remain significant after correction for the false discovery rate. Thus, in general, there were smaller gender differences than expected. All women were over age 55 years and probably postmenopausal, which may explain these small gender differences. Gender does not seem to provide an explanation for the conflicting results regarding the associations with cortical surface area.
versus cortical thickness in our study compared to that of Kremen et al. (170).

Cortisol levels were not associated with cognitive test results except for working memory performance, which was negatively associated with AUC for cortisol levels in men but not women. This possible gender difference should be further investigated. Nevertheless, cortical surface area of the left caudal anterior cingulate gyrus and the right rostral middle frontal gyrus were positively related to visuospatial abilities. Because cortisol levels were not directly related to visuospatial abilities, it is unclear whether the negative effects of cortisol on these brain areas could lead to visuospatial impairments.

Neither weight status (normal weight/obese) nor T2DM were related to altered cortisol levels. To test whether weight status or T2DM affected the association among cortisol measures, brain morphology, and cognitive functions, we added the interaction terms [weight status × cortisol measure] and [T2DM × cortisol measure] in the analyses. This further analysis revealed that in T2DM, higher AUC for cortisol levels was related to impaired episodic memory function. No clear differences emerged, however, in the association between AUC for cortisol levels and brain morphology. This outcome corroborates previous findings of impaired declarative memory performance related to abnormal HPAA feedback inhibition in T2DM (68).

This study had some important limitations. The MRI examinations were performed almost one year after the cognitive tests, health examinations, and saliva cortisol measurements. It is possible that brain morphology was altered during this time period, especially in the older age groups. Nevertheless, all brain areas that we found to be negatively related to cortisol measures have been reported to be associated with the HPAA in both experimental and human research. Furthermore, the participants did not provide the exact time of saliva sampling, and we therefore could not evaluate if the samples were taken at the instructed timepoints (0700, 1100, 1600, and 2300 h). This factor may to some extent explain the lack of associations between cortisol levels at 2300 h and brain morphology and cognitive performance. The AUC was based on four samples and may have been more robust in that respect. The number of statistical tests may also have posed a problem by increasing the risk of type one errors; however, we restricted our analysis to two measures of cortisol levels: the AUC and the saliva cortisol level at 2300 h. These measures were chosen because they have been related to morphology of the prefrontal cortex and hippocampus (168, 170) and we could form a priori hypotheses about these relationships. Furthermore, we corrected our results for multiple comparisons with the false discovery rate. This method was used because in our opinion, it
provides a good trade-off between the risks of type one and type two statistical errors.

**Paper 2**

Our hypothesis in this study was that diet-induced weight loss would normalize the disturbed tissue-specific GC metabolism in obesity, i.e., reduce total excretion of urinary GC metabolites, increase the conversion of orally taken cortisone to cortisol, and decrease the expression of 11βHSD1 in subcutaneous adipose tissue. Moreover, we hypothesized that the PD would have greater effects on GC metabolism than would the NNR diet.

11βHSD1 expression in subcutaneous adipose tissue decreased in both diet groups at 24 months, without group differences (Figure 7A). This effect was related to change in body weight but not insulin sensitivity. On the other hand, total excretion of urinary GC metabolites in urine increased significantly, which was accounted for specifically by an increased excretion of 5α-reduced metabolites between 6 and 24 months (Figure 7B-C). The conversion of orally taken cortisone to cortisol increased slightly after 6 months but was unaltered after 2 years. Taken together, these results for the effect on adipose 11βHSD1 supported our hypothesis, but the increased excretion of 5α-reduced GC metabolites was an unexpected and novel result relative to previous studies.

![Figure 7](image-url) A. Change in expression of 11βHSD1 in subcutaneous adipose tissue. B. Change in 5α-THF/Cortisol (F). C. Change in 5α-THF/5β-THF. Mean (SEM). AU = arbitrary units.

This 2-year diet intervention was considerably longer than previous weight loss interventions reporting data on tissue-specific GC metabolism. The effects on both adipose 11βHSD1 expression and excretion of urinary GC metabolites became significant between 6 and 24 months, which highlights the importance of conducting long-term interventions.

The decreased expression of 11βHSD1 in subcutaneous adipose tissue is in line with other intervention studies achieving a substantial amount of weight loss (234, 235). Our study, however, extends these results in several aspects:
Our participants achieved the weight loss *ad libitum* (without caloric restriction) compared to the previous interventions consisting of gastric bypass (235) and a very low calorie diet (234); the decreased 11\(\beta\)HSD1 expression was sustained for 2 years; and there were no significant differences between the diets. Although the 11\(\beta\)HSD1 expression decreased mostly between baseline and 6 months in the PD group and 6 to 24 months in the NNR group, these differences were not significant (time \(\times\) diet interaction, \(p = 0.344\)).

11\(\beta\)HSD1 expression and activity are regulated by a range of factors related to obesity and metabolic dysfunction, including insulin (266), inflammation (179), lipid metabolism (267), and hormones such as estradiol (268, 269) and growth hormone (183, 270). In our study, the change in 11\(\beta\)HSD1 expression was related to change in BMI but not insulin levels. The role of adipose 11\(\beta\)HSD1 as a potential mediator of improved insulin sensitivity is therefore questionable, but the participants in this study were healthy obese women without major insulin resistance. Furthermore, more sensitive measures of insulin sensitivity may be needed to find this association *in vivo* (234, 235). Other factors such as a decreased low-grade inflammation in adipose tissue may also have mediated the decreased expression of adipose 11\(\beta\)HSD1 with weight loss.

Increased 5\(\alpha\)-reductase activity is considered a primary alteration of GC metabolism in obesity and insulin resistance (139, 140, 184) that can be reversed by short-term weight loss (231, 232). However, the adverse metabolic effects of increased 5\(\alpha\)-reductase activity have been questioned recently because 5\(\alpha\)-reductase inhibition causes increased peripheral insulin resistance in men (271) and knock-out of hepatic 5\(\alpha\)-reductase in rodents causes hepatic steatosis (272). Excretion of 5\(\alpha\)-reduced metabolites increased between 6 and 24 months in this work while concomitantly, body weight increased in 23 out of 41 participants, and mean BMI was stable. Indeed, the percent change in BMI between 6 and 24 months correlated with percent change in aTHF/THF (\(r = 0.338, p = 0.033\)) and aTHF/Cortisol (\(r = 0.453, p = 0.004\)). This result clearly suggests that the unexpected increased estimates of 5\(\alpha\)-reductase activity may have been a protective response related to weight gain during the later phase of the study (see further below).

The decreased activity of hepatic 11\(\beta\)HSD1 in obesity and T2DM as first reported (140, 177) has been increasingly questioned as more advanced studies have been conducted. These studies have mainly found unaltered hepatic 11\(\beta\)HSD1 activity in obesity and T2DM (146, 181, 182). What effect might be expected on cortisone conversion after weight loss is thus unclear. We found that the AUC for plasma cortisol levels after oral cortisol increased slightly at 6 months, but this result was explained by increased plasma cortisol levels at the later timepoints of the test (Figure 2, paper 2). If this increase were driven by increased first-pass conversion of cortisone to
cortisol, the effect would probably have been present during the first hour after taking cortisone rather than at the later timepoints (183). The enrichment of plasma cortisol depends on the activity of hepatic $11\beta$HSD1 and on the uptake of cortisone acetate from the intestines, as well as first-pass degradation by $5\alpha$-reductase and $5\beta$-reductase in the liver. To further complicate the interpretation, $11\beta$HSD1 has been suggested to be bidirectional in vivo, and the direction of this conversion can obviously not be elucidated by this test (190). Nevertheless, the test has been widely used as an index of hepatic $11\beta$HSD1 activity.

Despite a significantly lower reported intake of carbohydrates and higher reported intake of protein and mono- and polyunsaturated fatty acids in the PD group, there were no significant group differences in the measures of tissue-specific GC metabolism. Although the PD group reported an increased intake of protein, this increase was not reflected by increased urinary nitrogen excretion, suggesting that the PD group was over-reporting protein intake. The specificity of the self-reported food diaries can be questioned, and perhaps the real differences in dietary intake were smaller than actually reported. This possibility may, at least in part, explain the lack of group differences in tissue-specific GC metabolism. Furthermore, the increase in measures of $5\alpha$-reductase activity took place between 6 and 24 months, and during this time period, there were no major differences in anthropometric or biochemical effects between the diets.

An important limitation in this study was the use of indirect estimates of $11\beta$HSD1 and A-ring reductase activities. The increased ratio of $5\alpha$-reduced to $5\beta$-reduced GC metabolites inevitably confounded the interpretation of the ratio of cortisol to cortisone metabolites reflecting systemic $11\beta$HSD1 activity. Nevertheless, because there were no substantial changes in cortisone conversion, this result indicates that there were probably limited effects on systemic $11\beta$HSD1 activity because the majority of systemic $11\beta$HSD1 is present in the liver (146, 273).

**Paper 3**

The hypothesis in paper 3 was that diet-induced weight loss would lead to improved episodic memory function and altered functional brain responses during episodic memory testing. The effects on anthropometric and biochemical variables were similar to those reported in paper 2, i.e., both groups lost a substantial amount of weight, decreased waist circumference, and lowered levels of total serum cholesterol and plasma free fatty acids. However, there were no statistically significant differences between the subgroups included in this study. Nevertheless, the PD group reported a significantly higher intake of protein, fat, and unsaturated fatty acids and a
lower intake of carbohydrates after 6 months compared to the NNR group (Table 1 in paper 3).

Episodic memory performance increased by about 10% in both diet groups after the intervention. The BOLD responses during memory encoding increased in the superior temporal gyrus bilaterally, left insula, right supplementary motor area, and right middle frontal gyrus. In contrast, the BOLD response decreased in the right fusiform gyrus. During memory retrieval, the BOLD responses decreased in the left and right inferior frontal gyrus, left middle frontal gyrus, and right visual cortex. On the other hand, the BOLD response increased in the left middle and superior temporal gyrus (Table 2 and Figure 1, paper 3). The ROI analysis of the hippocampus revealed a significantly increased BOLD response during memory encoding after the intervention. There were no group differences in BOLD response alterations (Figure 8).

The decreased BOLD response in the fusiform gyrus correlated with improved memory performance. Otherwise, there were no significant correlations between changes in memory performance and BOLD responses. The increased BOLD response in the hippocampus correlated with decreased levels of free fatty acids after the interventions (Figure 2, paper 3).

This study confirmed our hypothesis that diet-induced weight loss would improve episodic memory function and alter functional brain responses during memory testing. However, the lack of a weight-stable control precluded the possibility of excluding that some of these effects were related to repeated testing.

The increased hippocampal BOLD response during memory encoding is particularly interesting because the hippocampus is important for episodic memory function (255), hippocampal atrophy is a hallmark of Alzheimer’s disease (274), and even in non-demented older persons, a rapid decline in cognitive performance is associated with a decline in hippocampal BOLD responses during memory testing (275).

The altered BOLD responses in the prefrontal cortex were task-specific, i.e., during memory encoding, there were mainly increased BOLD responses whereas the BOLD responses decreased during memory retrieval. This distinction may indicate that the participants could recruit more prefrontal brain resources during memory encoding after weight loss, perhaps improving attention during the encoding task. Such a relationship could explain the decreased prefrontal BOLD responses during memory retrieval because the BOLD response in the inferior frontal gyrus has been associated with retrieval effort (276).

The most important drawback of this intervention study was the lack of a weight-stable control group, which limited the possibility of accounting for the effects of repeated testing on both memory performance and BOLD responses. Repeated testing is often associated with decreased BOLD
responses (277), in contrast to the increased BOLD responses in the hippocampus and prefrontal cortex after this intervention. A strength of the work, though, was the possibility of comparing our results with those from a large population-based cohort performing the same memory task. All regions displaying altered BOLD responses after this intervention were within the encoding and retrieval networks previously reported (254).

**Figure 8.** Percent BOLD signal change for the encoding – baseline contrast in the right anterior hippocampus [MNI coordinates: X 24, Y -8, Z -18]. Open circles = Paleolithic-type diet (PD) group in paper 3. Closed circles = Diet according to Nordic Nutrition Recommendations in paper 3. Open squares = PD group in paper 4. Closed squares = PD with high-intensity exercise in paper 4. Open triangles = weight-stable control group in paper 4. BL = baseline measurement, m = months, w = weeks.

**Paper 4**

In paper 4, we tested the effects on episodic memory function and functional brain responses of a PD combined with supervised high-intensity exercise or a PD with recommendations of increased physical activity.

Both interventions led to a substantial decrease in body weight, waist circumference, and percent body fat. There were major improvements in insulin sensitivity and blood glucose levels, as evidenced by decreased fasting insulin and HbA1c levels. Furthermore, both groups improved aerobic capacity. These effects were similar in both groups (Table 1 in paper 4).

After the interventions, during the second fMRI session, participants remembered about one more face than at baseline, an increase that was similar in all three groups and not significant.

During memory encoding, the intervention groups (PD + PD-EX) displayed increased BOLD responses within the bilateral lingual gyri and right medial prefrontal cortex. Moreover, the BOLD response in the left
inferior and middle frontal gyrus increased during memory retrieval (Figure 1 in paper 4). These effects were significantly different from the OG, in which the BOLD responses decreased (Figure 2 in paper 4).

The ROI analysis of the hippocampus revealed no significant changes over time within each group. However, the mean BOLD responses increased in the intervention groups and decreased in the OG; these group differences in change over time were significant in the right anterior (p = 0.044) and posterior (p = 0.043), hippocampus (Figure 4 in paper 4). On an individual level, 13/24 participants in the intervention groups, but no one (0/6) in the OG displayed clear increases in BOLD response in the right anterior hippocampus (Figure 8). Furthermore, the increased BOLD response in right posterior hippocampus correlated with increased BDNF levels in the intervention groups (Figure 9, r = 0.670, p < 0.001).

These results suggest that a PD, by inducing substantial improvements in metabolic regulation, is associated with increased BOLD responses in several brain regions suggested to be affected by T2DM including the lingual gyri, medial and lateral prefrontal cortex as well as the hippocampus (67, 76, 278, 279). Furthermore, the results of this study indicate that the addition of high-intensity exercise to the PD does not potentiate these effects.

In contrast to findings from paper 3, memory performance did not improve significantly after these interventions. Thus, the potential benefits of these alterations in functional brain responses are unclear. However, others have found that increased BOLD responses in the lingual gyri, left lateral prefrontal cortex (280), left lateral prefrontal cortex (255, 281) and the right hippocampus (254) is associated with higher memory performance in individuals without T2DM.

As evident in figure 8, the individual changes in hippocampal BOLD responses were variable and about half of the participants in the intervention groups displayed increased BOLD responses whereas it decreased or were unchanged in all participants in the OG. Alterations in BDNF levels may provide an explanation to this variability since it was clearly correlated with BOLD responses in the right posterior hippocampus (Figure 9). Notably, the val^{66}met polymorphism in the BDNF gene, causing impaired BDNF secretion (282), has previously been associated with lower BOLD response in the right hippocampus during the same episodic memory test as used in our study (283).

Although this study included a weight-stable OG, the sample sizes were small because of unexpected drop-outs. Thus, as evident by the low statistical thresholds used, the power to find significant effects within and between groups was rather low, limiting the possibility of drawing firm conclusions. Nevertheless, this study provides an indication that altered brain function in T2DM can possibly be reversed by lifestyle changes, which should be confirmed in future studies.
Figure 9. Correlation between change in %BOLD signal change for the encoding – baseline contrast in the right posterior hippocampus [MNI coordinates: X 38, Y -32, Z -4] with change in BDNF levels in paper 4. Closed circles = Paleolithic-type diet (PD, n = 10). Open circles = PD with high-intensity exercise (n = 11). $R^2$ was calculated for both groups combined.
General discussion & future perspectives

The main findings in this thesis are as follows:

- Diet-induced weight loss lowers the expression of 11βHSD1 in adipose tissue, regulating cortisol exposure.
- Weight maintenance and/or weight gain shifts the balance of GC metabolism from 5β-reductase towards 5α-reductase.
- Higher cortisol exposure for one day is related to smaller cortical surface areas in several prefrontal brain regions, with the strongest relationship with the left anterior cingulate gyrus in both women and men. Furthermore, high cortisol exposure may impair working memory function in men and episodic memory performance in patients with T2DM.
- Diet-induced weight loss increases functional brain responses in the prefrontal cortex and hippocampus during memory encoding, which may improve episodic memory function in obese postmenopausal women.
- A Paleolithic-type diet increases BOLD responses in the bilateral lingual gyrus and the right medial prefrontal cortex during memory encoding and the left prefrontal cortex during memory retrieval in T2DM. High-intensity exercise does not potentiate these effects.

Decreased 11βHSD1 in adipose tissue – a mediator of improved metabolic regulation?
The active GC cortisol is essential to meet the demands of everyday life. This requirement is particularly evident in patients with Addison’s disease (chronic adrenal insufficiency) requiring lifelong substitution with hydrocortisone to avoid a life-threatening adrenal crisis in stressful situations (284). However, when there is too much of a good thing, e.g., in chronic stress (285) or long-term drug treatment with GCs (286), hypercortisolemia has widespread negative effects throughout the body, including metabolic dysregulation, increased risk for cardiovascular disease (287), and impaired brain function.

The HPAA is the major determinant of circulating cortisol levels (134). Furthermore, cortisol exposure is regulated specifically in tissues. Within this system, 11βHSD1 is considered to play a major role, especially in relation to obesity and metabolic dysfunction (183). In a simplified view, the reduced expression of 11βHSD1 in adipose tissue after diet-induced weight loss would cause decreased production of cortisol in adipose tissue, which in turn would lead to increased insulin sensitivity. This improved insulin sensitivity in adipose tissue would then lower lipid mobilization and increase uptake of
lipids into adipose tissue from lipoproteins in the circulation, thus lowering circulating levels of triglycerides and free fatty acids (288). Furthermore, local cortisol production by 11βHSD1 in subcutaneous adipose tissue has been suggested to represent as much as one third of the total systemic cortisol production in obese humans and could obviously impair insulin sensitivity in other tissues as well (178). However, we found no evidence of a link between decreased 11βHSD1 expression and improved insulin sensitivity. This result could be related to several factors, including that the participants were rather healthy obese without major insulin resistance, the use of crude measures of insulin sensitivity, or a dissociation between 11βHSD1 expression and 11βHSD1 activity (although these measures are usually well correlated (244)). Another, more complex explanation is possible, however. In vitro studies have consistently shown that 11βHSD1 is bidirectional, interconverting cortisone and cortisol, but in vivo 11βHSD1 is primarily a reductase converting cortisone to cortisol (183). This pattern implies that increased 11βHSD1 expression and activity equals increased cortisol regeneration. However, results of a study using a dual tracer technique with labeled cortisone and cortisol infusions suggested that 11βHSD1 is bidirectional in vivo and that the balance between reductase and dehydrogenase activity may be tissue-specific (190). Future studies will benefit from either estimates of 11βHSD1 reductase and dehydrogenase activity specifically or cortisol and cortisone levels within the target tissue. If 11βHSD1 does not contribute to increased cortisol levels exclusively, this feature may explain the lack of association between 11βHSD1 expression and insulin sensitivity in our study as well as in previous studies (234, 235).

Although the effects of decreased adipose 11βHSD1 expression on insulin sensitivity in paper 2 were questionable, 11βHSD1 may have other beneficial effects in relation to obesity and metabolic dysregulation. For example, inhibiting or decreasing 11βHSD1 activity in macrophages may reduce the risk of atherosclerosis (289). Inhibition of 11βHSD1 in cardiomyocytes may also improve outcome after myocardial infarction (290). Future studies will elucidate whether lifestyle modification could reduce the conversion of cortisone to cortisol by 11βHSD1 in the brain and thereby lower the risk of cognitive impairment and dementia in T2DM. Addressing such a question is, however, a tricky business because 11βHSD1 activity in the brain is not measureable with the standard cortisol tracer technique (198).

**Increased 5α-reductase activity – a protective mechanism?**

The shifted balance towards 5α- rather than 5β-reduction of GCs after a long-term diet intervention has not been reported previously. The increased 5α-reductase activity in obesity and insulin resistance has mainly been considered an adverse effect, activating the HPAA as a consequence of the increased metabolic clearance rate of GCs (146, 232). However, recent
studies have highlighted a more complex role for 5α-reductase in metabolic disease (291). 5α-reductases type 1 (5α-R1) and type 2 (5α-R2) are expressed in liver, but 5α-R2 appears to be the predominant enzyme in GC metabolism. On the other hand, only 5α-R1 is expressed in adipose tissue. Consequently, although treatment with the dual 5α-reductase inhibitor dutasteride and the 5α-R2 inhibitor finasteride decreases urinary excretion of 5α-reduced GCs to a similar extent, dutasteride causes insulin resistance in adipose tissue (291). This effect suggests that 5α-R1 actually protects adipose tissue from increased cortisol levels. Obviously, because we analyzed excretion of urinary GC metabolites, we could not tie the increased excretion of 5α-reduced metabolites to either type of 5α-reductase or specific tissues. Nevertheless, these studies may indicate that the shifted balance towards 5α-reduction of GCs, possibly related to weight gain, could be protective rather than detrimental to metabolic regulation.

**Higher cortisol levels – a cause or consequence of altered brain structure?**

A dysregulated HPAA and increased cortisol levels have been suggested to play a role in the development of several psychiatric diseases causing memory impairments (157, 292, 293) as well as memory impairments in T2DM (156, 174, 195). Furthermore, hypercortisolemia resulting from Cushing’s disease is associated with impaired function in several cognitive domains, including verbal memory, as well as smaller brain volumes, especially in the cingulate gyrus and the hippocampus. These adverse effects are partly but not totally reversible after correction of the hypercortisolemia (138, 294). Thus, although paper 1 was a cross-sectional study, precluding the possibility of drawing conclusions regarding causality, results of previous studies clearly suggest that high cortisol levels cause both structural and functional brain alterations. Furthermore, even though the MRI examinations were performed almost one year after the saliva sampling, the negative associations with cortical surface areas still were present in brain areas previously implicated in regulation of the HPAA (295) and stress response (296), as well as negatively associated with impaired feedback inhibition of the HPAA (171). This association could be explained in three possible ways: 1) The smaller cortical surface areas are inherited and decrease the negative input from the prefrontal cortex on the HPAA, leading to increased cortisol levels (262, 297); 2) the increased cortisol exposure has induced non-reversible structural alterations to the prefrontal cortex (294, 298); or 3) a hypersensitive HPAA is a relatively stable trait determined by genetic and epigenetic factors (299), exposing the individual to high cortisol levels repeatedly (300, 301). Future longitudinal studies with repeated measurements and including more aspects of HPAA regulation could shed light on these potential relationships. Furthermore, studies on HPAA dynamics suggest that diurnal rhythmicity is a crude proxy for an underlying
ultradian rhythm with hourly cortisol pulses (302). If and how this ultradian rhythm is altered in metabolic dysfunction and whether it relates to altered brain structure and function are currently unknown.

Cortisol levels, brain structure, and cognition in men and women – similarities and differences
This thesis provides limited evidence of gender differences in the association between cortisol levels and brain structure. It was only in the left rostral middle frontal gyrus that cortisol levels were negatively related to surface area among women but not men. Thus, although we extended previous research in men, to include women as well, we could not confirm the research findings in rodents suggesting that females are protected from the adverse effects of corticosterone and chronic stress on prefrontal cortex morphology (258). However, MRI may not be sensitive enough to pinpoint differences at a neuronal level such as altered dendritic morphology, and the majority of women in our sample were postmenopausal. This factor is important because estradiol has been proposed to play a key role in mediating these gender differences. On the other hand, higher cortisol levels were associated with lower working memory performance among men, but not women. These differences could thus not be explained by different brain structure, in contradiction to previous studies indicating improved working memory performance after acute stress in men but not women (303). However, the positive effects of cortisol have been described only in relation to reaction times and not the number of correct responses in the working memory task. Moreover, acute vs. chronic effects of cortisol on cognitive functions may be different (304).

Does the HPAA constitute a target to reduce the risk of diabetes-associated cognitive impairments?
The clinical importance of the findings from paper 1 can be debated because of the limited association between higher cortisol levels and impaired cognitive function among women and men in general. However, the fact that the cortical surface area in the left anterior cingulate cortex and right rostral middle frontal gyrus was related to visuospatial abilities, with high demands on prefrontal brain function, highlights these brain regions as potential links between states of elevated cortisol levels and impaired cognition (305). Moreover, stress or other causes of elevated cortisol levels could be detrimental to memory functions in T2DM (156, 195). Thus, it may be particularly important to reduce cortisol levels and normalize HPAA function in T2DM to reduce the risk of cognitive impairments. The non-selective 11βHSD inhibitor carbenoxolone has indeed been suggested to improve verbal memory performance in T2DM (196), possibly by decreased conversion of inactive cortisone to cortisol in the brain. Whether similar
effects can be achieved by using selective 11\(\beta\)HSD1 inhibitors or other approaches to lower cortisol exposure, e.g., by stress-reducing interventions, remains to be elucidated.

**Lifestyle interventions as potential modifiers of hippocampal function**

Memory research is focused on the hippocampus for several reasons. By 1957, it already had been reported that medial temporal lobectomy causes a loss of the ability to encode new memories (306). Today, we know that atrophy of the medial temporal lobe is a hallmark of Alzheimer’s dementia (274, 275, 307) and that hippocampal atrophy has been linked to several other diseases associated with memory impairments, such as depression (308) and T2DM (67). Lifestyle factors like physical exercise (127), diet (227), and cognitive training (309) may have the potential to improve hippocampal function. In our first intervention study (paper 3), we found that 6 months with a PD or a diet according to the NNR led to increased hippocampal BOLD responses during memory encoding. This finding was partly replicated in our second intervention study (paper 4) in which the change in hippocampal BOLD response differed significantly between the groups; it increased in the intervention groups (PD + PD-EX) and decreased in the OG. We find it unlikely that the increased BOLD response was a test-retest effect since it decreased in the OG. However, although the memory performance increased in paper 3, it was unchanged in paper 4 and did not correlate with hippocampal BOLD responses in either study. Thus, the potential benefits from the increased hippocampal activity on memory performance can be questioned. But notably, other studies have found that a high brain response in the hippocampus is associated with a high episodic memory performance (254).

In both study 3 and 4 the BOLD response in the right anterior hippocampus increased clearly in about half of the participants in the intervention groups. On the other hand, no participants in the weight-stable OG (study 4) displayed increased BOLD responses. Thus, it would be interesting to investigate potential differences between responders and non-responders. Alterations in BDNF levels may be one factor since it was strongly correlated with alterations in BOLD responses in the right posterior hippocampus in study 4 (figure 9). The val\(^{66}\)met polymorphism in the BDNF gene causes impaired intracellular trafficking and secretion of BDNF in the hippocampus (282) and has been associated with lower encoding-related BOLD responses in the right posterior hippocampus (283) and impaired memory performance (310). However, most studies indicate that this polymorphism does not affect circulating BDNF levels (311). Furthermore, although several studies suggests that BDNF levels increase after acute exercise, the results of studies on long-term exercise is mixed (312) and to my knowledge, studies examining the potential influence of BDNF gene
polymorphism on circulating BDNF levels after lifestyle interventions including diet and exercise are lacking. Other gene variants such as the Apoε4 may also affect hippocampal BOLD responses (283). Moreover, factors related to more severe brain damage in T2DM such as insulin resistance, hyperglycemia, longer diabetes duration and hypertension could also differentiate the responders from non-responders.

In summary, the results presented in papers 3 and 4 indicate that lifestyle modifications can increase encoding-related hippocampal BOLD responses, and the absence of group differences suggests that these effects are probably related to improvements in metabolic regulation rather than to the specific interventions. All participants in the studies described in papers 3 and 4 were recommended to perform at least 30 minutes of moderate physical activity per day in addition to the dietary interventions. Thus, although total physical activity did not increase significantly in either study (313), it cannot be excluded that the measurements missed a potential increase in activity levels. Because relatively low-intensity physical activity has been associated with increased hippocampal and prefrontal cortex volume, the potential effects of everyday physical activity should not be underestimated (126-128). Furthermore, this suggestion may explain the absence of additive effects of high-intensity exercise on hippocampal function in study 4 even though the adherence to the high-intensity exercise was high and aerobic fitness tended to improve more in the high-intensity exercise group.

Future studies should try to find markers that predict the individual response to an intervention; this will be critical to give personalized recommendations on diet and exercise that can improve brain health and cognitive functions.

Prefrontal cortex – more or less?

There is no clear answer as to whether increased or decreased BOLD responses are beneficial with respect to cognitive function. Those who preserve memory function with age have higher BOLD signals in the left prefrontal cortex during memory encoding (255) and memory retrieval (281) than low-performing individuals. Two explanations have been proposed for these differences: 1) They could reflect that those with preserved memory function during aging compensate for impairments in other brain regions by increasing prefrontal brain activity (281); or 2) individuals with maintained prefrontal brain function throughout the lifespan have preserved memory function (35, 314, 315). In either circumstance, the increased BOLD response in the left inferior frontal gyrus during memory retrieval (paper 4) could represent beneficial changes. On the other hand, other parts of the left inferior and middle frontal gyrus have been related to retrieval effort (316). From this point of view, the lowered prefrontal BOLD response during
memory retrieval in paper 3 could also represent beneficial changes (276). However, changes in BOLD signal and memory performance showed no correlation in either study. One important explanation may be that the memory task included in these studies was not sensitive enough to detect small changes in memory function. Moreover, the interventions may have improved other cognitive domains impaired in obesity and T2DM that require intact prefrontal cortex functions such as working memory, processing speed, or executive function (22-24, 29). Nevertheless, our findings suggest that functional brain responses in the prefrontal cortex can be altered by lifestyle modifications associated with improved metabolic regulation. Future studies testing a wider range of cognitive functions, and how these develop over a longer time span, could reveal the potential long-term effects of these alterations in prefrontal cortex function.

*Increased BOLD responses in T2DM – reversal of diabetes-associated brain changes?*

A main finding in paper 4 was an increased BOLD response in the right lingual gyrus and right medial prefrontal cortex during memory encoding. Previous resting-state fMRI studies in T2DM have suggested that decreased spontaneous brain activity in the bilateral lingual gyrus correlates with worse executive function (75). Furthermore, others have found decreased functional connectivity within the default mode network, including the medial and inferior prefrontal cortices (77, 317), as well as decreased spontaneous brain activity in the medial prefrontal cortex in T2DM (76). A recent study also showed decreased BOLD responses in the left prefrontal cortex during memory encoding in T2DM compared to healthy controls but no differences in the prefrontal cortex under retrieval conditions (279). In summary, although most functional imaging in T2DM has been made under resting-state conditions, these studies have consistently found altered connectivity in the same areas where we found increased BOLD signal during memory encoding after the intervention. Thus, it can be hypothesized that the connectivity in these brain areas improved after the interventions described in paper 4. Indeed, it was recently proposed that aging causes increased functional connectivity between the left and right hippocampus during rest and that this increase is related to impaired connectivity between the hippocampus and prefrontal cortex during memory encoding (318). The increased encoding- and retrieval-related functional brain responses after the Paleolithic-type diet interventions in study 4 may thus represent a reversal of the impaired functional connectivity in T2DM.

*Methodological issues in papers 3 and 4*

The episodic memory paradigm used in papers 3 and 4 has been used previously in a large population-based cohort. A critical point is that the
encoding and retrieval networks (i.e., brain areas activated more during memory encoding and retrieval than during the control task) in our interventions overlapped with those presented in these previous studies (254, 255). Hence, the task was adequate for assessing functional brain responses related to episodic memory function.

The sample sizes included in these interventions were rather small, with limited power to find group differences. According to simulations, increasing the sample sizes to about 20 participants per group would probably have substantially increased the power for making group comparisons (257). This point should be taken into consideration in future studies. Furthermore, paper 3 did not include a weight-stable control group, limiting the possibility of excluding test–retest effects on BOLD responses. This weakness was at least in part corrected in paper 4, although the weight-stable control group included only six participants.

At first glance, it might be surprising that the effects on BOLD responses described in paper 4 (p < 0.005 uncorrected in global brain analysis) were much weaker than those found in paper 3 (p < 0.05 corrected for multiple comparisons) because the sample size was larger in paper 4 (n = 24 vs. n = 20 in paper 3). However, the duration of the interventions may be one critical factor explaining these results because previous findings on physical exercise and nutrients suggest that it may take at least 6 months before significant effects on brain function emerge (125-128, 223, 227). Future studies including several measurements over time could perhaps elucidate the time spans that are needed to affect cognition and brain function. Furthermore, T2DM increases the amount and severity of changes in brain structure and function associated with obesity (see Introduction). Thus, these changes may be less reversible, requiring longer interventions and more sensitive methods to find improvements.

fMRI is a widely used brain imaging method to study underlying functional brain responses to cognitive tasks (319) and how these are affected by, for example, Alzheimer’s disease (320). However, because fMRI measures the BOLD signal, which is dependent on blood flow, blood volume, and oxygenized hemoglobin, specific conclusions cannot be drawn regarding whether our interventions had effects on these parameters or on neuronal signaling per se (321). The BOLD signal is, however, correlated with synaptic activity in general (253). Future studies could involve measurement of cerebral blood flow with specific MRI sequences (322). Furthermore, other imaging modalities such as positron emission tomography could reveal underlying changes in neurotransmitter systems. This approach would be particularly interesting because the results of animal studies have suggested an increased turnover of dopamine related to insulin resistance and that this increase may contribute to cognitive impairments and a heightened risk of depression in T2DM (323).
Can the results from this thesis be transferred to other settings?
One important aspect when interpreting these results is their external validity — whether they can be transferred to the general population from which the study subjects were recruited. In paper 1, a population-based sample of women and men was assessed. However, although this selection should be unbiased, the possibility of skewed participation that could affect the sample in both directions cannot be excluded (324). Subjects with bad health or chronic diseases may be less likely to participate in this type of study. On the other hand, individuals with an increased risk of developing dementia may be more likely to participate in a study focusing on brain function. In the intervention studies (papers 2–4), a range of exclusion criteria was applied to increase the homogeneity of the samples. Consequently, a large number of individuals were excluded from participating (66% in papers 2 and 3 and 90% in paper 4). The included participants were probably more motivated and perhaps healthier than their respective population, which could lead to an overestimation of the putative effects of lifestyle interventions in an everyday clinical setting. Nevertheless, the results provide an example of the possible powerful effects of lifestyle changes on obesity and associated metabolic dysfunction. Furthermore, applying a mechanistic approach when studying the organ- and tissue-specific effects of lifestyle interventions makes it important to use a homogeneous sample, decreasing the number of potential confounders.
Concluding remarks

The rapid increase in obesity and associated T2DM is of great concern for future health and longevity, including cognitive impairments leading to dementia. This thesis and previous research suggest that increased demands in society along with exposure to chronic stress may potentiate these negative effects even further. However, this thesis also provides an example of the potential for lifestyle improvement to counteract obesity-related morbidity. This potential includes effects putatively mediated by altered cortisol exposure within tissues, ultimately decreasing the risk of developing dementia (131). Nevertheless, several questions remain to be answered, perhaps the most important of which is how to achieve these improvements in an everyday clinical setting and sustain them over time (325). Realizing this goal will require multiple approaches on the societal and individual levels to promote a healthy lifestyle throughout the lifespan (30).
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