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Analysis of selected genes in murine brain under different dietetic conditions

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# 1 Introduction

The structure of Medium-chain triglycerides (MCTs) consists of glycerol bound to three fatty acids (FA), each with an aliphatic chain containing 8 to 12 carbon atoms, classified as medium-chain fatty acid (MCFA). In comparison to MCTs, long-chain triglycerides (LCTs) differ in the length of their long-chain fatty acids (LCFAs), containing 13 or more carbon atoms (**Figure 1**). MCTs are naturally occurring in commodities like coconut oil, while LCTs are commonly found in products such as butter or breast milk. MCTs and LCTs also differ in their metabolic pathways and transport mechanisms. While LCTs are broken down into LCFAs that are incorporated into chylomicrons and transported via the lymphatic system, MCTs bypass the chylomicron pathway almost entirely and are transported directly through the hepatic portal vein (Cheng et al., 2024; Friedman et al., 1990; Papamandjaris et al., 1998).

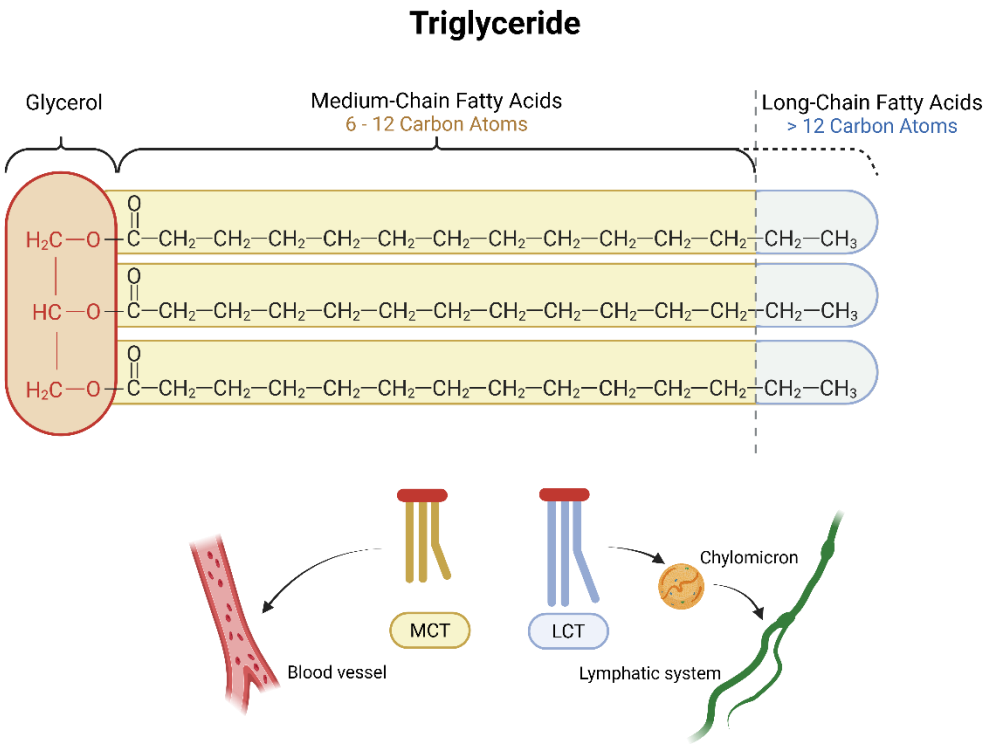


***Figure 1: Graphical illustration of the structural differences between a medium-chain triglyceride (MCT) and a long-chain triglyceride (LCT)*** *and their main transport mechanisms.*
*Created in https://BioRender.com*

LCFAs require the carnitine shuttle, involving CPT I and CPT II, to cross the mitochondrial membrane. In contrast, MCFAs are converted into medium-chain acylcarnitines and are able to directly enter the mitochondria, therefore they are used primarily for energy production via β-oxidation (Dambrova et al., 2022). This results in a much lower incorporation of triglycerides from MCTs into chylomicrons, thus MCTs are rapidly metabolized for energy or ketone production, whereas LCTs are more often stored or used for sustained energy release. Ketone bodies produced in the liver, mainly β-hydroxybutyrate (β-HB) and acetoacetate, can be transported to extrahepatic tissue, including the brain. There they undergo ketolysis and are converted back into acetyl-CoA to fuel the Krebs-cycle. This is especially important in the brain when glucose is limited. The brain then relies on ketone bodies for energy rather than directly metabolising FA via β-oxidation, even though the brain is capable of taking up certain FA. This can either occur through the so called “flip-flop” mechanism, which lets unesterified and uncharged FA directly pass the brain’s lipid bilayers, or by a protein-mediated diffusion. In the case where FA are transported through proteins, it is also possible for LCFAs to use this mechanism to pass the BBB. This metabolic preference is thought to be a protective strategy, as neurons possess only limited capacity for mitochondrial fatty acid oxidation (FAO), probably to reduce the production of reactive oxygen species (ROS) (Mitchell et al., 2011; Mitchell & Hatch, 2011; Schönfeld & Reiser, 2013). Nonetheless, once inside, both LCFA- and MCFA-derived acyl-CoA are oxidized to produce acetyl-Coenzyme A (acetyl-CoA), which drives ketone body synthesis (Friedman et al., 1990; Papamandjaris et al., 1998).

## 1.1 The Brain

The human brain is a complex organ characterized by its intricate structure and specialized metabolic processes, setting it apart from other organs. Anatomically, it consists of several key regions (**Figure 2**), each with distinct functions. The largest part is the cerebrum, responsible for higher cognitive functions such as reasoning, sensory perception, and voluntary muscle movements. The cerebellum, located posteriorly, coordinates balance and fine motor control. The brainstem, connecting the brain to the spinal cord, regulates essential autonomic functions like heartbeat and respiration. The cerebral cortex, a layer of neural tissue covering the

cerebrum, is important for processing information. (Brain Anatomy and How the Brain Works, 2021; Brain Basics, n.d.) Within the brain's deeper structures, the hypothalamus, thalamus, and hippocampus play pivotal roles in regulating emotions, memory processing, and autonomic functions. These regions are interconnected through complex neural pathways and are often involved in neurodegenerative diseases, where their dysfunction can lead to symptoms such as tremors. The brain's cellular network primarily consists of neurons and basal ganglia cells, which rely on neurotransmitters to transmit signals. (Brain Basics, n.d.; Dienel, 2019)

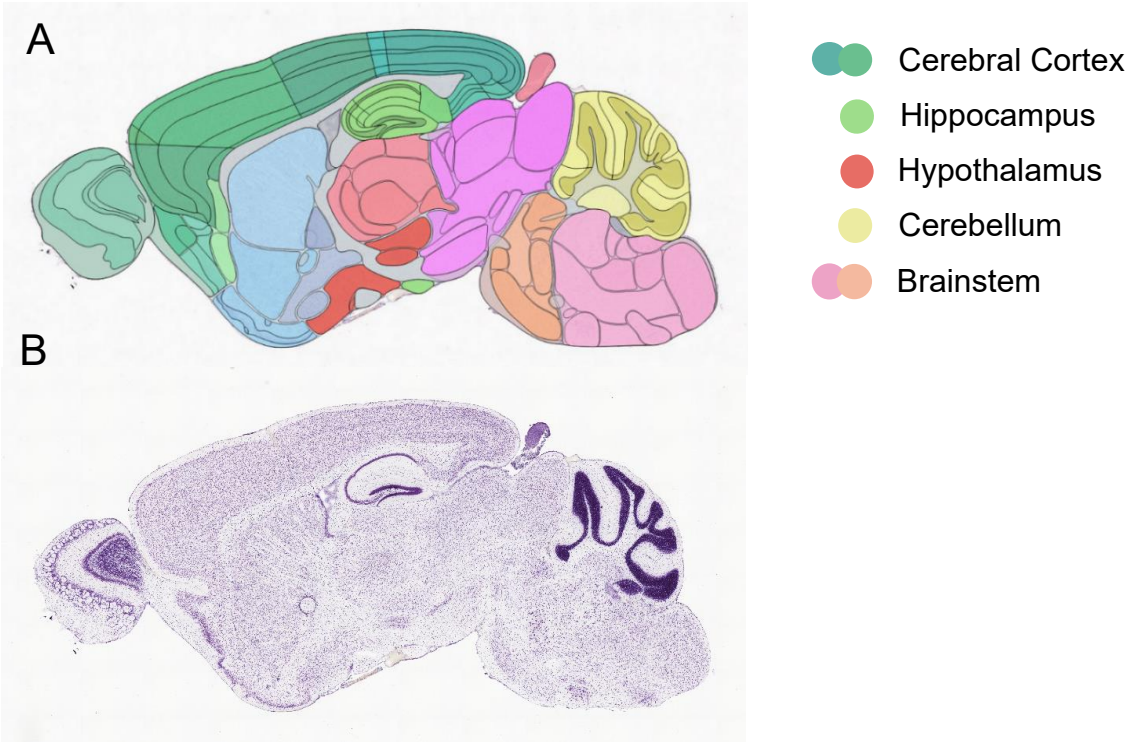


***Figure 2: Sagittal cut through the brain of an adult mouse. A*** *Schematic overview illustrating the segmentation into specific brain areas and* ***B*** *a Nissl-stained histological tissue section showing neuronal distribution across these areas. For this study the mouse cortex and hippocampus were used but the hypothalamus, striatum, cerebellum and brainstem were also taken separately for further experiments.*
*Pictures are taken from https://atlas.brain-map.org/*

A distinctive feature of the brain is the blood-brain barrier (BBB), a selective permeability barrier that maintains the brain's microenvironment by controlling the passage of certain substances from the bloodstream. In contrast to LCFAs, MCFAs and free fatty acids (FFA) can easily cross the BBB, presumably with the help of transport proteins. (Dunn et al., 2023; Mitchell & Hatch, 2011) The BBB's tight junctions are more restrictive compared to endothelial cells in other organs, limiting the types of molecules that can enter the brain. Metabolically, the brain mainly relies on glucose for energy under normal conditions. The astrocyte-neuron lactate shuttle hypothesis proposes that astrocytes are converting glucose to lactate and thereby fuel neurons. Glutamate released from neurons is taken up by astrocytes and triggers glycolytic activity, the lactate is then taken up by neurons via MCT2 and once inside the neurons it is converted to pyruvate and enters the TCA-cycle. But studies suggest that neurons do also uptake and phosphorylate glucose to sustain synaptic activity and neurotransmission especially during increased activity (Dienel, 2019; Kim et al., 2025; Patel et al., 2014). However, during periods of low carbohydrate availability or increased energy demand, the brain can adapt by using alternative energy substrates such as lactate or ketone bodies, which are also able to cross the BBB. This metabolic flexibility helps maintain neuronal function when glucose metabolism is impaired (Dienel, 2019; Mitchell & Hatch, 2011; Raichle, 2010).

These abilities have been observed in both humans and animal models such as mice. It is important to emphasize that there are certain differences between a murine and a human brain. These include structural and anatomical variations, such as overall size but also the proportional amount of gray to white matter, which is lower in humans than in mice. The surface of the human brain also differs and shows more folds compared to that of mice, which tend to have a smoother surface. Additionally, there are certain differences in gene expression, which should especially be taken into account when translating findings to humans, as not all of them overlap with those of rodents and some are expressed only in one of the two species (Hagan et al., 2012; Treuting et al., 2012; Ventura-Antunes et al., 2013; N. Xu et al., 2022).

## 1.2 Ketogenic Diet

The definition of a KD varies somewhat between studies and papers, but fundamentally, a KD is intended to promote an increased production of ketone bodies, which is typically associated with elevated concentrations of β-HB, acetone, and acetoacetate, eventually leading to ketosis. However, this state can be achieved not only through a diet with a very high fat content (60-90% of consumed calories) but also through prolonged fasting or calorie restriction.

### 1.2.1 Health Aspects of a Ketogenic Diet

Besides the use of KDs as a treatment for epilepsy, they have also been shown to offer many other benefits. In type 2 diabetes and weight management, especially when combined with MCTs, KD can be effective in lowering blood glucose levels and promoting weight loss, thereby also reducing the risk of cardiovascular diseases (Adafer et al., 2020; Tisdale & Brennan, 1988; Yang et al., 2021). Positive effects of KD or fasting are also being investigated in autoimmune diseases such as multiple sclerosis, with some studies already showing beneficial effects on autoimmunity, as well as on mental health (Brenton et al., 2019; Choi et al., 2016). Interest in research on KD and fasting or calorie restriction is growing due to its great potential in various areas, including migraine, ADHD, depression, schizophrenia, and more (Danan et al., 2022; Di Lorenzo et al., 2019; Dyńka et al., 2022; Sethi et al., 2024).

#### 1.2.1.1 History of Ketogenic Diet in Healthcare

Fasting has been practiced for a long time across many cultures and for a wide variety of reasons. In nearly all religions, fasting plays a role, whether for spiritual purposes or as a form of "cleansing". But fasting has also had a place in medical practice. Physicians in the 19th century documented and recommended fasting and caloric restriction ("starvation") as a healthy and effective method for treating overweight and digestive issues. However, dietary changes like these had already been practiced much earlier for various reasons. That said, complications were much more common back then, probably because caloric intake was often far too low, maintained for too long, and no attention was paid to covering the essential nutrients. Complications included gout attacks, kidney issues, cardiac arrhythmias,

anaemia, vitamin deficiencies and the refeeding syndrome. Still, it was hard to ignore the many positive aspects of fasting when done under the right conditions (Kerndt et al., 1982).

Before it was discovered that ketosis could also be induced by a high-fat diet, the associated benefits were attributed solely to fasting. There are also indications that fasting may have been used as a treatment for epilepsy long before the first recorded study conducted by physicians in 1911. After the discovery of diphenylhydantoin as an epilepsy treatment in 1938, the use of KD became much less common and lost significance in this field. This changed rapidly as reports of drug-resistant epilepsy increased, leading to a resurgence of KD as an effective alternative therapy, particularly for infants and young children, a practice that continues to this day. The exact mechanisms underlying its effects remain unclear, as KD can influence metabolic pathways through multiple mechanisms, but several hypotheses have been proposed and will be briefly discussed in the following section (Sampaio, 2016; Wheless, 2008).

### 1.2.2 Pathways Linked to Ketogenic Benefits

Particularly interesting in the context of neurological disorders is the impact of KD on neurotransmitters and the resulting changes in metabolism within neurons and glial cells. In animals experiencing ketosis, it has been shown that the brain may preferentially metabolizes acetate due to increased glutamate and glutamine concentrations (Yudkoff et al., 2005). Astrocytes are located in regions where the regulation of synaptic plasticity is essential, such as the hippocampus and cortex, as they are also responsible for maintaining the stability of the blood-brain barrier (BBB). During ketosis, the metabolism of astrocytes adapts, particularly in the cortex, where they tend to accumulate acetate. This occurs because astrocytes preferentially process and transport acetate compared to neurons. (Waniewski & Martin, 1998; Yudkoff et al., 2005).

These astrocytes are also GABA-receptive cells, and ketosis leads to an increase in glutamate, which can enhance GABA synthesis (**Figure 3**). The elevation of GABA could be one of the reasons for the antiepileptic effect of a KD. GABA plays a role in various other processes, for instance, it may have protective effects for Alzheimer’s and Parkinson’s disease and is also used as a treatment for mental

disorders. (Rho, 2017; Yudkoff et al., 2005) The increased GABA synthesis during KD occurs because glutamate can be utilized for either GABA or aspartate production. However, aspartate formation is reduced since it requires oxaloacetate. During ketosis, oxaloacetate is increasingly needed in the Krebs cycle, where it combines with the elevated levels of acetyl-CoA (resulting from the KD) for further metabolism. Consequently, glutamate is more likely to be converted into GABA via glutamate decarboxylase. (Rho, 2017; Yudkoff et al., 2005)

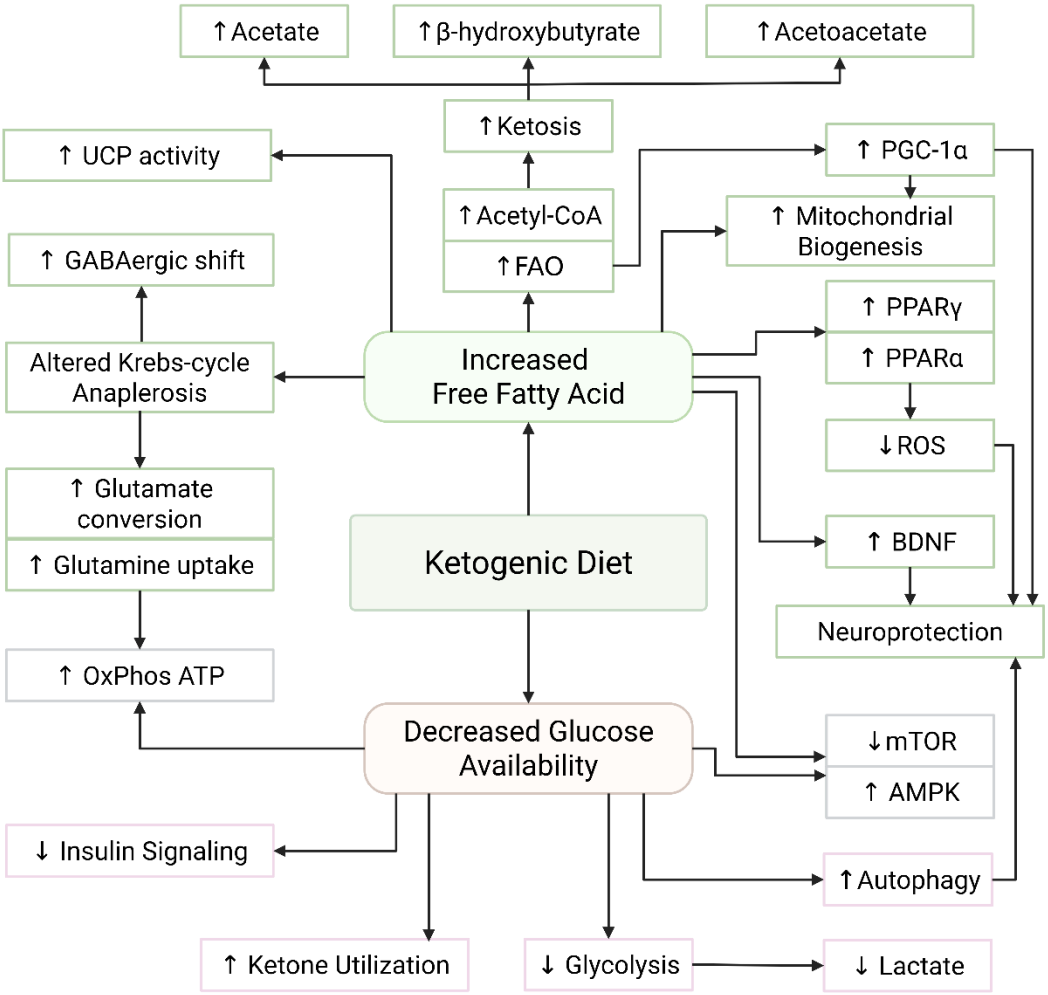


***Figure 3: Schematic illustration of key metabolic pathways potentially induced by ketogenic diet****, reflecting mechanisms suggested by current literature, with a focus on energy homeostasis and neuroprotection.*
*Created in https://BioRender.com*

However, a KD has additional effects that could partly explain its positive impact on epilepsy. Studies have shown that KD promotes mitochondrial biogenesis through increased expression of certain genes, as well as an elevated phosphocreatine/creatine ratio in the hippocampus (Bough et al., 2006). This implies greater ATP availability, but also a reduction in reactive oxygen species (ROS) has been observed with this dietary approach. These effects are partly attributed to pyruvate, which is thought to be both neuroprotective and anticonvulsant, and may contribute to synaptic stabilization (Bough et al., 2006; D. Y. Kim et al., 2010). In a low-carbohydrate diet, pyruvate is increasingly produced as an end product of glycolysis, which is subsequently decarboxylated to acetyl-CoA (Dobritzsch et al., 1998). There are also other effects of KD, such as anaplerosis, reduced histone deacetylase activity, among others, which may also contribute to these positive outcomes (Rho, 2017; Sternberg et al., 2023).

## 1.3 MCT Effects in Murine Models

In recent years, MCTs have gained attention for their potential neuroprotective effects and have been studied for their ability to influence cognitive function and neuroinflammation in animal models. The following study highlights some of these findings that focused on MCTs.

In the study from Lin et al. (2022), which aimed to induce ketogenesis, the primary goal was to assess cognitive function in mice. It included a control and an intervention group, both of which were fed a high-fat and high-cholesterol diet (HFHC) for 16 weeks. After this period, a part of the intervention group was switched to a high-fat diet containing mostly MCTs, continuing until week 24. Cognitive testing was conducted at week 16 and 24 using the Morris water maze. Mice that received MCTs performed significantly better in the cognitive test compared to those in the HFHC and control groups (D.-T. Lin et al., 2022). Similar interventions also demonstrated that KD-MCT fed mice achieved better cognitive outcomes (Sun et al., 2024), and improvements were also noted in motor performance. Especially in cases of acute keto-exposure with MCTs, better results were observed in the accelerating rotarod test. Even during longer interventions, MCT mice outperformed both KD and control groups. Results in WR-mice were quite similar, particularly when ketosis was induced using ketone esters rather than

ketone salts (Ari et al., 2020). Positive outcomes with MCTs were also seen in pathological murine models, such as mice with Parkinson's disease or GLUT1 deficiency, and a variety of MCT supplementation strategies yielded comparable improvements (Ari et al., 2020; Shcherbakova et al., 2022; Zhang et al., 2023).
Studies have shown that a KD can have rather negative effects on cardiac health. In mouse cardiomyocytes, exposure to β-HB led to increased apoptosis and consequently to fibrosis of these cells (S. Xu et al., 2021) . In vivo, an eight-week KD intervention based on LCTs also induced cardiac cell fibrosis and caused accumulation of 4-hydroxy-2-nonenal, a marker of lipid peroxidation linked to fibrotic remodelling. Additionally, an MCT-KD was tested too, which showed a more favourable effect on mitochondrial size but did not reduce 4-hydroxy-2-nonenal accumulation nor did it prevent collagen deposition and thus likewise induced cardiac fibrosis (Sternberg et al., 2023) .

### 1.3.1 Neurological Effects of MCT Supplementation in Murine Models

Several studies have pointed out the potential health benefits of ketones and MCTs, particularly in neurological contexts. In some medical fields KD has already been used for a long time as a medical nutrition therapy such as epilepsy, by providing an alternative energy source for neurons (Zupec-Kania & Zupanc, 2008). Research demonstrates that brain glucose metabolism declines with age, in Alzheimer's disease (AD) and other degenerative disorders, leading to cognitive impairment. Ketone bodies serve as an alternative energy source for the brain, bypassing deficits in glucose metabolism. This metabolic shift has been linked to improved mitochondrial function, reduced oxidative stress, enhanced synaptic activity, and may even reduce β-amyloid accumulation, underlining the potential of MCTs to support brain energy metabolism under conditions of impaired glucose utilization (D.-T. Lin et al., 2022; Cunnane et al., 2016; Kashiwaya et al., 2000).
In two mouse studies, a diet rich in MCTs reduced inflammation and increased protective factors in the brain. In one experiment, young mice were fed a high-fat diet for 16 weeks, which increased their ketone bodies similar to a KD. Then for the next eight weeks they were switched onto an MCT-based KD. Protein expression was measured in their hippocampus and cortex and the MCT-fed mice

showed lower levels of the inflammatory markers NF-κB and TNF-α and a higher expression of brain-derived neurotrophic factor (BDNF) compared to mice that stayed on the classic high-fat chow (D.-T. Lin et al., 2022). In a second study, mice with a Parkinson's-like condition were put on either a standard diet or an MCT-KD diet for five weeks. Here the assessments included histological and biochemical readouts of brain cell health, as well as changes in gene expression. Again, the MCT-KD mice showed reduced NF-κB and TNF-α, higher BDNF and healthier mitochondrial function. BDNF is essential for neuron protection and plasticity, and low BDNF levels are often associated with stress and an increased risk of psychiatric disorders such as depression (Sasi et al., 2017). Together these studies demonstrate that adding MCT to a high-fat or KD can lower brain inflammation and boost BDNF, even in animals with Parkinson's like symptoms or diet-related stress (Zhang et al., 2023). The reduced neuroinflammation may also contribute to the decreased apoptosis and neuronal damage observed in the hippocampus and cortex (Shcherbakova et al., 2022; Sun et al., 2024). Glial Fibrillary Acidic Protein (GFAP) was notably elevated in the HFHC group and is considered a potential indicator of various neurological disorders, such as AD. Studies have shown that, in addition to Tau and β-amyloid, GFAP levels are significantly elevated in individuals with Alzheimer's and could potentially serve as a biomarker (K. Y. Kim et al., 2023; D.-T. Lin et al., 2022). Studies further indicate that MCT supplementation can boost ketone availability in the brain, which could potentially improve memory and cognitive function in aging and AD. When combined with docosahexaenoic acid (DHA) supplementation, an essential omega-3-FA for brain development, MCTs seem to have even stronger effects. This includes the inhibition of neurocyte apoptosis, reducing neurocyte damage, and slow down age-related cognitive decline (Sun et al., 2024). Even in Parkinson's Disease, improvements in motor skills and other symptoms have been observed in both animal models and humans through the ketogenic diet (KD) (Phillips et al., 2018; Shaafi et al., 2016). Other recent studies have shown the positive effects of KDs may have on brain health include the possible restoration of cognitive function lost due to brain injury (Har-Even et al., 2021), alleviation of mood disturbances (Robbins & Solito, 2022), and improvements in memory (Acuña-Catalán et al., 2024)

Taken together, the ability of MCFAs to cross the BBB, along with their beneficial effects on mitochondrial function, neuroinflammation and enhanced neuronal survival underscores their potential as neuroprotective agents (Cunnane et al., 2016; Dunn et al., 2023; Sun et al., 2024).

### 1.3.2 PPAR-γ

One molecular pathway that is associated with the metabolic and neuroprotective benefits of KD involves the activation of peroxisome proliferator-activated receptor gamma (PPAR-γ) by specific MCTs. PPAR-γ is a nuclear receptor that regulates gene expression in response to lipid-derived ligands and is highly expressed in adipose tissue. It plays an important role in adipogenesis, lipid metabolism, glucose homeostasis, energy storage and the modulation of inflammatory responses (Malapaka et al., 2012).

MCFAs, particularly decanoic acid (C10:0), have been identified as direct ligands of PPAR-γ, acting as partial activators of the receptor. Crystallographic analyses reveal that decanoic acid binds specifically within the small ligand-binding pocket of PPAR-γ, inducing conformational changes, which are necessary for its partial activation. This binding pocket is structurally unsuitable for LCFAs, due to their length, or short-chain fatty acids, pointing out its specificity for C10:0. When activated by C10:0, PPAR-γ can modulate glucose metabolism without promoting adipogenesis, offering a safer profile for therapeutic interventions. This selective activation may explain some of the observed metabolic benefits of MCTs, like improved insulin sensitivity in obese and weight neutrality in diabetes (Liberato et al., 2012; Malapaka et al., 2012). Additionally, positive effects have been shown in cardiovascular and Alzheimer's diseases, where they may have protective effects by reducing inflammation (Malapaka et al., 2012; Steinke et al., 2023) Jeong et al. (2011) demonstrated that a KD increases PPAR-γ expression in the hippocampus, both in the mouse brain and in vitro. In the normal adult brain, PPAR-γ is expressed at low levels, primarily in hippocampal neurons, with additional expression in the basal ganglia, thalamus, and piriform cortex.

## 1.4 Caloric Restriction

With increasing age, maintaining health and preventing age-related disease becomes more important. Caloric restriction (CR) is a promising approach that may support healthy aging and longevity. CR refers to a dietary pattern that involves reducing caloric intake without causing malnutrition. In animal studies, CR typically involves a reduction of 10% to 40% of the usual *ad libitum* caloric consumption. CR has been widely recognized for its potential to extend both life- and healthspan across various species, including murines and primates ( A.-L. Lin et al., 2015; Mehrabani et al., 2020; Weindruch et al., 1986). Research also indicates that CR enhances the utilization of ketone bodies as an alternative energy source, ensuring steady energy metabolism in the brain. CR can preserve cerebral blood flow, which usually declines with age, showing stable vascular function. (A.-L. Lin et al., 2015) One of the largest and most well-known studies that has extensively investigated caloric restriction is “CALERIE” (Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy). This study included over 200 participants and was conducted over a period of two years, which, unlike many other studies, focused on long-term effects and also included a control group. Originally, the goal was to reduce calorie intake by 25%, but it eventually settled at 11.9%. However, this seems to result in significantly higher adherence over time and appears to be more compatible with modern lifestyles. In addition to the classical anthropometric data, this experiment primarily focused on investigating the impact on inflammatory markers, oxidative stress, cardiometabolic health, and biological aging processes. The results showed significant differences after just one year, and even more so after two years. As expected, there was a significant reduction in weight, waist circumference, and fat mass, although muscle mass also decreased slightly. Nevertheless, the CR group still had a higher percentage of muscle mass compared to fat mass after two years, in contrast to the control group (Das et al., 2017). There were positive effects on lipid levels, such as a decrease in cholesterol and an increase in HDL-cholesterol levels, as well as a reduction in blood pressure. Additionally, more significant differences were observed in other health parameters. These included improved insulin sensitivity, lower blood glucose levels, and a reduction in C-reactive protein, which may indicate lower inflammation in the body. Generally, CR had positive effects on cardiovascular health and

metabolic syndrome but unfortunately neurological impacts like memory were not tested (Kraus et al., 2019).

In murines and mammals, CR has been shown to improve both motor and cognitive functions such as memory. Beyond these effects, this dietary pattern also led to reduced inflammation and improved mitophagy – a specific form of autophagy, which selectively removes mitochondria. Long-term CR may also reduce the accumulation of amyloid β, potentially contributing to further positive effects on neuronal health (Lobo et al., 2022; Mehrabani et al., 2020). Another interesting area of application for CR, KD, or a combination of both (CRKD), is the potential effect on brain tumors. Tumors in the brain tend to rely heavily on glucose for energy due to the Warburg effect. As previously mentioned, healthy brain cells do prefer glucose, but they are able to adapt and use ketones for energy. Researchers therefore investigated whether CRKD could actually affect brain tumors by lowering glucose availability. Results in mice showed that under CRKD, the tumors in the brain had a significantly reduced growth rate and glucose uptake. These findings provide a basis for future therapies, despite limited human data (Seyfried et al., 2009; Voss et al., 2020). It is worth noting that certain tumors rely on FAs as their preferred energy source and may even enhance and may even promote tumor growth. Some studies have linked higher FASN levels with specific cancer types and poorer outcomes. Tumors that appear to prefer FA include, for example, liver and prostate cancer (Currie et al., 2013; Nath et al., 2015; Röhrig & Schulze, 2016). Furthermore, CR could reduce glucose metabolism in aging brains, as seen in reduced glucose uptake and lactate production. These effects generally promote improved mitochondrial function, mitigate cognitive decline, support neuroprotection, and reduce the production of reactive oxygen species (ROS) (Cullingford et al., 2002; Jensen et al., 2020; A.-L. Lin et al., 2015).

## 1.5 Intermittent Fasting

Intermittent fasting (IF), compared to CR, aligns food intake with the body's circadian rhythm by restricting eating to certain time windows, which can vary from hours to days. Although IF can lead to caloric restriction in some cases, it has demonstrated metabolic benefits even without reducing the calorie intake, such as enhancing ketogenesis and β-oxidation. IF is a term that can cover a broad range of approaches, even though some studies use more specific labels depending on the variant such as "alternate day fasting" or the "fasting-mimicking diet". For example, it could be 8 hours of eating followed by 16 hours of fasting, but in some studies where IF was observed, the participants would have a few days out of the week where they would fast (very low calorie intake) and the other days would be regular eating days. The greatest weight loss has been observed with fasting for several days within a week. On the other hand, the highest adherence rates were seen with IF methods like time-restricted eating (e.g., 8h/16h) and the fasting-mimicking diet (cyclical, with several days of low-calorie intake every few weeks) (Fanti et al., 2021). Certain fasting variants are associated with increased AMPK activation and enhanced FAO – which tend to rise with longer fasting durations – as well as elevated ketogenesis and autophagy. Inflammation and related markers appear to decrease, along with reduced mTOR activity (Antoni et al., 2017; Longo & Panda, 2016; Vasim et al., 2022).

A recent study which focused on IF and brain responses involved two groups of elderly people: one, which was eating a healthy diet according to the USDA dietary guidelines, while the other group was following the same recommendations, but included two consecutive days of fasting per week. This study was conducted over the time period of 8 weeks, and all the participants were insulin resistant. The "healthy living" group was encouraged to reduce their calorie intake based on USDA guidelines, which may have led some individuals to eat less than their energy requirements – thus resulting in caloric restriction, which has itself been shown to have positive effects. All participants were overweight, and by the end of the eight-week period, both groups had achieved notable reductions in BMI and waist circumference, as well as reduced fasting insulin and a significant reduction of HbA1c in the IF-group only. Ketone bodies rose in both groups, in the IF group, however, β-HB and acetoacetate levels rose significantly higher compared to the

first measurement in week 0. While both interventions contributed to healthier weight and metabolic markers, IF showed even better outcomes in terms of anthropometrics, insulin resistance, and lipid levels such as cholesterol. But perhaps even more interesting was its effect on the brain. MRI scans were used to determine the brain age, showing a reduction of 2.6 years in the IF and 2.4 years in the “healthy eating” group. Additionally, IF showed to have a significant positive impact on memory and cognitive function, which was not observed in the other intervention group (Kapogiannis et al., 2024).

But there is much more research exploring the variety of impacts this dietary pattern can have on the body. Some of these studies have looked at how IF can influence hormone levels, which may be especially relevant for women with polycystic ovary syndrome (PCOS) or other menstrual-related issues, as it appears to help regulate testosterone and androgens (Cienfuegos et al., 2022). Interestingly, there are even studies examining its role in hair follicle regeneration. However, those studies concluded that prolonged fasting may actually have a negative impact on hair follicle stem cells, as these cells lack strong antioxidant defences and are highly sensitive to circulating FFA and cortisol. This can lead to slower regeneration and reduced hair growth the longer the fasting period lasts (H. Chen et al., 2025).

Overall, the positive outcomes of IF include weight loss, reduced fat mass, improved blood glucose levels, and increased insulin sensitivity. IF has the potential to improve both metabolic and cognitive health as shown in the studies mentioned above and many more (Adafer et al., 2020; Robbins & Solito, 2022).

## 1.6 Mitochondrial Dynamics and Metabolism

It is well known that mitochondria are the powerhouses of the cell, but they are also incredibly adaptable and fulfil many more functions beyond merely providing energy. At the center of energy production is the Krebs-cycle, with acetyl-CoA serving as one of its most important substrates. However, there are several pathways through which acetyl-CoA can be supplied. The three main pathways that make acetyl-CoA available for further metabolic processes are, first, carbohydrates via glycolysis. Second, acetyl-CoA supply is also possible through amino acids, by their conversion into pyruvate. The third pathway, which is the focus of this work, is FAO, also known as β-oxidation. As mentioned earlier, this energy-generating pathway is activated when carbohydrates are scarce, but plenty FAs are available, which can be broken down and processed into acetyl-CoA units (Berg et al., 2015; Osellame et al., 2012).

Mitochondria are also essential for the efficient functioning and overall health of the cell, and they are capable of regulating cell growth, differentiation and apoptosis through various signalling pathways. Additionally, mitochondria themselves can adapt their morphology according to the needs of the cell, for example through fusion or fission. This may be necessary to separate damaged parts for mitophagy and thereby better maintain mitochondrial quality. Some metabolic pathways also increase their functionality, for instance, when mitochondrial elongation occurs. All of these processes can be influenced by different lifestyle and dietary interventions, including KD and CR, leading to metabolic shifts that can result in different transcriptional changes (Kyriazis et al., 2022; Twig et al., 2008).

### 1.6.1 Target Genes Involved in MCT Metabolism

#### 1.6.1.1 Acc1 and Acc2

Acetyl-CoA carboxylase exists in two isoforms: *Acaca* gene (encodes ACC1/ACC α) and *Acacb* gene (encodes ACC2/ACC β), each playing distinct roles in FA metabolism. ACC1 is mainly located in the cytosol, where it catalyses the carboxylation of acetyl-CoA to malonyl-CoA, making it the first rate-limiting step in de novo FA biosynthesis. In contrast, ACC2 can be found in the outer mitochondrial membrane and produces malonyl-CoA that regulates FA β-oxidation by inhibiting

carnitine palmitoyltransferase 1 (CPT1), thereby controlling the entry of FAs into mitochondria for oxidation (Wang et al., 2022). While KDs are known to alter lipid metabolism, direct effects on ACC1 and ACC2 expression or activity have not been demonstrated. However, high-fat-diets have shown to affect ACC expression very differently compared to high-carbohydrate diets, likely due to the changed needs for FAs and therefore affecting lipogenesis (Dankel et al., 2021).

#### 1.6.1.2Acadm

Medium-Chain-Acyl-CoA Dehydrogenase (MCAD) is a mitochondrial enzyme encoded by the Acyl-CoA Dehydrogenase Medium Chain (*Acadm*) gene. It plays an important role in the β-oxidation pathway by catalysing the initial step in the breakdown of MCFAs from MCTs. This process is essential for turning dietary fats and stored body fat into acetyl-CoA, which enters the citric acid cycle to produce energy. Deficiency in MCAD activity impairs the body's ability to metabolize MCTs, leading to an accumulation of unmetabolized FAs and a shortage of ketone bodies during periods of fasting or increased energy demand. This metabolic disorder, known as MCAD-deficiency, can result in hypoglycaemia, neurological damage, and other severe complications if untreated (ACADM Gene, n.d.; Chang et al., 2024). Research on the regulation of MCAD during ketogenic or high-fat diets is very limited but there may be some evidence suggesting that MCAD expression may be downregulated while consuming a diet high in fat (Zheng & Cai, 2019).

#### 1.6.1.3 Crat

Carnitine acetyltransferase (CRAT) is a mitochondrial enzyme that facilitates the transfer of acetyl groups between acetyl-CoA and carnitine, forming acetylcarnitine. CRAT plays a role in managing mitochondrial acetyl-CoA levels, which influences the tricarboxylic acid (TCA) cycle and overall energy homeostasis (Seiler et al., 2014). KDs enhance the production of ketone bodies through increased FAO, leading to an accumulation of acetyl-CoA. The higher acetyl-CoA concentration may influence the activity of CRAT, as it is crucial in regulating the transport of acetyl groups. However, studies investigating the impact of KDs on CRAT activity are still limited (Mezhnina et al., 2020).

#### 1.6.1.4 Fasn

During de novo lipid synthesis, the enzyme fatty acid synthase (FASN) plays a central role in catalysing the production of palmitate from malonyl-CoA and acetyl-CoA. FASN is essential in various tissues, including the liver, brain, and adipose tissue, where FA synthesis is critical for maintaining physiological processes. Dysregulation of FASN has been linked to metabolic disorders and diseases such as cancer and is associated with apoptosis and reduced proliferation of healthy cells, potentially through mechanisms like cytochrome c release (Bruning et al., 2018). In relation to decanoic acid (C10:0), the activity of FASN has impacts on the synthesis of MCFAs. Studies have demonstrated that inhibiting *Fasn* expression results in a reduction in cellular MCFA levels, including decanoic acid, which underlines FASN's role in their biosynthesis (Zhu et al., 2014).

Although direct evidence linking *Fasn* regulation to specific dietary interventions, such as KDs, remains limited, recent studies indicate that *Fasn* expression can be modulated by dietary patterns. For instance, KDs have been found to reduce *Fasn* expression in diabetic and *ob/ob* mice (Cao et al., 2023; Yang et al., 2021). On the other hand, *Fasn* expression is upregulated in high-fat diet-induced obese mice, suggesting that its regulation depends on the overall dietary context (Dong et al., 2023).

#### 1.6.1.5 Trp53

p53 (which equals p53α), a tumor suppressor protein encoded by the *Trp53* gene, plays a very essential role in regulating cellular metabolism, cell proliferation, apoptosis, and senescence. It counteracts many metabolic changes associated with cancer, such as increased glycolysis and FA synthesis, thereby maintaining energy balance and cellular homeostasis. In lipid metabolism, p53 inhibits key pathways, including the mevalonate pathway, by repressing SREBP2. This is leading to reduced cholesterol and lipid synthesis, processes that are critical for tumor proliferation (Humpton & Vousden, 2019).

Studies suggest that MCFAs can affect the cellular metabolic states and potentially impact p53 functions by inducing oxidative stress and apoptosis in cancer cells, where p53 acts as a key regulator (Lappano et al., 2017). Additionally, dietary interventions such as KDs, have been shown to enhance p53 activity. Recent

research indicates that β-OHB can modify p53 through β-hydroxybutyrylation (Liu et al., 2019; Wei et al., 2024).

In my work, two different isoforms of p53 were used as primers. In addition to p53α, the Δ122p53 isoform was also utilized, which corresponds to the human Δ133p53 isoform and exhibits distinct properties compared to the full-length variant. Due to an alternative splicing site at exon 3 in mice and exon 4 in humans, an isoform is produced that lacks the first 122 (murine) or 133 (human) amino acids. According to studies, Δ122p53 (Δ133p53) deviates from the classical tumor suppressor function and can instead initiate processes that attenuate the suppressor activity, apoptosis, and senescence associated with p53α, while also promoting inflammation through pro-inflammatory molecules such as interleukin-6 (Roth et al., 2016; Slatter et al., 2015). In the following, p53α – the full-length isoform – will be abbreviated as **l** (long), whereas Δ122p53 (Δ133p53), due to its shorter gene sequence, will be abbreviated as **s** (short).

### 1.6.2 Fission and Fusion

Mitochondria are dynamic organelles which are able to adapt their morphology and function through the processes of fusion and fission, depending on the cellular demands (**Figure 4**). These dynamic changes have functional implications, particularly influencing energy metabolism, such as the processing of FAs (Kyriazis et al., 2022).

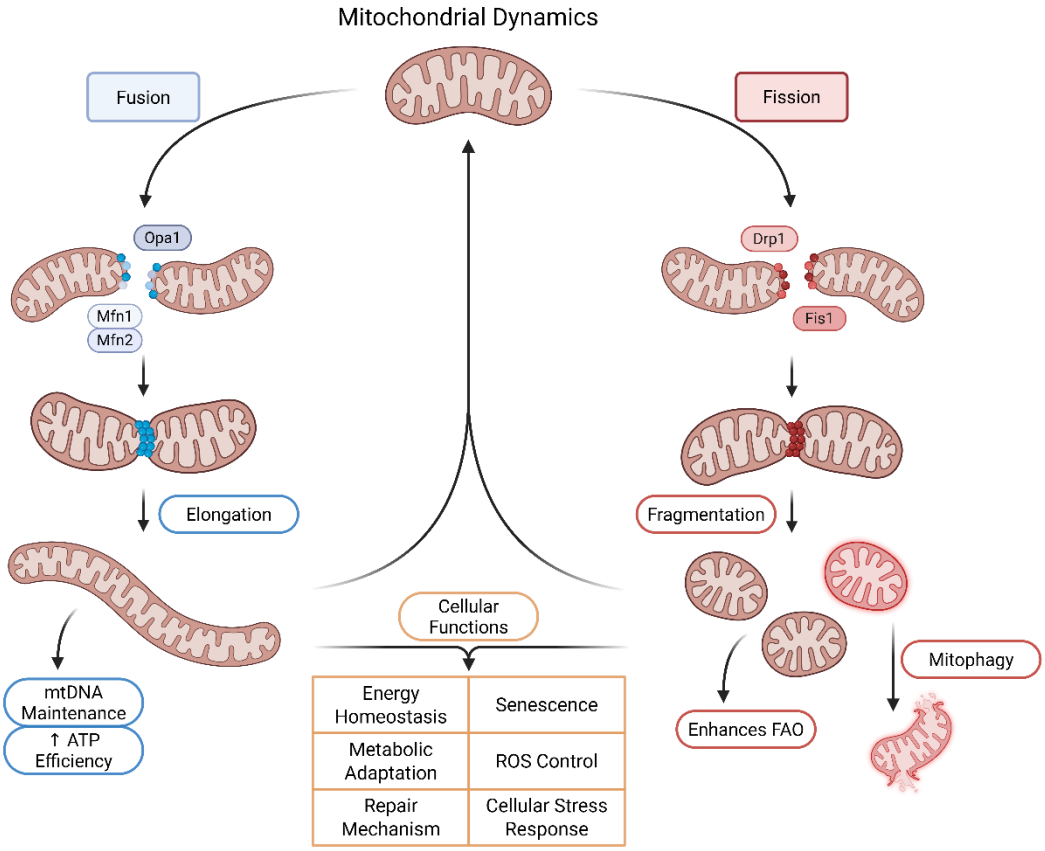


***Figure 4: Mitochondrial dynamics*** *with the key regulators Fis1 and Drp1 for fission and Opa1, Mfn1 and Mfn2 for fusion. Fragmentation (fission) caused by partly damaged mitochondria may lead to mitophagy but also enhances β-oxidation in the healthy mitochondria.*
*Created in [https://BioRender.com](https://BioRender.com)*

### 1.6.2.1 Mfn1, Mfn2 and Opa1

Fusion happens in stages, beginning with the tethering of two mitochondria. The outer mitochondrial membranes start to merge, followed by the inner membranes. This process is mainly mediated by the proteins Mitofusin 1 and 2 (MFN1 and MFN2) and optic atrophy 1 (OPA1). These structural modifications are important for maintaining mitochondrial respiration, ATP production, and the repair of damaged mitochondria (Sharma et al., 2021; Tilokani et al., 2018).

### 1.6.2.2 Fis1

Fission refers to the division of mitochondria into smaller fragments, a process which is driven by proteins such as dynamin-related protein 1 (DRP1) and mitochondrial fission protein 1 (Fis1). Fission is involved in regulating ROS

accumulation, removing dysfunctional mitochondria, and facilitating mitophagy during cellular stress (Sharma et al., 2021; Tilokani et al., 2018).

Studies have shown that high-fat diets can increase the expression of *Fis1*, *Drp1* and *Opa1*, while decreasing *Mfn2* expression. In contrast, KDs have been seen to decrease *Drp1* and *Mfn2* expression while increasing mitochondrial volume. However, the effects of these diets can vary depending on the organ being examined (D. Chen et al., 2018; Kang et al., 2020; Kyriazis et al., 2022).

## 1.7 Housekeeping Genes

Ribosomal Protein L4 (RPL4) is a component of the 60S subunit of ribosomes, playing an important role in protein synthesis. It is involved in ribosome assembly, ensuring structural stability and the efficient synthesis of proteins necessary for cellular function and growth (PubChem, n.d.). Due to its fundamental role in basic cellular operations, RPL4 has been consistently evaluated across various tissues and experimental conditions, demonstrating solid stability as a housekeeping gene (HKG) across different species. Its stable expression has made *Rpl4* a reliable reference gene in studies involving mice, including those involving dietary interventions (Dankel et al., 2021; de Jonge et al., 2007; Shin et al., 2022). These characteristics make *Rpl4* useful for normalization in quantitative PCR (qPCR).

The primary function of the HPRT enzyme (hypoxanthine-guanine phosphoribosyltransferase) is to recycle guanine and hypoxanthine into purine nucleotides through the purine salvage pathway. In the absence of HPRT, these purine bases are instead converted into uric acid. This enzyme is particularly critical in the brain, where purine nucleotide demand is high. The activity of HPRT is primarily regulated by the cellular requirements for ATP and GTP (Sekine et al., 2024). *Hprt* has been validated as a stable HKG for RT-qPCR studies in the hypothalamus, cortex and hippocampus (Feria-Romero et al., 2021; Li et al., 2014). Research analysing *Hprt* stability under various experimental conditions, including dietary restrictions such as high-fat diets and fasting shows that *Hprt* expression remains stable under most of these conditions (Li et al., 2014; Tanic et al., 2007).

## 1.8 Aim of the Study

MCTs exert rapid metabolic effects in peripheral tissues, yet their impact on brain metabolism remains unclear. Despite significant transcriptional changes in cortex tissue, we found no detectable increase in C8/C10 fatty acids following LCT/MCT feeding compared to LCT alone, nor differences in β-HB levels – raising questions about regional metabolism or detection limits (Sternberg et al., in submission). Given the brain's energy demands and regional plasticity, this study aims to examine how MCT-containing diets versus LCT-rich KDs, as well as fasting (IF) and caloric restriction (CR), affect gene expression related to fatty acid metabolism and mitochondrial function in the mouse cortex and hippocampus. We focus on key genes including *Acaca*, *Acacb*, *Acadm*, *Crat*, *Fasn*, *Fis1*, *Mfn1*, *Mfn2*, *Opa1*, and *Trp53*.

## 1.9 Hypothesis

A key focus of this study is to determine whether the addition of MCTs to a classical ketogenic LCT diet leads to transcriptional changes in the brain, reflecting enhanced metabolic flexibility. Given their rapid uptake and oxidation, we expect the LCT/MCT diet to induce gene expression patterns that differ from those seen with LCT alone, and to potentially overlap with the metabolic signatures observed under IF or CR, as these can lead to similar state of ketosis.

Based on these findings, we hypothesize that adding MCT to an LCT high-fat diet would lead to measurable differences in metabolic gene expression compared to LCT in the brain. Furthermore, we anticipate that these effects will reflect the brain's region-specific metabolic adaptability.

# 2 Materials

## 2.1 Mice and Diets

Male C57BL/6 mice were obtained from the Jackson Laboratory and housed under specific-pathogen-free conditions in a controlled environment with a 12-hour light/12-hour dark cycle, constant temperature, and regulated humidity. Water was provided *ad libitum* throughout the study for all groups and all animals were maintained under the same housing conditions. Detailed information regarding the housing and diet composition of the mice are available in previously published studies by Gregor et al. (2022) and Sternberg et al. (2023). The mice were randomly assigned to different dietary groups, each group consisting of eight mice (**Table 1**).

| Diets | Product number | Company |
|---|---|---|
| Standard Diet | S9139-E028 | ssniff |
| LCT | S9139-E025 | ssniff |
| LCT/MCT | S9139-E032 | ssniff |
| IF | V153x R/M-H auto | ssniff |
| CR | V153x R/M-H auto | ssniff |

***Table 1: Product number and company name from all five diets*** *used in this study.*

## 2.2 Chemicals and Kits

| Kits and Chemicals | Product number | Company |
|---|---|---|
| 4 x gDNA wiper Mix | R323-01-AB | Vazyme |
| 5 x HiScript III qRT SuperMix | R323-01-AC | Vazyme |
| 5 x No RT Control Mix | R323-01-AD | Vazyme |
| 50 x TAE-Electrophoresis Buffer | B49 | Thermo Fisher Scientific |
| 6 x DNA Loading Dye | R0611 | Thermo Fisher Scientific |
| Agarose | A6013-5G | Sigma-Aldrich |
| ChamQ Universal SYBR qPCR Master Mix | Q711-03 | Vazyme |
| Chloroform | n. a. | Sigma-Aldrich |
| GeneRuler 100bp DNA Ladder | SM0241 | Thermo Fisher Scientific |
| Glycogen | AM9510 | Thermo Fisher Scientific |
| Isopropanol | n. a. | Sigma-Aldrich |
| NF-H2O | E476-500ML | VWR |
| peqGreen DNA/RNA Dye | 37-5000 | peqlab |
| RNase AWAY | 7002 | Molecular BioProducts |
| RNase-free ddH2O | R323-01-AA | Vazyme |

***Table 2: Chemicals and Assay Kits*** *that were used throughout the experiments in alphabetic order, including the product number and company name.*
*"n. a." refers to products, where product number was not available.*

## 2.3 Primers

| Primer | Direction | Sequence 5'-3' | GC (%) | Tm (°C) | Amplicon length (bp) | Company |
|---|---|---|---|---|---|---|
| *mAcaca* | forward | GCCGCCAGCCTGAGTTCTT | 63.16 | 62.19 | 112 | Microsynth |
| | reverse | TGGCCAACGGAGATGGTTCA | 55.00 | 61.77 | | |
| *mAcacb* | forward | CCCCAGCCAACAAGGAGCTT | 60.00 | 62.72 | 106 | Microsynth |
| | reverse | GCAAGACCATTCAGGCAACTGTG | 52.17 | 62.70 | | |
| *mAcadm* | forward | GGGGGAAAGGCCAACTGGTAT | 57.14 | 62.10 | 151 | Microsynth |
| | reverse | AGCATCGCTGGCCCATGTTT | 55.00 | 63.13 | | |
| *mCrat* | forward | TGACTCGGCGACCTTGAACCTA | 54.55 | 63.36 | 168 | Microsynth |
| | reverse | CCCAGGGGTTTTACCACGGTTC | 59.09 | 63.36 | | |
| *mFasn* | forward | AACACGATCTGGTGCTGCCC | 60.00 | 63.09 | 105 | Microsynth |
| | reverse | TGGGGGCTGTCGTGTCAGTA | 60.00 | 63.01 | | |
| *mFis1* | forward | GCTGGTTCTGTGTCCAAGAGCA | 54.55 | 63.02 | 144 | Microsynth |
| | reverse | GACATAGTCCCGCTGTTCCTCT | 54.55 | 61.53 | | |
| *mMfn1* | forward | CCAGGTACAGATGTCACCACAG | 54.55 | 60.35 | 149 | Microsynth |
| | reverse | TTGGAGAGCCGCTCATTCACCT | 54.55 | 63.98 | | |

*Table continues at next page.*

| Primer | Direction | Sequence 5'-3' | GC (%) | Tm (°C) | Amplicon length (bp) | Company |
|---|---|---|---|---|---|---|
| *mMfn2* | forward | TCCCTCTGACACCTGCCAAC | 60.00 | 61.77 | 155 | Microsynth |
| | reverse | TGCCTTCCACACCACTCCTC | 60.00 | 61.77 | | |
| *mOpa1* | forward | TCCACCACAGGAAGCTCAAGAAATC | 48.00 | 63.01 | 141 | Microsynth |
| | reverse | TTTTCCCAGCACTCTGATCTCCAAC | 48.00 | 63.01 | | |
| *mTrp53 (l)* | forward | GCAGGGCTCACTCCAGCTAC | 65.00 | 62.59 | 124 | Microsynth |
| | reverse | GTGATGGGGACGGGATGCAG | 65.00 | 62.95 | | |
| *mTrp53 (s)* | forward | TTCCGGGAGCTGAATGAGGC | 60.00 | 62.26 | 93 | Microsynth |
| | reverse | TCTAGGCTGGAGGCTGGAGTG | 61.90 | 63.04 | | |
| *mRpl4* | forward | GTATGGCACTTGGCGGAAGG | 60.00 | 61.40 | 124 | Microsynth |
| | reverse | TGCTCGGAGGGCTCTTTGG | 63.16 | 62.00 | | |
| *mHprt* | forward | GCAGTCCCAGCGTCGTGATTAG | 59.09 | 63.51 | 174 | Microsynth |
| | reverse | TGTGATGGCCTCCCATCTCCT | 57.14 | 62.75 | | |

***Table 3: DNA Primer sets used throughout the study*** *including their sequences and company name.*

# 3 Methods

## 3.1 Experimental Setup

All experiments with animals were approved by the Austrian national authority according to §§ 26ff. of the Animal Experiments Act, Tierversuchsgesetz 2012-TVG 2012 (BMWFW-66.006/0008-V/3b/2018). All the procedures were in alignment with the directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes.

Each intervention and the control group consisted of 8 C57BL/6 male mice, which were housed as previously described in the Materials section.

All animals were fed under the same dietary conditions for the first twelve weeks after their birth. The animals were then separated into five groups. The group which served as the control was maintained on a standard chow diet (SD) *ad libitum*, with Carbohydrates making up 64% of the kcal intake, throughout the whole study. One group received an LCT-based KD (LCT) for eight weeks. This diet was formulated to supply around 94.5% of its total energy from fat, 4.5% from protein and 1% from carbohydrates. The LCT/MCT group was fed a similar diet for eight weeks, but the fat-derived energy consisted of around 30% MCT, of which 25% were composed of C8:0 and C10:0 fatty acids (**Figure 5**). Mice of the CR group received 80% of the calories provided to SD fed mice once daily and continued this diet for two weeks. To be able to match the age with IF, CR started at week 14.

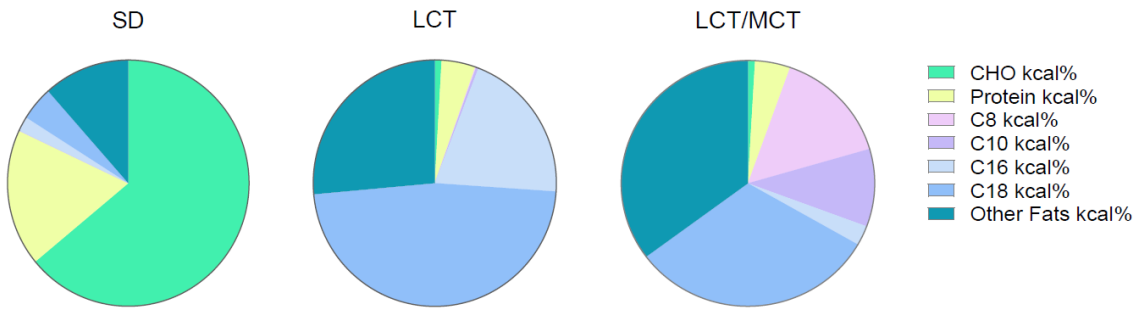


***Figure 5: Dietary composition of SD, LCT and LCT/MCT expressed as percentage of total caloric intake (kcal%).*** *Fats are separated according to their carbon chain length. CHO = Carbohydrates.*

IF mice underwent a cycle of 24-hours of complete fasting followed by 24-hours of *ad libitum* refeeding. IF mice were fed continuously like described for four weeks, starting at the age of 12 weeks. IF and CR mice were age-matched, and both sacrificed at the age of 16 weeks. Water was provided *ad libitum* for all groups.
The dietary interventions started at the age of 12 weeks for LCT and LCT/MCT (**Figure 6**). SD, LCT and LCT/MCT were 20 weeks old at the time of tissue collection. Euthanasia for all animals was performed via anaesthesia overdose. All tissue samples were collected and processed as previously described by Gregor et al. (2022) and Sternberg et al. (2023) and the isolated RNA was stored and used for this experiment.

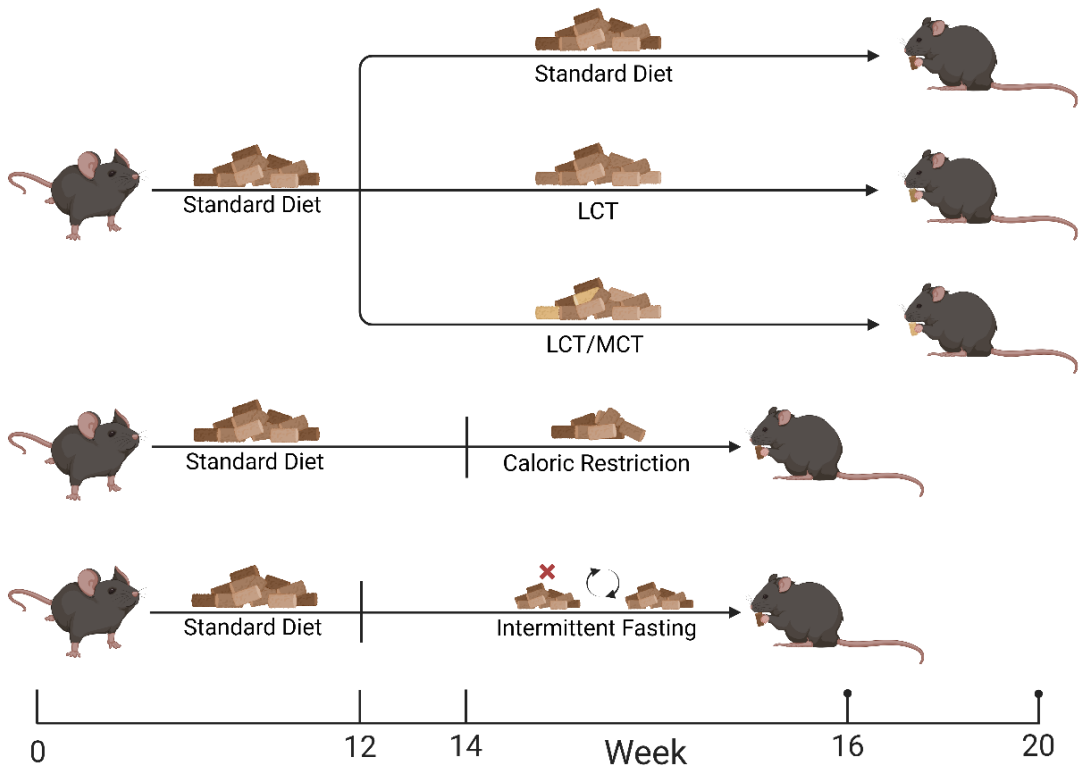


***Figure 6: Graphical representation of the timeline of dietary interventions in male C57/BL6NRJ mice.*** *All mice were initially maintained on a standard diet (SD) prior to the start of the intervention period. Long-chained ketogenic diet (LCT), mixed LCT and middle-chained diet (LCT/MCT) and intermittent fasting (IF) were introduced at week 12, while the caloric restriction (CR) intervention started at week 14. One control group continued receiving the SD throughout the study. The CR and IF animals were age-matched and sacrificed at week 16. SD, LCT and LCT/MCT were sacrificed after 8 weeks, at the age of 20 weeks. Nutrient compositions of the diets are detailed in the Supplement.*
*Created in https://BioRender.com*

Several brain regions were dissected separately. These include the hippocampus, which was further used for this study, as well as the hypothalamus and cerebellum. Some regions, such as the striatum and the brainstem, were removed and the remaining brain tissue was pooled and is referred to here as the “cortex”. Due to this pooling, the cortex tissue is inhomogeneous, which may lead to fluctuating gene expression levels.

## 3.2 DNA digestion and reverse transcription to cDNA

DNase digestion, reverse transcription into cDNA, and all following steps were performed under a laminar flow hood that was thoroughly cleaned with an RNase decontamination solution. Sterile filter tips were used for all pipetting procedures.
Samples were maintained on dry ice at all times to minimize the risk of genomic DNA contamination or RNA degradation. Enzymes were stored on cooling racks to preserve their activity. For reverse transcription, the “HiScript III RT SuperMix for qPCR (+gDNA wiper)” from Vazyme was used.
The RNA concentration of all samples was measured using a NanoDrop spectrophotometer, with nuclease-free water (NF-$H_2O$) serving as the blank. Prior to proceeding, the RNA concentrations of all samples were calculated to achieve a final RNA concentration of approximately 0.5 µg/µL when diluted with RNase-free dd$H_2O$. The calculated volumes of each RNA sample and RNase-free dd$H_2O$ were combined in PCR tubes, and 4× gDNA wiper mix was added to eliminate genomic DNA contamination, ensuring more reliable quantitative results. The final reaction volume was adjusted to be 16 µL per tube.
The samples were incubated at 42°C for 3 minutes, after which 4 µL of 5× HiScript III qRT SuperMix was added to complete the reverse transcription reaction, following the Vazyme protocol. This buffer contains dNTPs, HiScript III Reverse Transcriptase, RNase inhibitor, random primers, and an oligo(dT)VN primer mix. A no-RT control reaction was included once to detect any residual genomic DNA in the RNA templates.
cDNA synthesis was performed using the PCR thermal cycler “Rotor-Gene”, with reaction times specified in the following protocol (**Table 4**).

| Temperature protocol for RT of mRNA to cDNA | | | | | |
|---|---|---|---|---|---|
| Step | Incubation | 1 | 2 | 3 | 4 |
| Time | 3 min | 10 min | 10 min | 10 min | 5 min |
| Temperature | 42 °C | 25 °C | 37 °C | 50 °C | 85 °C |

***Table 4: Temperature protocol for reverse transcription of mRNA to cDNA** used for thermal cycler "Rotor-Gene".*

The resulting 20 µL cDNA were diluted with 80 µL NF-$H_2O$, creating a 1:5 dilution. The diluted cDNA was transferred into separate tubes and stored at -20°C until further use.

## 3.3 Primer design and validation

To design the primer sequences, the NCBI Nucleotide database and NCBI Primer-BLAST tool were utilized. Gene sequences were restricted to *Mus musculus* (taxid: 10090), and the maximum PCR product size was set to 200 base pairs to optimize primer efficiency. The primers were designed to span exon-exon junctions to minimize genomic DNA (gDNA) amplification.

The melting temperatures (Tm) were defined as follows: a minimum of 60°C, an optimum of 63°C, and a maximum of 66°C. These parameters were selected to reduce the likelihood of nonspecific primer binding and gDNA interference. No additional settings were modified during the design process.

In cases where multiple primers met the design criteria, all candidate primers were tested experimentally, and the most efficient primer pair was selected. For the *Trp53* gene, two distinct isoforms were targeted: a longer transcript variant (*Trp53 (l)*) and a shorter variant *Trp53 (s)*) with differing C-terminal regions.

The following murine primer sets from **Table 5** were included in this study:

| Primer name | Abbreviation |
|---|---|
| Acetyl-CoA-carboxylase-α | *Acaca* |
| Acetyl-CoA carboxylase β | *Acacb* |
| Acyl-CoA Dehydrogenase Medium Chain | *Acadm* |
| Carnitine acetyltransferase | *Crat* |
| Fatty acid synthase | *Fasn* |
| Mitochondrial fission protein 1 | *Fis1* |
| Mitofusin 1 | *Mfn1* |
| Mitofusin 2 | *Mfn2* |
| Optic atrophy 1 | *Opa1* |
| Transformation-related protein 53 α | *Trp53 (l)* |
| Transformation-related protein Δ122p53 | *Trp53 (s)* |
| Ribosomal Protein L4 | *Rpl4* |
| Hypoxanthine-guanine-phosphoribosyltransferase | *Hprt* |

***Table 5: murine DNA Primer names used in this study*** *including their abbreviations.*

Upon receipt, primers were solubilized in NF-$H_2O$ according to the manufacturer's instructions. Stock solutions were prepared at a concentration of 100 µM. Working solutions were created by combining 10 µL of the forward primer and 10 µL of the reverse primer for each gene and diluting to a final volume of 1 mL with NF-$H_2O$, resulting in a working concentration of 1 pmol/µL. These aliquots were subsequently used for experimental applications.

For the evaluation of the primer efficiency, pooled cDNA samples were used in a serial dilution. The initial PCR master mix consisted of 5 µL ChamQ and 2 µL cDNA, with the fist tube containing 36 units of these master mixes. The nine subsequent dilutions contained 24 master mix units, where the cDNA was replaced by 2 µL of nuclease-free water to achieve the aimed concentrations, while maintaining the 5 µL of ChamQ. These volumes were prepared to be sufficient for 7 primers at once and would vary, when prepared for different quantities. The concentrations

were then serially diluted at a 1:3 ratio. These serial dilutions were repeated, until all primers were successfully tested for their efficiency.

For the primer efficiency test 3 µL of each primer and 7 µL of the corresponding master mix dilution were added to the PCR well plates, carried out in triplicates. The Primer efficiency was assessed using the QuantStudio Real-Time PCR System, following the cycling protocol viewed below (**Table 6**).

| Step | Temperature | Time (m:s) | Nr. of Cycles | Scan |
|---|---|---|---|---|
| Initial Denaturation | 95 °C | 03:00 | | |
| Denaturation | 95 °C | 00:10 | | |
| Annealing | 62 °C | 00:20 | | |
| Extension | 72 °C | 00:20 | 40 | yes |
| Melting Curve | | 00:15 | | yes |

***Table 6: Real time-qPCR temperature protocol*** *used for QuantStudio Real-Time PCR Systems.*

## 3.4 RT qPCR

Based on efficiency tests, the optimal dilution for cDNA was determined to ensure that the Ct values for all primers fell within their respective linear amplification ranges. The cDNA from Cortex samples was diluted 1:4, while Hippocampus cDNA samples were diluted 1:4 or 1:5 when sample volume was limited. Despite the variation in dilution, the results indicated that amplification initiated with approximately equal amounts of cDNA in each well, as visualized by the standard curves of the primer efficiency test (Figure 4).

Master mixes were prepared by combining 1 µL of diluted cDNA, 1 µL of NF-$H_2O$, and 5 µL of ChamQ SYBR Master Mix, following manufacturers guidelines. For the no-template control (NTC), NF-$H_2O$ was used in place of cDNA. All primer-cDNA reactions, NTCs, and controls were prepared in duplicates. Samples were pipetted into a 384-well PCR plate, with each well containing 3 µL of primer solution and 7 µL of the master mix, resulting in a total reaction volume of 10 µL per well.

Pipetting and the RT-qPCR thermal cycling program were performed as described in Table 6 in “Primer design and validation”.

## 3.5 Statistical analysis and Language Support

To support the translation of selected academic literature from Englisch and to assist with some translations into Englisch, AI-based language tools were used in a few cases. The translated content was subsequently reviewed for accuracy.

To analyse the gene and protein expression of the selected target genes, the ΔCT method was first calculated. Expression differences were determined using GraphPad Prism software and the 2^-ΔCT method. To identify extreme outliers, the Grubbs test (α = 0.05) was applied, and any detected outliers were excluded from further calculations. The data were then analysed with the use of one-way ANOVA for nonparametric data, specifically with the Kruskal-Wallis test and the Bonferroni correction, which allowed for the comparison of more than two groups. The mean of each intervention group was compared to the mean of every other group using the Dunn’s test for multiple comparison, as the data may not be distributed normally.

The results were graphically presented as mean values ± Standard Error of Mean (SEM), with significant differences between groups indicated by the following symbols: * vs. SD, # vs. LCT, ° vs. LCT/MCT, and ^ vs. IF.

The significance levels were represented by the number of symbols, depending on the group being compared, as follows: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

# 4 Results

## 4.1 Primer

After evaluating the result, a standard curve for each primer was generated by plotting Ct values on the y-axis against the decadic logarithm of the dilution factor on the x-axis. Using the slope, primer efficiency was being calculated. To calculate the individual primer efficiencies, the slope of the corresponding primers was used, using the formula shown in **Figure 7 A**. If the primer efficiency was evaluated to be between 90% and 105%, they were considered to be acceptable. The data demonstrate consistent linear amplification across a wide range of dilutions, indicating high sensitivity and efficiency, even at low template concentrations, which can be seen in **Figure 7 B**.

A

$$\text{Efficiency (\%)} = -1 + 10 \times (-1 / \text{slope})$$

B

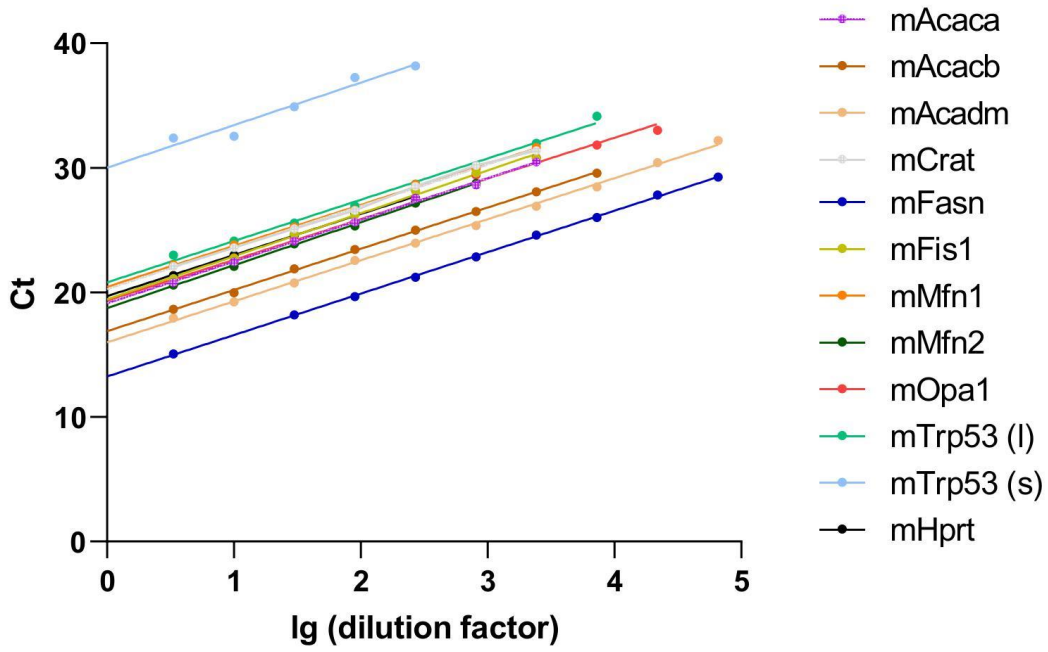


***Figure 7: Standard curve of primer efficiency test and formula of primers used in subsequent experiments.*** ***A*** *Formula used for the primer efficiency calculations and* ***B*** *standard curves of the primer efficiency test. Ct values are plotted against the logarithmic cDNA dilution factor for each primer. All primers demonstrated a linear range. Regression lines are included to illustrate the linearity of each primer's performance.*

## 4.2 Gel electrophoresis

Following the primer efficiency test, gel electrophoresis was performed to analyse the amplicons corresponding to each primer. A 50× TAE electrophoresis buffer (Thermo Scientific) was diluted with deionized $H_2O$ to prepare a 1× TAE running buffer. For the agarose gel, 110 mL of 1× TAE buffer was combined with 2 g of agarose and 5 µL of peqGREEN dye (Peqlab). The 2% agarose gel was poured into a gel tray and allowed to solidify. Once set, the gel was prepared for electrophoresis by submerging it in 1× TAE buffer within the electrophoresis chamber.

For sample loading, 5 µL of each sample were mixed with 1 µL of 6× DNA loading dye. The GeneRuler 100 bp DNA ladder was used as a molecular weight marker. Gel electrophoresis was conducted at 120 V for 75 minutes. Visualization of the results was performed using the Bio-Rad ChemiDoc MP Imaging System with the preset "SYBR Green" program for fluorescence imaging (**Figure 8.1 and 8.2**).

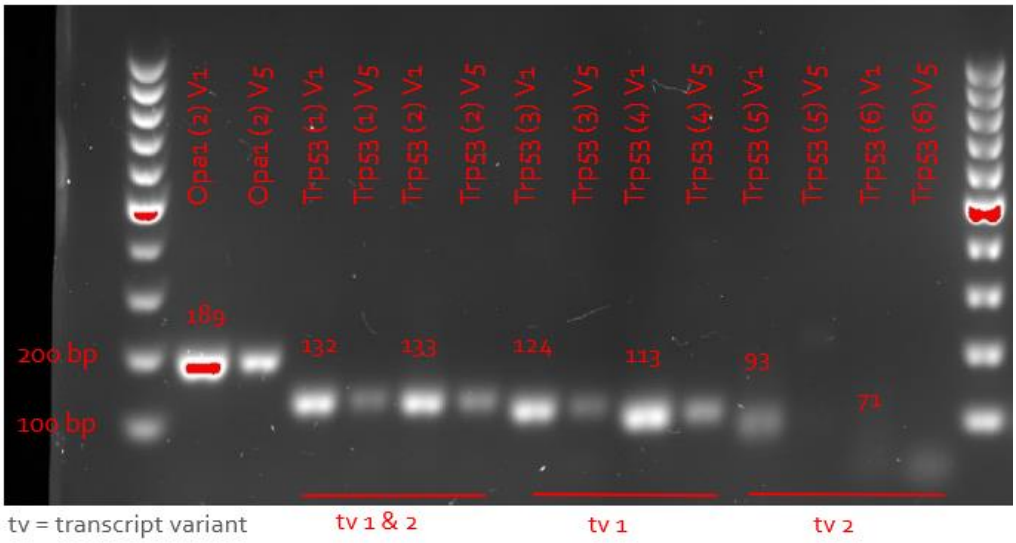


***Figure 8.1: Image of PCR products after agarose gel electrophoresis with different dilutions used for the PCR reaction ("V").*** *The labels in parentheses indicate alternative primer versions. The gel image displays the predicted amplicon sizes for each primer. The actual sizes can be approximated by comparing the heights of the bands to the corresponding markers. Rpl4 has been tested on previous study.*

*The Trp53 gene expresses multiple transcripts variants (isoforms), which were tested to assess primer specificity and amplification performance.*

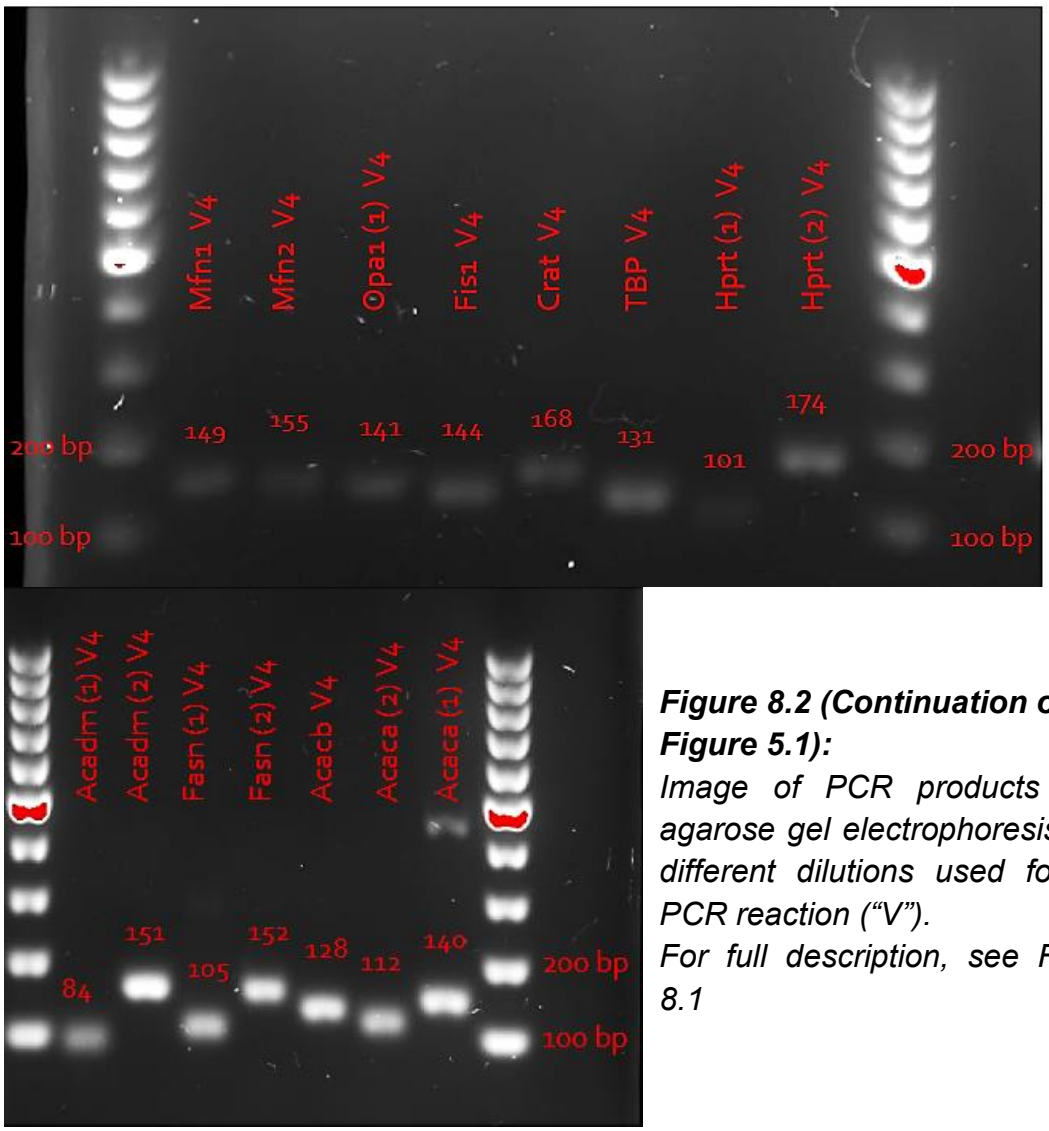


***Figure 8.2 (Continuation of Figure 5.1):***
*Image of PCR products after agarose gel electrophoresis with different dilutions used for the PCR reaction ("V").*
*For full description, see Figure 8.1*

After calculating the primer efficiency and considering the results of the gel electrophoresis, it was possible to determine which primers were best suited for the subsequent analyses. The following primers in **Table 7** were ultimately selected for this purpose:

| Primer | Direction | Sequence 5'-3' | Mean melting Tm (°C) | Amplicon length (bp) | Slope | Efficiency (%) |
|---|---|---|---|---|---|---|
| *mAcaca (2)* | forward | GCCGCCAGCCTGAGTTCTT | 79.63 | 112 | 3.35 | 98.87 |
| | reverse | TGGCCAACGGAGATGGTTCA | | | | |
| *mAcacb* | forward | CCCCAGCCAACAAGGAGCTT | 85.15 | 106 | 3.31 | 100.80 |
| | reverse | GCAAGACCATTCAGGCAACTGTG | | | | |
| *mAcadm (2)* | forward | GGGGGAAAGGCCAACTGGTAT | 82.06 | 151 | 3.29 | 101.18 |
| | reverse | AGCATCGCTGGCCCATGTTT | | | | |
| *mCrat* | forward | TGACTCGGCGACCTTGAACCTA | 88.67 | 168 | 3.33 | 99.50 |
| | reverse | CCCAGGGGTTTTACCACGGTTC | | | | |
| *mFasn (1)* | forward | AACACGATCTGGTGCTGCCC | 85.82 | 105 | 3.33 | 99.79 |
| | reverse | TGGGGGCTGTCGTGTCAGTA | | | | |
| *mFis1* | forward | GCTGGTTCTGTGTCCAAGAGCA | 84.64 | 144 | 3.45 | 94.97 |
| | reverse | GACATAGTCCCGCTGTTCCTCT | | | | |
| *mMfn1* | forward | CCAGGTACAGATGTCACCACAG | 80.97 | 149 | 3.29 | 101.35 |
| | reverse | TTGGAGAGCCGCTCATTCACCT | | | | |
| *mMfn2* | forward | TCCCTCTGACACCTGCCAAC | 86.70 | 155 | 3.44 | 95.15 |
| | reverse | TGCCTTCCACACCACTCCTC | | | | |
| *mOpa1 (1)* | forward | TCCACCACAGGAAGCTCAAGAAATC | 79.43 | 141 | 3.27 | 102.34 |
| | reverse | TTTTCCCAGCACTCTGATCTCCAAC | | | | |
| *mTrp53 (l) (3)* | forward | GCAGGGCTCACTCCAGCTAC | 83.93 | 124 | 3.32 | 100.23 |
| | reverse | GTGATGGGGACGGGATGCAG | | | | |
| *mTrp53 (s) (5)* | forward | TTCCGGGAGCTGAATGAGGC | 83.03 | 93 | 3.41 | 96.30 |
| | reverse | TCTAGGCTGGAGGCTGGAGTG | | | | |
| *mRpl4** | forward | GTATGGCACTTGGCGGAAGG | 82.76 | 124 | 3.54 | 91.57 |
| | reverse | TGCTCGGAGGGCTCTTTGG | | | | |
| *mHprt (2)* | forward | GCAGTCCCAGCGTCGTGATTAG | 81.08 | 174 | 3.30 | 101.02 |
| | reverse | TGTGATGGCCTCCCATCTCCT | | | | |

***Table 7: DNA primer versions used for the study*** *including their efficiency (%).*
** mRPL4 was tested in previous study.*

## 4.3 Impact of diets on the housekeeping gene expression

Both primers, *mRpl4* and *mHprt*, were evaluated for stability throughout the different dietary conditions. *Rpl4* demonstrated high consistency across all diets in the hippocampus and especially in the cortex (**Figure 9**) and was therefore selected for normalization.

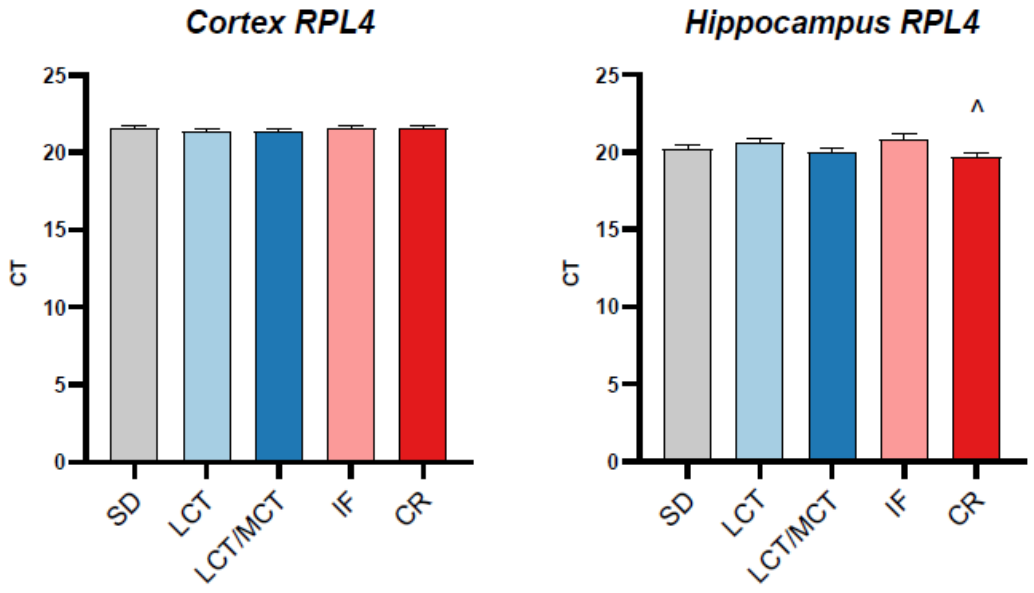


***Figure 9: Ct values of murine Rpl4 housekeeping gene upon different diets in the cortex and hippocampus.*** *Each bar represents the mean ± SEM from duplicate measurements per sample. Statistical comparisons were performed using one-way ANOVA followed by post hoc testing to compare all diet groups with each other.*

*Significance symbols refer to group comparison: * vs. SD, # vs. LCT, ° vs. LCT/MCT, and ^ vs. IF. Significant differences (p-values) are marked with: *p ≤ 0.05, ** p ≤ 0.01 and ***p ≤ 0.001 (symbols depending on the group being compared to). Abbreviations: SD – standard diet, LCT – long-chain triglycerides, LCT/MCT – long- and medium-chain triglycerides, CR – caloric restriction, IF – intermittent fasting.*

As expected, *Hprt* remained consistently stable in the hippocampus and also in the cortex under standard high-fat-diet (keto diet) conditions. However, HPRT showed significant variability in the cortex (**Figure 10**) under the LCT/MCT and IF conditions compared to SD. Although it remained stable under the other dietary setups and in the different tissue, *Hprt* had to be excluded as a normalization gene. For better accuracy, normalization was therefore performed using RPL4 alone.

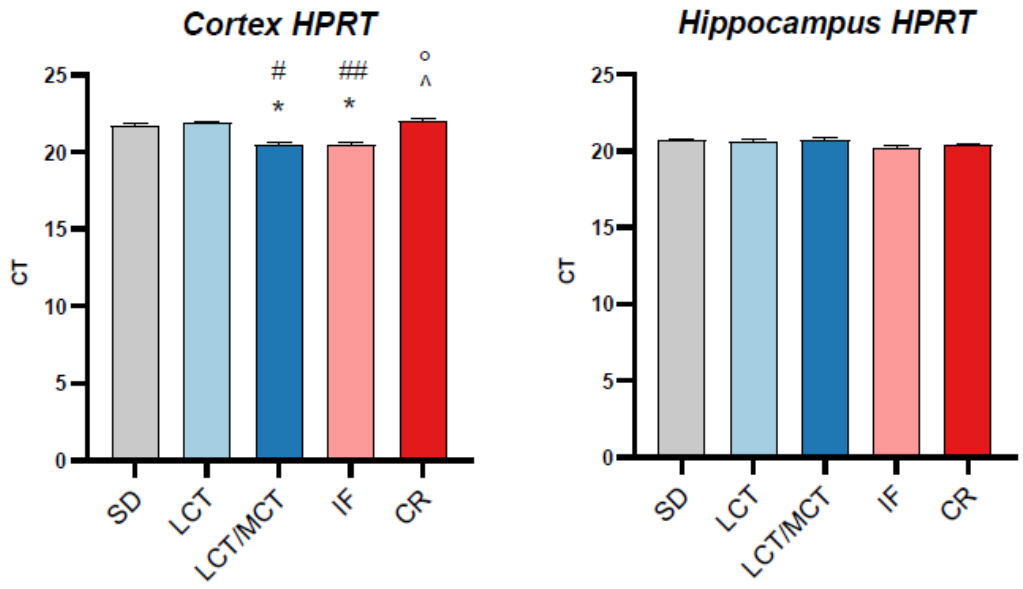


***Figure 10: Ct values of murine Hprt housekeeping gene upon different diets in the cortex and hippocampus.*** *Each bar represents the mean ± SEM from duplicate measurements per sample. Statistical comparisons were performed using one-way ANOVA followed by post hoc testing to compare all diet groups with each other.*
*Significance symbols refer to group comparison: * vs. SD, # vs. LCT, ° vs. LCT/MCT, and ^ vs. IF. Significant differences (p-values) are marked with: *p ≤ 0.05, ** p ≤ 0.01 and ***p ≤ 0.001 (symbols depending on the group being compared to). Abbreviations: SD – standard diet, LCT – long-chain triglycerides, LCT/MCT – long- and medium-chain triglycerides, CR – caloric restriction, IF – intermittent fasting.*

One possible reason for the fluctuation in *Hprt* expression in the Cortex could be its involvement in purine metabolism. This pathway can be influenced by a KD or by fasting, as these dietary approaches may increase purine levels and

consequently affect uric acid regulation. Accumulated uric acid could also promote oxidative stress, which may impact certain metabolic pathways (Sekine et al., 2024). Since HPRT is directly involved in the recycling of purines for energy production, this might indirectly lead to altered *Hprt* gene expression through changes in purine metabolism.

## 4.4 Weight gain and Body fat

The epididymal white adipose tissue in percent (%-eWAT) to bodyweight ratio (**Figure 11 B**) and the mouse body weight (**Figure 11 A**) in CR mice was the lowest among all the intervention groups, followed by IF.

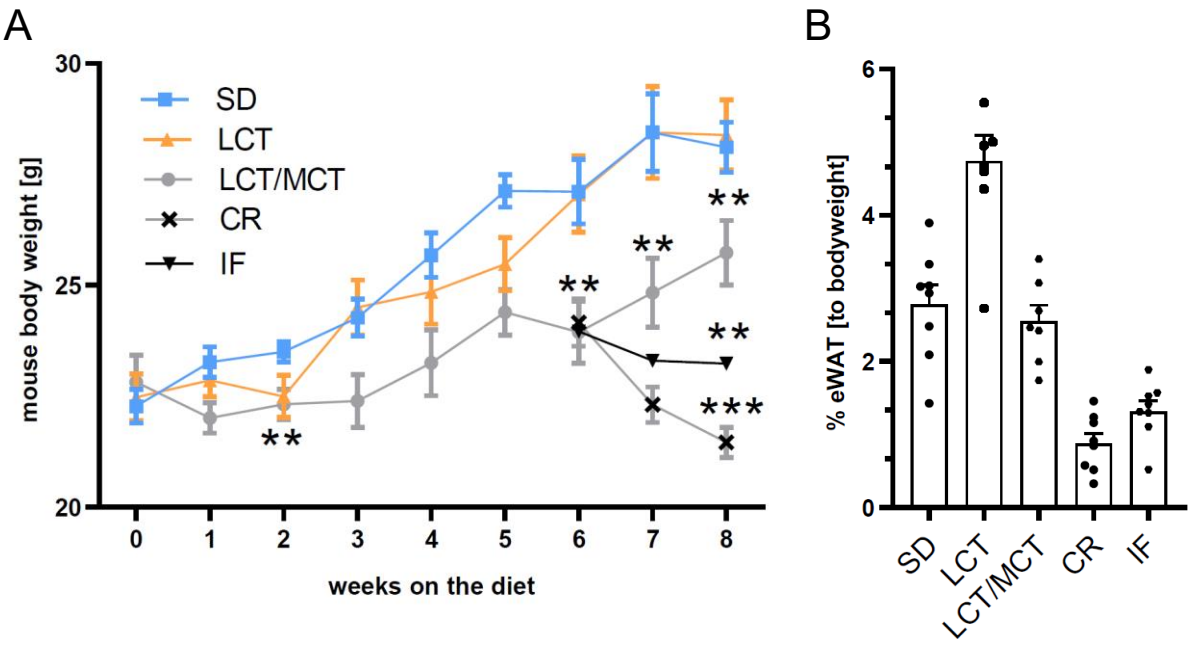


***Figure 11: A*** *Body weight in grams of mice over the course of the dietary intervention. IF and CR were initiated at week 6, as indicated.* ***B*** *Percentage of epididymal white adipose tissue (eWAT) relative to body weight at the end of the intervention period.*
*Bars and data points represent mean ± SEM. Abbreviations: SD – standard diet, LCT – long-chain triglycerides, LCT/MCT – long- and medium-chain triglycerides, CR – caloric restriction, IF – intermittent fasting.*

It is important to mention, that when CR and IF intervention was started, these mice already had a lower body weight than SD, even though they were fed the same SD diet until they started the dietary intervention. LCT mice had approximately the

same body weight but showed the highest %-eWAT amongst all the groups. LCT/MCT gained significantly less weight than SD but showed the same amount of %-eWAT compared to the body weight.

## 4.5 Target Genes

To assess how different dietary interventions influence genes involved in FA metabolism and mitochondrial function, qPCR analyses were conducted in the cortex and hippocampus of mice subjected to five dietary conditions: SD, LCT, LCT/MCT, IF and CR. Gene expression levels were normalised using RPL4 as the reference gene and relative quantification was calculated using the 2^-ΔCt method. The selected targets genes include markers of lipid synthesis, FAO, mitochondrial fusion and fission and stress response. These genes were chosen based on their roles in metabolic adaptation under ketogenic conditions and their potential sensitivity to MCT-based dietary modulation. Since a previous study (publication in process) has demonstrated systemic effects of MCT in various peripheral tissues but have failed to detect them in the brain, this study focused on exploring whether these metabolic shifts would be indicated by changes in gene expression in brain regions with high energy demand and plasticity.
The following results show gene- and region-specific expression patterns across dietary intervention groups.

### 4.5.1 Cortex

The fact that *Acaca* does not increase under high-fat diets was expected, as this would otherwise inhibit CPT1, a key enzyme required for the mitochondrial uptake of LCFAs. A slight increase in *Acaca* expression observed with the LCT/MCT (**Figure 12 A**) diet was expected due to the lower proportion of LCTs present.
In the cortex, *Acaca* showed a significant increase under IF compared to SD (**Figure 12 A**) and the other dietary conditions, although we initially expected IF and CR to behave similarly in this context. However, *Acaca* can also be influenced by insulin, and insulin levels fluctuate more frequently under IF than CR.
*Acab*, which is also increased in LCT/MCT and IF compared to LCT (**Figure 12 B**), has similar functions to that of *Acaca.* They both lead to higher levels of malonyl-CoA, which could inhibit CPT1 and thereby affect FA uptake into the cell.

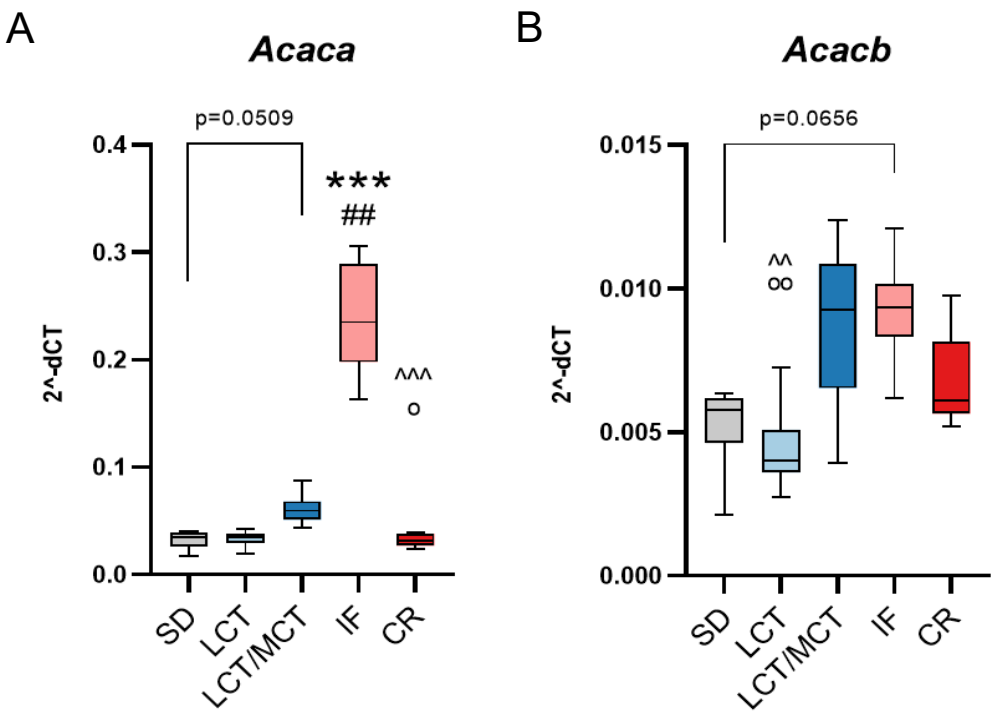


***Figure 12: Relative gene expression levels** of **A** Acaca and **B** Acacb in the cortex of mice subjected to different diets, normalised to the housekeeping gene Rpl4.*
*Data are presented as mean ± SEM. Significance symbols refer to group comparison: * vs. SD, # vs. LCT, ° vs. LCT/MCT, and ^ vs. IF. Significant differences (p-values) are marked with: *p ≤ 0.05, ** p ≤ 0.01 and ***p ≤ 0.001 (symbols depending on the group being compared). Abbreviations: SD – standard diet, LCT – long-chain triglycerides, LCT/MCT – long- and medium-chain triglycerides, CR – caloric restriction, IF – intermittent fasting.*

Unexpectedly, *Acadm* did not show significant changes compared to SD (**Figure 13 A**). We had anticipated increased β-oxidation in high-fat diets, especially in LCT/MCT, which would have resulted in *Acadm* upregulation. *Acadm* is involved in the β-oxidation and an important key regulator for breaking down MCFAs.
We expected *Fasn* to be downregulated in KDs, particularly in LCT and LCT/MCT, because it is involved in FA synthesis. However, it makes sense that a diet rich in MCTs might still require the synthesis of palmitate and other long-chain fatty acids. In the LCT group, *Fasn* was significantly downregulated (**Figure 13 C**), likely due to the reduced need for *de novo lipogenesis*, as the diet is already rich in C16 fatty acids.

One possible explanation is that since palmitate and other long-chain triglycerides are already present in LCT, additional synthesis becomes unnecessary.
Following an increase in *Acaca*, we also expected a rise in *Crat*, which facilitates the mitochondrial uptake of acyl-CoA. Although the increase was not statistically significant, *Crat* expression under IF was notably higher than under CR (**Figure 13 B**).

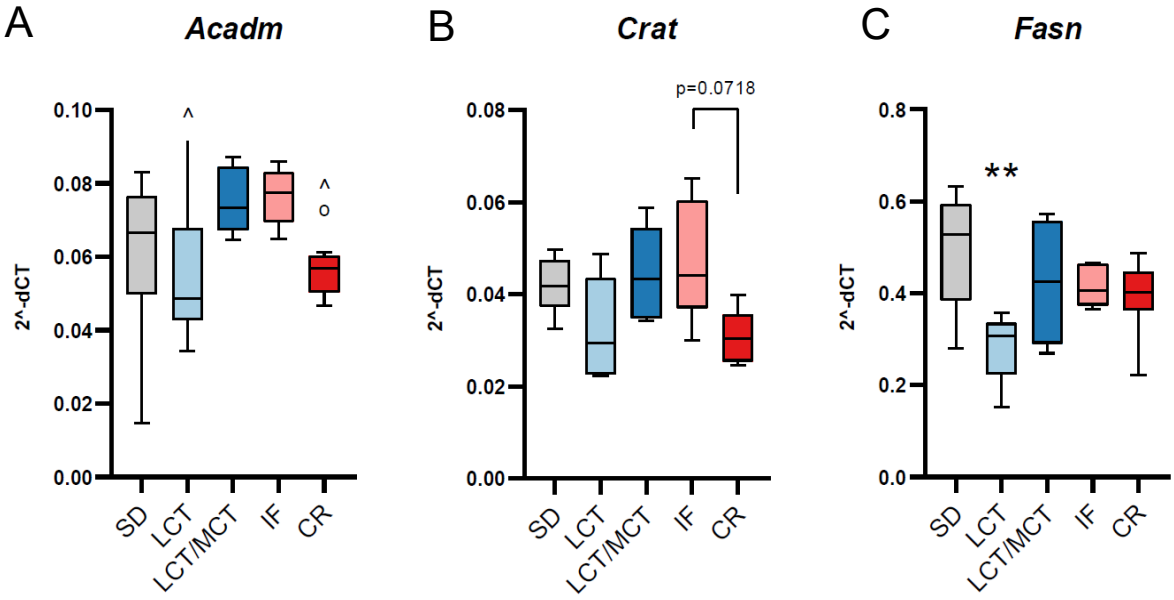


***Figure 13: Relative gene expression levels** of **A** Acadm, **B** Crat and **C** Fasn in the cortex of mice subjected to different diets, normalised to the housekeeping gene Rpl4.*
*Data are presented as mean ± SEM. Significance symbols refer to group comparison: * vs. SD, # vs. LCT, ° vs. LCT/MCT, and ^ vs. IF. Significant differences (p-values) are marked with: *p ≤ 0.05, ** p ≤ 0.01 and ***p ≤ 0.001 (symbols depending on the group being compared). Abbreviations: SD – standard diet, LCT – long-chain triglycerides, LCT/MCT – long- and medium-chain triglycerides, CR – caloric restriction, IF – intermittent fasting.*

LCT/MCT, IF and CR showed higher *Fis1* expression (**Figure 14 A**), while fusion-related target genes such as *Mfn1* and *Mfn2* show a significant decrease (**Figure 14 B, C**), indicating increased mitochondrial fission. This fragmentation into smaller mitochondria may allow for more efficient FAO, potentially reducing the need for strong upregulation of *Acadm* to support β-oxidation. *Mfn1* is a key protein required for mitochondrial fusion, often initiated when increased ATP production is needed. *Opa1* showed almost no significant changes – except in LCT vs. IF (**Figure 14 D**) – and has similar functions like *Mfn1* and *Mfn2*.

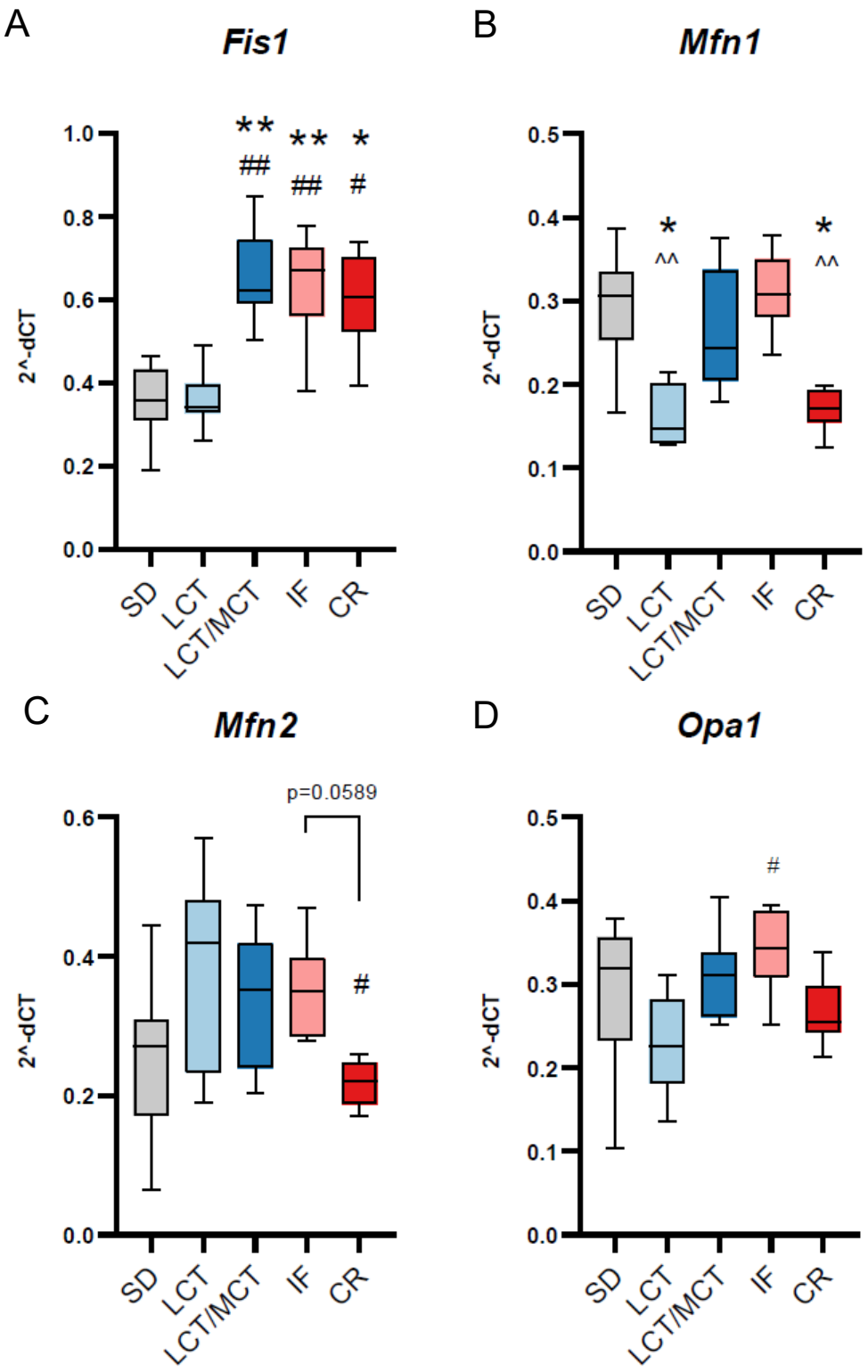


***Figure 14: Relative gene expression levels*** *of* ***A*** *Fis1,* ***B*** *Mfn1,* ***C*** *Mfn2 and* ***D*** *Opa1 in the cortex of mice subjected to different diets, normalised to the housekeeping gene Rpl4.*

*Data are presented as mean ± SEM. Significance symbols refer to group comparison: * vs. SD, # vs. LCT, ° vs. LCT/MCT, and ^ vs. IF. Significant differences (p-values) are marked with: *p ≤ 0.05, ** p ≤ 0.01 and ***p ≤ 0.001 (symbols depending on the group being compared). Abbreviations: SD – standard diet, LCT – long-chain triglycerides, LCT/MCT – long- and medium-chain triglycerides, CR – caloric restriction, IF – intermittent fasting.*

In the Cortex the expression of *Trp53 (l)* was significantly reduced in the LCT and LCT/MCT dietary groups compared to IF (**Figure 15 A**). The significant increase in the *Trp53 (s)* variant has been observed under CR compared to SD, IF and LCT/MCT diets (**Figure 15 B**). *Trp53 (s)* has been associated with senescence and cells where DNA repair is required.

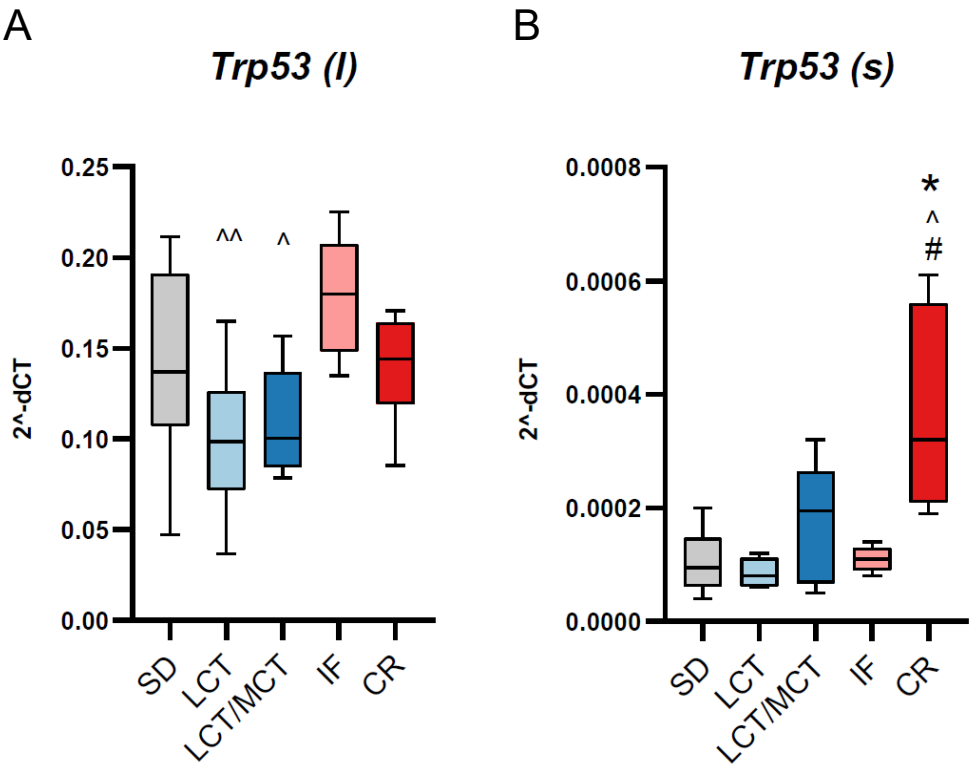


***Figure 15: Relative gene expression levels** of **A** Trp53 (l), which refers to Trp53 α and **B** Trp53 (s), which refers to Trp Δ122p53, in the cortex of mice subjected to different diets, normalised to the housekeeping gene Rpl4.*
*Data are presented as mean ± SEM. Significance symbols refer to group comparison: * vs. SD, # vs. LCT, ° vs. LCT/MCT, and ^ vs. IF. Significant differences (p-values) are marked with: *p ≤ 0.05, ** p ≤ 0.01 and ***p ≤ 0.001 (symbols depending on the group being compared). Abbreviations: SD – standard diet, LCT – long-chain triglycerides, LCT/MCT – long- and medium-chain triglycerides, CR – caloric restriction, IF – intermittent fasting.*

### 4.5.2 Hippocampus

As in the cortex, *Acaca* and *Acacb* expression in the hippocampus remained largely unchanged across most dietary conditions. However, in the CR group, *Acaca* was significantly upregulated and *Acacb* expression was also altered (**Figure 16 A, B**). This change may reflect a neuroprotective adaptation in response to a CR in the hippocampus. Since CPT1 is one of the key transport mechanisms for LCFAs, it is important not to upregulate *Acaca* excessively in high-fat diets, as this would inhibit FA uptake into cells and potentially disrupt β-oxidation.

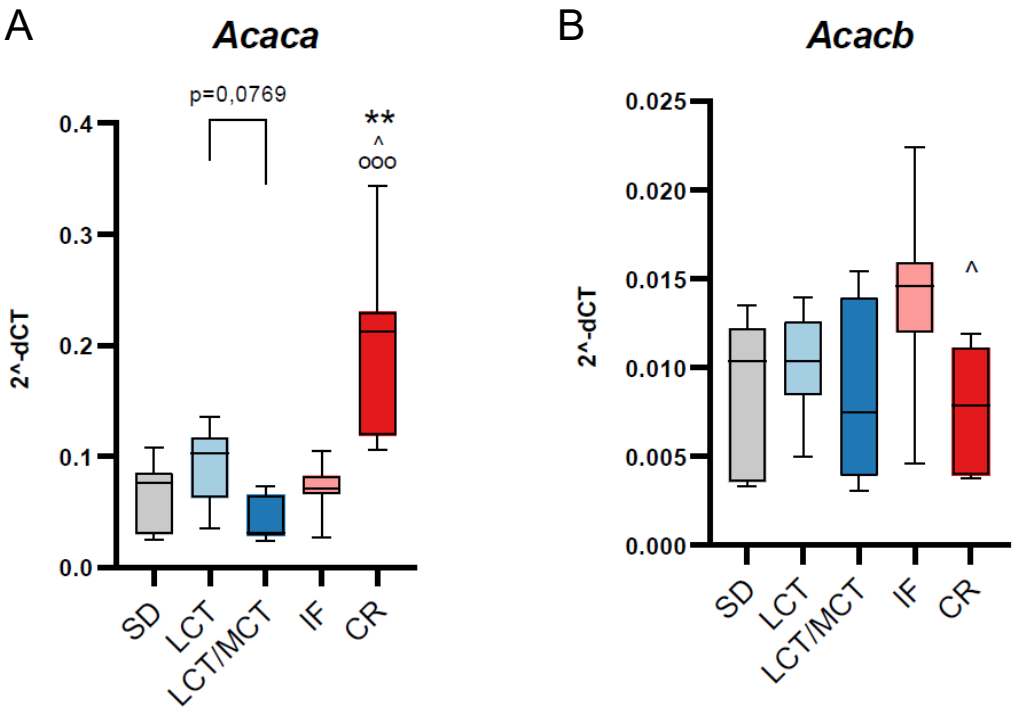


***Figure 16: Relative gene expression levels*** *of* ***A*** *Acaca and* ***B*** *Acacb in the hippocampus of mice subjected to different diets, normalised to the housekeeping gene Rpl4.*
*Data are presented as mean ± SEM. Significance symbols refer to group comparison: * vs. SD, # vs. LCT, ° vs. LCT/MCT, and ^ vs. IF. Significant differences (p-values) are marked with: *p ≤ 0.05, ** p ≤ 0.01 and ***p ≤ 0.001 (symbols depending on the group being compared). Abbreviations: SD – standard diet, LCT – long-chain triglycerides, LCT/MCT – long- and medium-chain triglycerides, CR – caloric restriction, IF – intermittent fasting.*

*Acadm* showed no significant changes in the hippocampus (**Figure 17 A**). Unexpectedly, *Crat* expression in the hippocampus did not increase across all KDs, but only in the LCT group (**Figure 17 B**). A closer look suggests this might be because LCTs generate more acetyl-CoA during breakdown than MCTs. Over time, this can lead to an acetyl-CoA surplus in the brain, and upregulation of *Crat* could serve as a compensatory mechanism to convert the excess into acetyl-carnitine.

It was anticipated that *Crat* would be significantly higher in the LCT group than in LCT/MCT, but also higher than in the other diets, because MCFAs do not require carnitine transport to enter mitochondria.

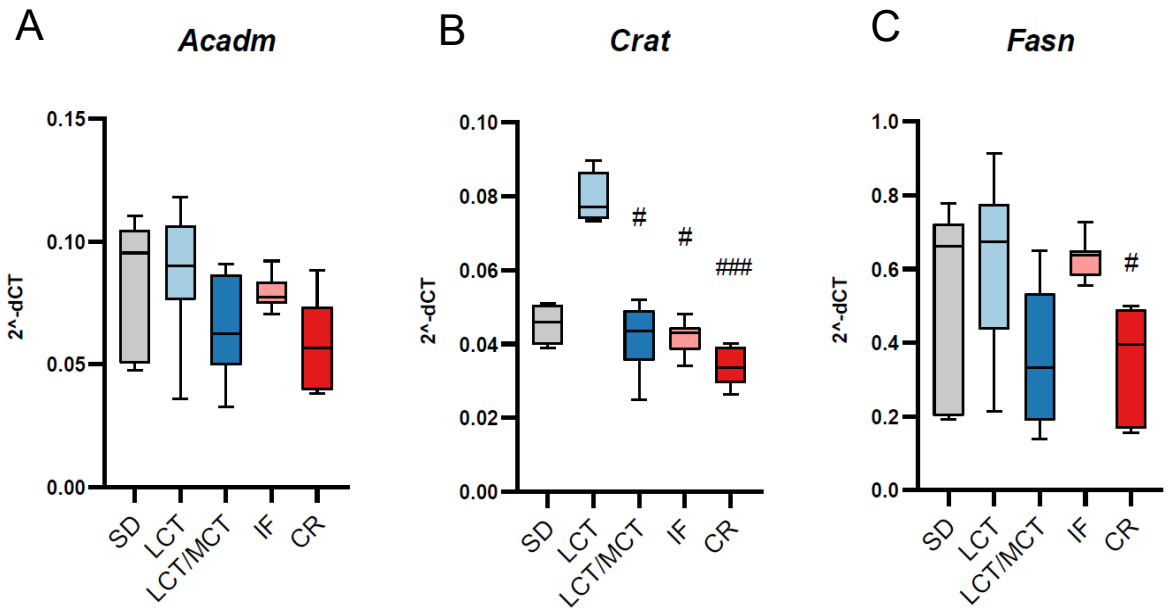


***Figure 17: Relative gene expression levels** of **A** Acadm, **B** Crat and **C** Fasn in the hippocampus of mice subjected to different diets, normalised to the housekeeping gene Rpl4.*
*Data are presented as mean ± SEM. Significance symbols refer to group comparison: * vs. SD, # vs. LCT, ° vs. LCT/MCT, and ^ vs. IF. Significant differences (p-values) are marked with: *p ≤ 0.05, ** p ≤ 0.01 and ***p ≤ 0.001 (symbols depending on the group being compared). Abbreviations: SD – standard diet, LCT – long-chain triglycerides, LCT/MCT – long- and medium-chain triglycerides, CR – caloric restriction, IF – intermittent fasting.*

Due to the hippocampus's high plasticity and continuous role in learning and memory, it may rely more heavily on adaptations in mitochondrial dynamics. *Fis1* was significantly upregulated in the LCT/MCT diet (**Figure 18 A**), promoting mitochondrial fragmentation and thereby improving FAO. The increase in *Mfn1* expression under LCT in the hippocampus (**Figure 18 B**) supports the formation of larger mitochondria, which may enhance energy production and reduce the need for mitophagy. This is further supported by the concurrent upregulation of *Crat*, facilitating acyl-CoA formation, which is an essential molecule for β-oxidation. In IF, neither fusion- nor fission-related genes were upregulated in the hippocampus, suggesting that energy demands are met without excessive ROS production, which would otherwise trigger mitochondrial fission.

The gene expression of *Mfn2* and *Opa1* have similar functions like *Mfn1* and showed no significant changes (**Figure 18 C, D**) between the diets in the hippocampus.

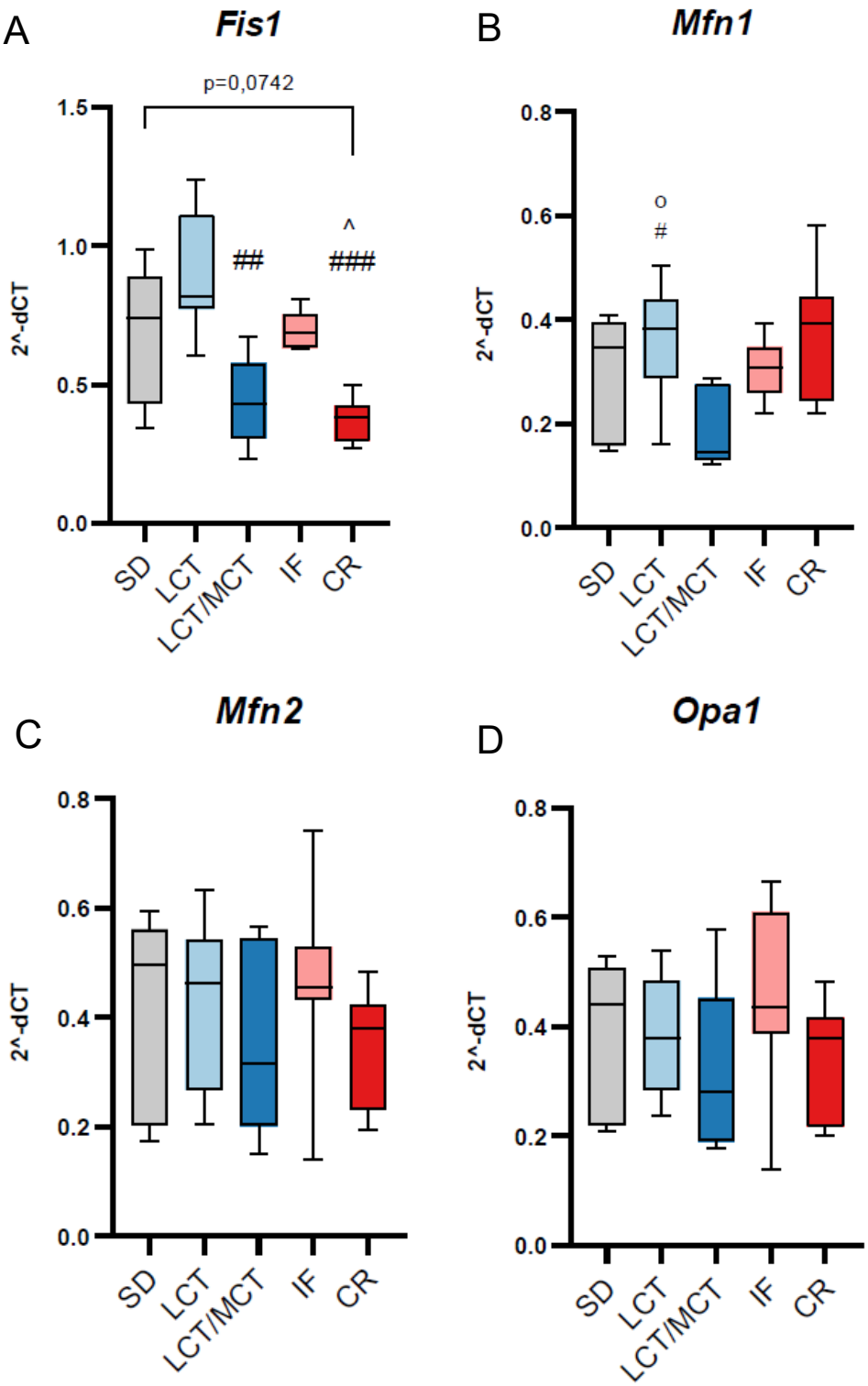


***Figure 18: Relative gene expression levels*** *of* ***A*** *Fis1,* ***B*** *Mfn1,* ***C*** *Mfn2 and* ***D*** *Opa1 in the hippocampus of mice subjected to different diets, normalised to the housekeeping gene Rpl4.*
*Data are presented as mean ± SEM. Significance symbols refer to group comparison: * vs. SD, # vs. LCT, ° vs. LCT/MCT, and ^ vs. IF. Significant differences (p-values) are marked with: *p ≤ 0.05, ** p ≤ 0.01 and ***p ≤ 0.001 (symbols depending on the group being compared). Abbreviations: SD – standard diet, LCT – long-chain triglycerides, LCT/MCT – long- and medium-chain triglycerides, CR – caloric restriction, IF – intermittent fasting.*

The significant downregulation of *Trp53 (l)* in the LCT/MCT group compared to SD and LCT (**Figure 19 A**) may reflect differences in cellular stress levels. Since MCTs are metabolized more efficiently and generate fewer ROS, this leads to reduced DNA damage. *Trp53 (s)* expression was notably higher under LCT compared to IF and CR (**Figure 19 B**), which showed the lowest levels of expression.

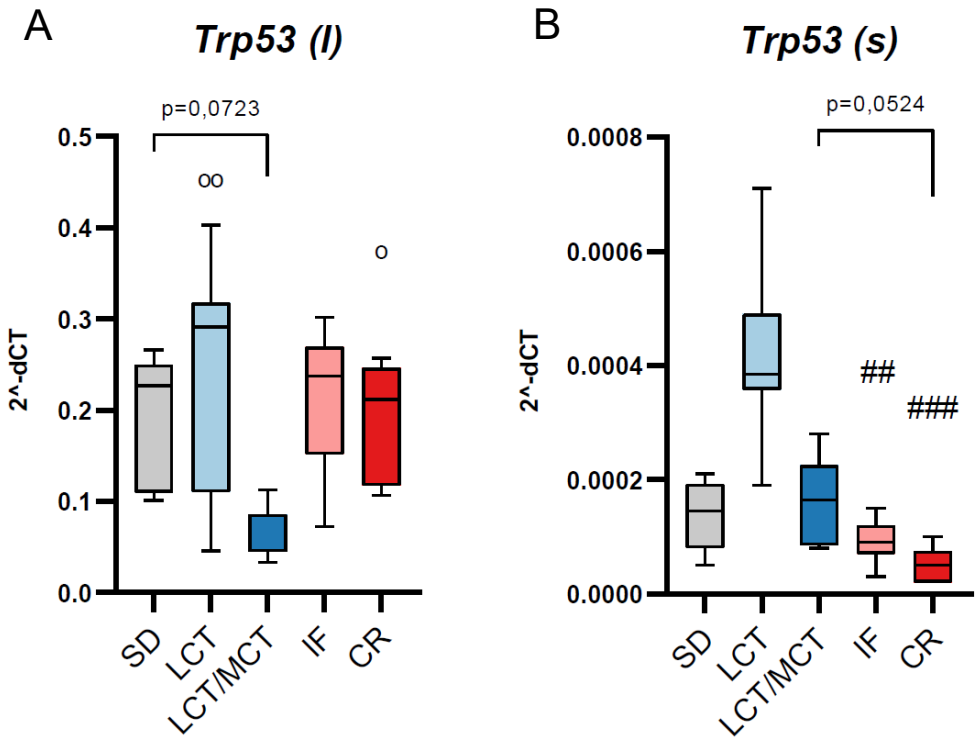


***Figure 19: Relative gene expression levels*** *of* ***A*** *Trp53 (l), which refers to Trp53 α and* ***B*** *Trp53 (s), which refers to Trp Δ122, in the hippocampus of mice subjected to different diets, normalised to the housekeeping gene Rpl4.*
*Data are presented as mean ± SEM. Significance symbols refer to group comparison: * vs. SD, # vs. LCT, ° vs. LCT/MCT, and ^ vs. IF. Significant differences (p-values) are marked with: *p ≤ 0.05, ** p ≤ 0.01 and ***p ≤ 0.001 (symbols depending on the group being compared). Abbreviations: SD – standard diet, LCT – long-chain triglycerides, LCT/MCT – long- and medium-chain triglycerides, CR – caloric restriction, IF – intermittent fasting.*

# 5 Discussion

In the present study we aimed to investigate how different dietary interventions – namely LCT, LCT/MCT, IF and CR and an additional control group (SD) – affect gene expression related to FA metabolism and mitochondrial function in the cortex and hippocampus. The results provide insights into some region-specific metabolic plasticity of the brain and how dietary components may modulate key regulatory pathways such as FAO, mitochondrial dynamics, and energy homeostasis. Consistent with existing literature, our data suggest that the hippocampus exhibits a distinct transcriptional response compared to the cortex, likely due to its heightened sensitivity to metabolic stress and its critical role in neuroplasticity.

The cortex and hippocampus have different metabolic demands and regional pathway activities due to the varying distribution of neurons, astrocytes, and glial cells, which may influence gene expression. This could explain the differences in gene expression observed in different brain regions. In general, key signalling pathways such as AMPK, PGC-1α, and SREBP are regulated differently in the hippocampus compared to the cortex, which may also contribute to these differences (Lettieri Barbato et al., 2012). This kind of metabolic plasticity – the ability of brain cells to adapt to metabolic shifts, such as those caused by a KD – is especially observable in glial cells and neurons. This might be due to the hippocampus' high metabolic activity and rapid cyclic renewal, which are closely linked to its role in learning and memory (Citri & Malenka, 2008; Zhou et al., 2019). But plasticity is not only relevant in response to diet. It is also critical in learning processes or after injury. This form of adaptation is rather unique to the brain and occurs quite differently than in most other organs, which tend to focus more on regeneration (Citri & Malenka, 2008; Düking et al., 2022) This could help explain why the brain shows distinct changes in metabolism and gene expression under the same dietary conditions compared to other organs.

During IF, the cortex may require increased energy production to maintain synaptic structures and neuronal membranes, whereas the hippocampus might rely more on alternative metabolic pathways, such as ketone bodies. This could explain why *Acaca* is not upregulated in the hippocampus as it is in the cortex. In conditions where *Acaca* or *Acacb* are upregulated, it may lead to reduced transport of

acyl-CoA into mitochondria via CPT1 (**Figure 20**). Since CPT1 is inhibited by malonyl-CoA and also plays a crucial role in transporting long-chain fatty acids, which cannot cross the membrane unaided, this inhibition could impair FAO. If *Acaca* is upregulated in high-fat diets, leading to increased malonyl-CoA levels, β-oxidation could be disrupted, which is particularly undesirable under ketogenic conditions (Cao et al., 2023; Wang et al., 2022).

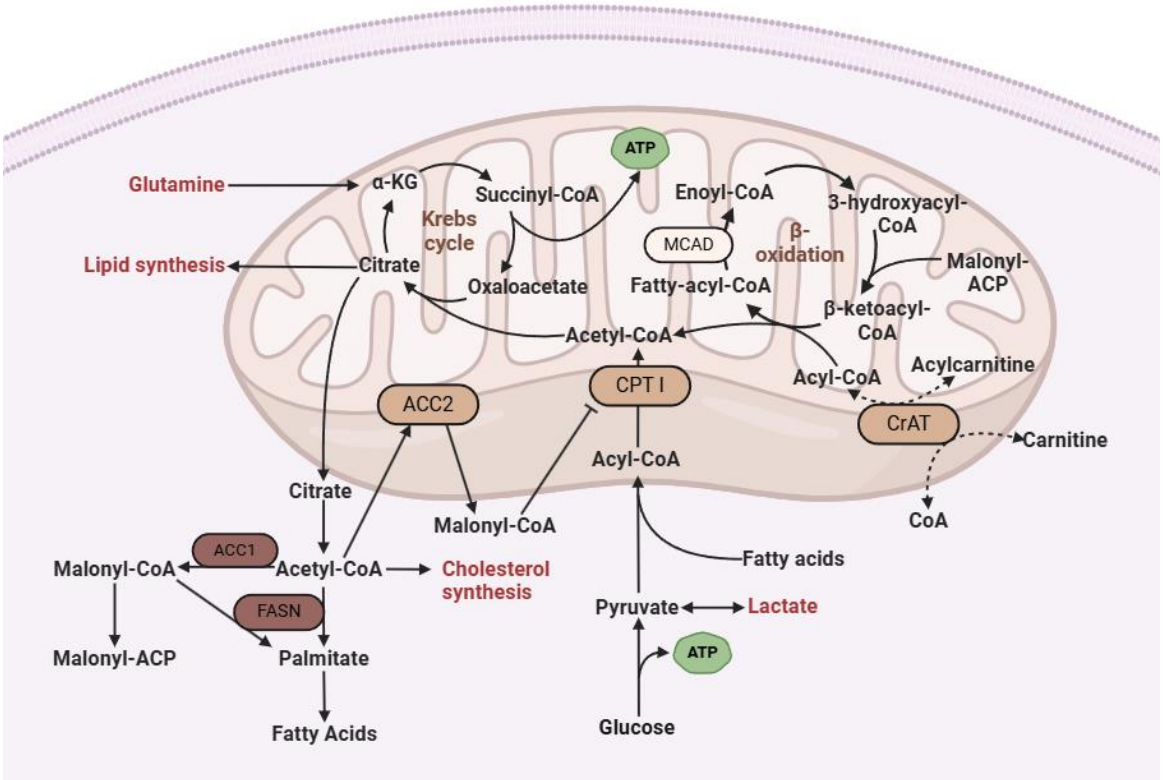


***Figure 20: Overview of mitochondrial pathways involved and related to fatty acid metabolism****, involving Acaca (ACC1), Acacb (ACC2), Acadm (MCAD), Crat and FASN.*
*Created in https://BioRender.com*

This finding aligns with previous studies showing that high-carbohydrate diets upregulate lipogenic enzymes like *Acaca* and *Fasn*, due to the promotion of *de novo* lipogenesis in the liver. In contrast, high-fat diets suppress these enzymes, since the external supply of FAs reduces the demand for endogenous lipid synthesis (Bruning et al., 2018; Zhu et al., 2014). Interestingly, in our current study, *Fasn* expression was downregulated only in the cortex under LCT, which may indicate that fat production in the cortex is reduced under ketogenic conditions.

Given that FASN catalyses palmitate trough acetyl-CoA and malonyl-CoA, lower FASN levels could help the brain save energy and prevent excess fat from building up in cortical cells.

Although both IF and CR were expected to elicit similar transcriptional responses due to their shared CR aspect, notable differences were observed across most of the target genes. Interestingly, IF often showed greater similarity to the LCT/MCT condition than CR, for instance in the cortex with genes such as *Acacb*, *Acadm, Mfn1* or *Mfn2.* This could also be due to MCTs providing energy more rapidly than LCTs alone, as they can pass directly into the brain, thereby potentially mimicking the metabolic substrate shifts observed under IF. In contrast, the slower breakdown of LCTs results in a more stable energy supply, reducing metabolic fluctuations.

A further explanation for these differences between IF and CR could relate to the timing of sample collection. IF and CR condition differences may be the phenomenon of food anticipation (or also known as feed-forward regulation), since the animals were sacrificed after their fasting phase. If feeding always occurred at the same time, certain pathways might already be up- or downregulated before actual food intake (Challet et al., 2009; Khapre et al., 2014; Skvortsova et al., 2021).

Khapre et al., (2014) addressed this by studying inter alia the mTOR signalling pathway, which can be affected by fasting. Mice were fed once every 24 hours for two weeks, and food anticipation was tested by sacrificing them at various time points after their last meal. A few downstream targets of the mTOR pathway were analysed and it showed that mice sacrificed closer to their habitual feeding time already exhibited altered expression levels of these targets. While ACC was not directly assessed in this study, its regulation is known to be influenced by both AMPK and mTOR signalling (Wang et al., 2022). It is also worth noting that CR mice had a lower percentage of eWAT and reduced body weight compared to IF mice, which could result in altered brain lipid metabolism due to limited energy resources.

Shifting focus to the hippocampus, it is structurally and functionally distinct from the cortex and is notably more sensitive to stress, especially during energy or nutrient deprivation. This leads to the activation of specific mechanisms to counteract stress and its effects. This sensitivity is likely due to both the high concentration of glucocorticoid receptors in the hippocampus and its high plasticity. This plasticity can affect not only metabolism but also structural aspects such as dendritic complexity (Albadawi, 2025; Beck & Luine, 1999; Rappeneau et al., 2023). This may help explain why certain targets in the hippocampus – such as *Trp53* or the elevated *Fis1* expression – are particularly affected by the LCT diet compared to the cortex, while appearing less affected by the LCT/MCT diet. This difference may be connected to the known anti-inflammatory properties of MCTs and their ability to reduce ROS. A similar pattern is observed with *Acaca* expression under CR. As previously reported, the hippocampus is notably sensitive to food deprivation, likely to its metabolic and stress-related vulnerabilities.

The observed changes in *Trp53* expression suggest that dietary composition exerts a differential impact in stress-related and regulatory signalling pathways in a region-specific manner. The significant downregulation in LCT and LCT/MCT (cortex) compared to IF may indicate a reduced metabolic or oxidative challenge in these groups, as p53 α activity has been linked to cellular response to stress and DNA damage. Interestingly, in the hippocampus only the LCT/MCT group showed a marked suppression of *Trp53 α*, which may demonstrate a region-specific sensitivity to MCTs or to a faster adaptation of hippocampal cells to alternative metabolic substrates provided by this diet (Humpton & Vousden, 2019; Wei et al., 2024).

The upregulation of *Trp Δ122p53* in the cortex under CR suggests that this diet may modulate p53-dependent transcriptional activity and apoptosis and shift cellular priorities under energy limitation. In the hippocampus *Trp Δ122p53* expression was increased only in the LCT group, which could indicate that LCFAs in contrast to MCFAs induce stress responses in hippocampal neurons. This could be due to an increased ROS or altered lipid metabolism, which could influence stress response, neuroprotection and cell fate decisions (Roth et al., 2016; Slatter et al., 2015).

Regarding mitochondrial dynamics, the increased expression of *Fis1* in the cortex suggests that mitochondria undergo fission more frequently compared to the SD

condition. One possible explanation could be that mitochondria are able to produce energy more efficiently and are thus able to manage accumulated ROS easily by fragmentation and mitophagy without disrupting their energy metabolism. This is further supported by the fact that *Acadm* is not upregulated, indicating that FAO does not increase. Studies suggest that fragmented mitochondria have enhanced FAO compared to elongated ones. This is largely due to the reduced inhibition of CPT1 by malonyl-CoA. The significantly downregulated *Mfn1* under LCT condition indicates less mitochondrial fusion, resulting in smaller mitochondria. Studies have shown that non-elongated mitochondria can oxidize LCFAs more efficiently. Another possible explanation could be an increase in ROS, which could trigger mitochondrial fission to facilitate mitophagy (Ngo et al., 2023; Seifert & Hajnóczky, 2023).

In summary, this study demonstrates that dietary interventions such as IF, CR, and particularly the inclusion of MCTs in a classical LCT-based KD can induce notable changes in the expression of genes involved in FA metabolism and mitochondrial dynamics. Targets like *Acaca, Acacb, Fasn, Fis1, Mfn1, Trp53* or *Crat* (hippocampus) revealed specific regulatory patterns, with MCT supplementation often altering expression changes otherwise observed under LCT conditions – possibly due to their faster metabolism and reduced oxidative stress. Moreover, IF showed unexpected overlap with the LCT/MCT group, supporting the idea that MCTs may partially mimic metabolic states observed during fasting. But the observed differences between the cortex and hippocampus emphasize the importance of considering brain region-specific functions and vulnerability when evaluating metabolic interventions. Altogether, these findings provide evidence that MCTs may offer a more favourable metabolic profile than LCTs alone, particularly by supporting efficient energy use and limiting damaging shifts in mitochondrial function.

# 6 Abstract

Ketogenic diets (KD), which elevate ketone body production, have gained attention for their ability to shift the energy metabolism away from glucose toward fatty acid oxidation (FAO). Fats, like medium-chain triglycerides (MCTs) play a crucial role in metabolic regulation, particularly in the context of KD and neuroprotection. Unlike long-chain triglycerides (LCTs), which require lymphatic transport and carnitine-dependent mitochondrial entry, MCTs are rapidly absorbed via the hepatic portal vein and a bulk of MCTs undergo direct mitochondrial β-oxidation in the liver. This is especially interesting for neuronal health, as the brain then utilizes ketones for energy and these shifts have been associated with neuroprotective effects such as improved synaptic function and reducing oxidative stress and inflammation.

Ketosis can be not only reached by a high-fat and low-carbohydrate diet, but also through intermittent fasting (IF) and caloric restriction (CR). These dietary interventions have demonstrated their ability to reduce reactive oxygen species (ROS) production and support metabolic homeostasis.

This thesis aimed to address the hypothesis that if MCFAs reach the brain tissue and mediate direct effects rather via β-hydroxybutyrate (β-HB), MCT addition to an LCT-KD should alter specific gene expression compared to LCT-KD alone accordingly investigate the effects of dietary interventions – including a standard diet (SD), an LCT-based diet, an LCT/MCT diet, CR, and IF – on key metabolic pathways in murine brain tissue by employing quantitative PCR.

To investigate this, a specific focus is placed on the expression of genes involved in fatty acid oxidation and mitochondrial dynamics, including *Acaca, Acacb, Acadm, Crat, Fasn, Fis1, Mfn1, Mfn2, Opa1* and *Trp53*. The results showed that supplementing an LCT-KD with MCTs does alter gene expression in both the cortex and hippocampus. These differences could especially be seen in genes related to mitochondrial dynamics, such as *Fis1* and *Mfn1*. While expecting IF and CR to have similar outcomes, the results showed some differences. In IF several genes were either up- or downregulated compared to CR, which may be related to food-anticipation.

# 7 Zusammenfassung

Eine ketogene Ernährung (KD) führt zur gesteigerten Bildung von Ketonkörpern und kann so zu einer Verschiebung des Stoffwechsels zur Fettsäureoxidation (FAO) zur Energiegewinnung, anstatt dabei auf Glukose zurückzugreifen. Mittelkettige Triglyceride (MCT) spielen ebenfalls eine wesentliche Rolle in der Regulation des Stoffwechsels – insbesondere im Zusammenhang mit einer KD und neuroprotektiven Mechanismen. Anders als langkettige Triglyceride (LCT), die einen lymphatischen Transport sowie eine Carnitin-abhängige Aufnahme in die Mitochondrien erfordern, werden MCTs rasch über die Pfortader aufgenommen und direkt der mitochondrialen β-Oxidation zugeführt. Dies ist besonders interessant im Hinblick auf die neuronale Gesundheit, da diese Umstellung mit einer Verringerung des oxidativen Stresses und neuroprotektiven Effekten in Verbindung gebracht wird. Darüber hinaus haben diätetische Interventionen wie Kalorienrestriktion (CR) und intermittierendes Fasten (IF) gezeigt, dass sie die Produktion reaktiver Sauerstoffspezies (ROS) senken und zur Aufrechterhaltung der metabolischen Homöostase beitragen können. Diese Arbeit ging der Hypothese nach, dass mittelkettige Fettsäuren, sofern sie das Gehirngewebe erreichen und dort wirken, bei Ergänzung einer LCT-KD spezifische Veränderungen in der Genexpression bewirken, verglichen mit einer reinen LCT-KD. Ziel war es daher verschiedene Ernährungsinterventionen – darunter eine Standardernährung (SD), eine LCT-basierte Ernährung (LCT), eine kombinierte LCT/MCT-Ernährung, CR und IF – auf zentrale Stoffwechselwege im Gehirngewebe von Mäusen mittels quantitativer PCR zu untersuchen. Dabei liegt der Fokus auf der Expression von Genen, die an der FAO und der mitochondrialen Dynamik beteiligt sind, *darunter Acaca, Acacb, Acadm, Crat, Fasn, Fis1, Mfn1, Mfn2, Opa1* und *Trp53*.

Die Ergebnisse zeigten, dass eine Supplementierung der LCT-KD mit MCTs die Genexpression sowohl im Kortex als auch im Hippocampus verändert, besonders bezüglich der mitochondrialen Dynamik, wie bei *Fis1* und *Mfn1*. Während bei IF und CR ähnliche Effekte erwartet wurden, wiesen die Ergebnisse teils Unterschiede auf. Bei IF waren mehrere Gene im Vergleich zu CR entweder hoch- oder runterreguliert, was möglicherweise mit dem Phänomen der „food anticipation“ zusammenhängen könnte.

# 8 Abbreviations

| | |
|---|---|
| Acaca | Acetyl-CoA-carboxylase-α |
| Acacb | Acetyl-CoA carboxylase β |
| Acadm | Acyl-CoA Dehydrogenase Medium Chain |
| BDNF | Brain-derived neurotrophic factor |
| β-HB | β-hydroxybutyrate |
| CR | Caloric restriction |
| Crat | Carnitine acetyltransferase |
| FA | Fatty acid |
| FAO | Fatty acid oxidation |
| Fasn | Fatty acid synthase |
| Fis1 | Mitochondrial fission protein 1 |
| Hprt | Hypoxanthine-guanine-phosphoribosyltransferase |
| IF | Intermittent fasting |
| KD | Ketogenic diet |
| LCFA | Long chain fatty acids |
| LCT | Long chain triglycerides |
| MCFA | Medium chain fatty acids |
| MCT | Medium chain triglycerides |
| Mfn1 | Mitofusin 1 |
| Mfn2 | Mitofusin 2 |
| NF-$H_2O$ | Nuclease free water |
| Opa1 | Optic atrophy 1 |
| OxPhos | Oxidative phosphorylation |
| ROS | Reactive oxygen species |
| Rpl4 | Ribosomal Protein L4 |
| RT | Reverse transcription control |
| SD | Standard diet |
| TCA - cycle | Tricarboxylic acid cycle |
| Tm | Melting Temperature |
| Trp53 (l) | Transformation-related protein 53 α |
| Trp53 (s) | Transformation-related protein Δ122p53 |

# 9 References


*ACADM gene: MedlinePlus Genetics*. (n.d.). Retrieved 3 January 2025, from https://medlineplus.gov/genetics/gene/acadm/

Acuña-Catalán, D., Shah, S., Wehrfritz, C., Nomura, M., Acevedo, A., Olmos, C., Quiroz, G., Huerta, H., Bons, J., Ampuero, E., Wyneken, U., Sanhueza, M., Arancibia, F., Contreras, D., Cárdenas, J. C., Morales, B., Schilling, B., Newman, J. C., & González-Billault, C. (2024). Ketogenic diet administration later in life improves memory by modifying the synaptic cortical proteome via the PKA signaling pathway in aging mice. *Cell Reports Medicine*, *5*(6). https://doi.org/10.1016/j.xcrm.2024.101593

Adafer, R., Messaadi, W., Meddahi, M., Patey, A., Haderbache, A., Bayen, S., & Messaadi, N. (2020). Food Timing, Circadian Rhythm and Chrononutrition: A Systematic Review of Time-Restricted Eating's Effects on Human Health. *Nutrients*, *12*(12), 3770. https://doi.org/10.3390/nu12123770

Albadawi, E. A. (2025). Structural and functional changes in the hippocampus induced by environmental exposures. *Neurosciences Journal*, *30*(1), 5–19. https://doi.org/10.17712/nsj.2025.1.20240052

Antoni, R., Johnston, K. L., Collins, A. L., & Robertson, M. D. (2017). Effects of intermittent fasting on glucose and lipid metabolism. *Proceedings of the Nutrition Society*, *76*(3), 361–368. https://doi.org/10.1017/S0029665116002986

Ari, C., Murdun, C., Goldhagen, C., Koutnik, A. P., Bharwani, S. R., Diamond, D. M., Kindy, M., D'Agostino, D. P., & Kovacs, Z. (2020). Exogenous Ketone Supplements Improved Motor Performance in Preclinical Rodent Models. *Nutrients*, *12*(8), 2459. https://doi.org/10.3390/nu12082459

Beck, K. D., & Luine, V. N. (1999). Food deprivation modulates chronic stress effects on object recognition in male rats: Role of monoamines and amino acids. *Brain Research*, *830*(1), 56–71. https://doi.org/10.1016/S0006-8993(99)01380-3

Berg, J. M., Stryer, L., & Tymoczko, J. L. (2015). *Stryer Biochemie* (A. Held, C. Lange, K. Mahlke, G. Maxam, L. Seidler, N. Zellerhoff, B. Häcker, & B. Jarosch, Trans.; 7. Aufl. 2013). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-8274-2989-6

Bough, K. J., Wetherington, J., Hassel, B., Pare, J. F., Gawryluk, J. W., Greene, J. G., Shaw, R., Smith, Y., Geiger, J. D., & Dingledine, R. J. (2006). Mitochondrial biogenesis in the anticonvulsant mechanism of the ketogenic diet. *Annals of Neurology*, *60*(2), 223–235.

https://doi.org/10.1002/ana.20899

*Brain Anatomy and How the Brain Works*. (2021, July 14). https://www.hopkinsmedicine.org/health/conditions-and-diseases/anatomy-of-the-brain

*Brain Basics: Know Your Brain | National Institute of Neurological Disorders and Stroke*. (n.d.). Retrieved 6 January 2025, from https://www.ninds.nih.gov/health-information/public-education/brain-basics/brain-basics-know-your-brain

Brenton, J. N., Banwell, B., Bergqvist, A. G. C., Lehner-Gulotta, D., Gampper, L., Leytham, E., Coleman, R., & Goldman, M. D. (2019). Pilot study of a ketogenic diet in relapsing-remitting MS. *Neurology Neuroimmunology & Neuroinflammation*, *6*(4), e565. https://doi.org/10.1212/NXI.0000000000000565

Bruning, U., Morales-Rodriguez, F., Kalucka, J., Goveia, J., Taverna, F., Queiroz, K. C. S., Dubois, C., Cantelmo, A. R., Chen, R., Loroch, S., Timmerman, E., Caixeta, V., Bloch, K., Conradi, L.-C., Treps, L., Staes, A., Gevaert, K., Tee, A., Dewerchin, M., … Carmeliet, P. (2018). Impairment of Angiogenesis by Fatty Acid Synthase Inhibition Involves mTOR Malonylation. *Cell Metabolism*, *28*(6), 866-880.e15. https://doi.org/10.1016/j.cmet.2018.07.019

Cao, H., Cai, Q., Guo, W., Su, Q., Qin, H., Wang, T., Xian, Y., Zeng, L., Cai, M., Guan, H., Chen, S., Liang, H., & Xu, F. (2023). Malonylation of Acetyl-CoA carboxylase 1 promotes hepatic steatosis and is attenuated by ketogenic diet in NAFLD. *Cell Reports*, *42*(4). https://doi.org/10.1016/j.celrep.2023.112319

Challet, E., Mendoza, J., Dardente, H., & Pévet, P. (2009). Neurogenetics of food anticipation. *European Journal of Neuroscience*, *30*(9), 1676–1687. https://doi.org/10.1111/j.1460-9568.2009.06962.x

Chang, I. J., Lam, C., & Vockley, J. (2024). Medium-Chain Acyl-Coenzyme A Dehydrogenase Deficiency. In *GeneReviews® [Internet]*. University of Washington, Seattle. https://www.ncbi.nlm.nih.gov/sites/books/NBK1424/

Chen, D., Li, X., Zhang, L., Zhu, M., & Gao, L. (2018). A high-fat diet impairs mitochondrial biogenesis, mitochondrial dynamics, and the respiratory chain complex in rat myocardial tissues. *Journal of Cellular Biochemistry*, *119*(11), 9602–9602. https://doi.org/10.1002/jcb.27068

Chen, H., Liu, C., Cui, S., Xia, Y., Zhang, K., Cheng, H., Peng, J., Yu, X., Li, L., Yu, H., Zhang, J., Zheng, J.-S., & Zhang, B. (2025). Intermittent fasting triggers interorgan communication to suppress hair follicle regeneration. *Cell*, *188*(1), 157-174.e22. https://doi.org/10.1016/j.cell.2024.11.004

Cheng, X., Jiang, C., Jin, J., Jin, Q., Akoh, C. C., Wei, W., & Wang, X. (2024). Medium- and Long-Chain Triacylglycerol: Preparation, Health Benefits, and Food Utilization. *Annual Review of Food Science and Technology*, *15*(1), 381–408. https://doi.org/10.1146/annurev-food-072023-034539

Choi, I. Y., Piccio, L., Childress, P., Bollman, B., Ghosh, A., Brandhorst, S., Suarez, J., Michalsen, A., Cross, A. H., Morgan, T. E., Wei, M., Paul, F., Bock, M., & Longo, V. D. (2016). A Diet Mimicking Fasting Promotes Regeneration and Reduces Autoimmunity and Multiple Sclerosis Symptoms. *Cell Reports*, *15*(10), 2136–2146. https://doi.org/10.1016/j.celrep.2016.05.009

Cienfuegos, S., Corapi, S., Gabel, K., Ezpeleta, M., Kalam, F., Lin, S., Pavlou, V., & Varady, K. A. (2022). Effect of Intermittent Fasting on Reproductive Hormone Levels in Females and Males: A Review of Human Trials. *Nutrients*, *14*(11), 2343. https://doi.org/10.3390/nu14112343

Citri, A., & Malenka, R. C. (2008). Synaptic Plasticity: Multiple Forms, Functions, and Mechanisms. *Neuropsychopharmacology*, *33*(1), 18–41. https://doi.org/10.1038/sj.npp.1301559

Cullingford, T. E., Eagles, D. A., & Sato, H. (2002). The ketogenic diet upregulates expression of the gene encoding the key ketogenic enzyme mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase in rat brain. *Epilepsy Research*, *49*(2), 99–107. https://doi.org/10.1016/S0920-1211(02)00011-6

Cunnane, S. C., Courchesne-Loyer, A., Vandenberghe, C., St-Pierre, V., Fortier, M., Hennebelle, M., Croteau, E., Bocti, C., Fulop, T., & Castellano, C.-A. (2016). Can Ketones Help Rescue Brain Fuel Supply in Later Life? Implications for Cognitive Health during Aging and the Treatment of Alzheimer's Disease. *Frontiers in Molecular Neuroscience*, *9*. https://doi.org/10.3389/fnmol.2016.00053

Currie, E., Schulze, A., Zechner, R., Walther, T. C., & Farese, R. V. (2013). Cellular Fatty Acid Metabolism and Cancer. *Cell Metabolism*, *18*(2), 153–161. https://doi.org/10.1016/j.cmet.2013.05.017

Dambrova, M., Makrecka-Kuka, M., Kuka, J., Vilskersts, R., Nordberg, D., Attwood, M. M., Smesny, S., Sen, Z. D., Guo, A. C., Oler, E., Tian, S., Zheng, J., Wishart, D. S., Liepinsh, E., & Schiöth, H. B. (2022). Acylcarnitines: Nomenclature, Biomarkers, Therapeutic Potential, Drug Targets, and Clinical Trials. *Pharmacological Reviews*, *74*(3), 506–551. https://doi.org/10.1124/pharmrev.121.000408

Danan, A., Westman, E. C., Saslow, L. R., & Ede, G. (2022). The Ketogenic Diet for Refractory Mental Illness: A Retrospective Analysis of 31 Inpatients. *Frontiers in Psychiatry*, *13*, 951376. https://doi.org/10.3389/fpsyt.2022.951376

Dankel, S. N., Bjørndal, B., Lindquist, C., Grinna, M. L., Rossmann, C. R., Bohov, P., Nygård, O., Hallström, S., Strand, E., & Berge, R. K. (2021). Hepatic Energy Metabolism Underlying Differential Lipidomic Responses to High-Carbohydrate and High-Fat Diets in Male Wistar Rats. *The Journal of Nutrition*, *151*(9), 2610–2621. https://doi.org/10.1093/jn/nxab178

Das, S. K., Roberts, S. B., Bhapkar, M. V., Villareal, D. T., Fontana, L., Martin, C. K., Racette, S. B., Fuss, P. J., Kraus, W. E., Wong, W. W., Saltzman, E., Pieper, C. F., Fielding, R. A., Schwartz, A. V., Ravussin, E., & Redman, L. M. (2017). Body-composition changes in the Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy (CALERIE)-2 study: A 2-y randomized controlled trial of calorie restriction in nonobese humans123. *The American Journal of Clinical Nutrition*, *105*(4), 913–927. https://doi.org/10.3945/ajcn.116.137232

de Jonge, H. J. M., Fehrmann, R. S. N., de Bont, E. S. J. M., Hofstra, R. M. W., Gerbens, F., Kamps, W. A., de Vries, E. G. E., van der Zee, A. G. J., te Meerman, G. J., & ter Elst, A. (2007). Evidence Based Selection of Housekeeping Genes. *PLoS ONE*, *2*(9), e898. https://doi.org/10.1371/journal.pone.0000898

Di Lorenzo, C., Pinto, A., Ienca, R., Coppola, G., Sirianni, G., Di Lorenzo, G., Parisi, V., Serrao, M., Spagnoli, A., Vestri, A., Schoenen, J., Donini, L. M., & Pierelli, F. (2019). A Randomized Double-Blind, Cross-Over Trial of very Low-Calorie Diet in Overweight Migraine Patients: A Possible Role for Ketones? *Nutrients*, *11*(8), Article 8. https://doi.org/10.3390/nu11081742

Dienel, G. A. (2019). Brain Glucose Metabolism: Integration of Energetics with Function. *Physiological Reviews*, *99*(1), 949–1045. https://doi.org/10.1152/physrev.00062.2017

Dong, Y., Song, H., J. Holmes, A., Yan, J., Ren, C., Zhang, Y., Zhao, W., Yuan, J., Cheng, Y., Raubenheimer, D., & Cui, Z. (2023). Normal diet ameliorates obesity more safely and effectively than ketogenic diet does in high-fat diet-induced obesity mouse based on gut microbiota and lipid metabolism. *International Journal of Food Sciences and Nutrition*, *74*(5), 589–605. https://doi.org/10.1080/09637486.2023.2235899

Düking, T., Spieth, L., Berghoff, S. A., Piepkorn, L., Schmidke, A. M., Mitkovski, M., Kannaiyan, N., Hosang, L., Scholz, P., Shaib, A. H., Schneider, L. V., Hesse, D., Ruhwedel, T., Sun, T., Linhoff, L., Trevisiol, A., Köhler, S., Pastor, A. M., Misgeld, T., … Saher, G. (2022). Ketogenic diet uncovers differential metabolic plasticity of brain cells. *Science Advances*, *8*(37), eabo7639. https://doi.org/10.1126/sciadv.abo7639

Dunn, E., Zhang, B., Sahota, V. K., & Augustin, H. (2023). Potential benefits of

medium chain fatty acids in aging and neurodegenerative disease. *Frontiers in Aging Neuroscience*, *15*. https://doi.org/10.3389/fnagi.2023.1230467

Dyńka, D., Kowalcze, K., & Paziewska, A. (2022). The Role of Ketogenic Diet in the Treatment of Neurological Diseases. *Nutrients*, *14*(23), 5003. https://doi.org/10.3390/nu14235003

Fanti, M., Mishra, A., Longo, V. D., & Brandhorst, S. (2021). Time-Restricted Eating, Intermittent Fasting, and Fasting-Mimicking Diets in Weight Loss. *Current Obesity Reports*, *10*(2), 70–80. https://doi.org/10.1007/s13679-021-00424-2

Feria-Romero, I. A., Bribiesca-Cruz, I., Coyoy-Salgado, A., Segura-Uribe, J. J., Bautista-Poblet, G., Granados-Cervantes, A., & Guerra-Araiza, C. (2021). Validation of housekeeping genes as an internal control for gene expression studies in the brain of ovariectomized rats treated with tibolone. *Gene*, *769*, 145255. https://doi.org/10.1016/j.gene.2020.145255

Friedman, M. I., Ramirez, I., Bowden, C. R., & Tordoff, M. G. (1990). Fuel partitioning and food intake: Role for mitochondrial fatty acid transport. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *258*(1), R216–R221. https://doi.org/10.1152/ajpregu.1990.258.1.R216

Gregor, A., Huber, L., Auernigg-Haselmaier, S., Sternberg, F., Billerhart, M., Dunkel, A., Somoza, V., Ogris, M., Kofler, B., Longo, V. D., König, J., & Duszka, K. (2022). A Comparison of the Impact of Restrictive Diets on the Gastrointestinal Tract of Mice. *Nutrients*, *14*(15), 3120. https://doi.org/10.3390/nu14153120

Hagan, C. E., Bolon, B., & Keene, C. D. (2012). 20—Nervous System. In P. M. Treuting & S. M. Dintzis (Eds.), *Comparative Anatomy and Histology* (pp. 339–394). Academic Press. https://doi.org/10.1016/B978-0-12-381361-9.00020-2

Har-Even, M., Rubovitch, V., Ratliff, W. A., Richmond-Hacham, B., Citron, B. A., & Pick, C. G. (2021). Ketogenic Diet as a potential treatment for traumatic brain injury in mice. *Scientific Reports*, *11*, 23559. https://doi.org/10.1038/s41598-021-02849-0

Humpton, T., & Vousden, K. H. (2019). Taking up the reins of power: Metabolic functions of p53. *Journal of Molecular Cell Biology*, *11*(7), 610–614. https://doi.org/10.1093/jmcb/mjz065

Jensen, N. J., Wodschow, H. Z., Nilsson, M., & Rungby, J. (2020). Effects of Ketone Bodies on Brain Metabolism and Function in Neurodegenerative Diseases. *International Journal of Molecular Sciences*, *21*(22), Article 22. https://doi.org/10.3390/ijms21228767

Jeong, E. A., Jeon, B. T., Shin, H. J., Kim, N., Lee, D. H., Kim, H. J., Kang, S. S., Cho, G. J., Choi, W. S., & Roh, G. S. (2011). Ketogenic diet-induced peroxisome proliferator-activated receptor-γ activation decreases neuroinflammation in the mouse hippocampus after kainic acid-induced seizures. *Experimental Neurology*, *232*(2), 195–202. https://doi.org/10.1016/j.expneurol.2011.09.001

Kang, Y. S., Seong, D., Kim, J. C., & Kim, S. H. (2020). Low-Intensity Exercise Training Additionally Increases Mitochondrial Dynamics Caused by High-Fat Diet (HFD) but Has No Additional Effect on Mitochondrial Biogenesis in Fast-Twitch Muscle by HFD. *International Journal of Environmental Research and Public Health*, *17*(15), Article 15. https://doi.org/10.3390/ijerph17155461

Kapogiannis, D., Manolopoulos, A., Mullins, R., Avgerinos, K., Delgado-Peraza, F., Mustapic, M., Nogueras-Ortiz, C., Yao, P. J., Pucha, K. A., Brooks, J., Chen, Q., Haas, S. S., Ge, R., Hartnell, L. M., Cookson, M. R., Egan, J. M., Frangou, S., & Mattson, M. P. (2024). Brain responses to intermittent fasting and the healthy living diet in older adults. *Cell Metabolism*, *36*(8), 1668-1678.e5. https://doi.org/10.1016/j.cmet.2024.05.017

Kashiwaya, Y., Takeshima, T., Mori, N., Nakashima, K., Clarke, K., & Veech, R. L. (2000). D-β-Hydroxybutyrate protects neurons in models of Alzheimer's and Parkinson's disease. *Proceedings of the National Academy of Sciences of the United States of America*, *97*(10), 5440–5444.

Kerndt, P. R., Naughton, J. L., Driscoll, C. E., & Loxterkamp, D. A. (1982). Fasting: The History, Pathophysiology and Complications. *Western Journal of Medicine*, *137*(5), 379–399.

Khapre, R. V., Patel, S. A., Kondratova, A. A., Chaudhary, A., Velingkaar, N., Antoch, M. P., & Kondratov, R. V. (2014). Metabolic clock generates nutrient anticipation rhythms in mTOR signaling. *Aging (Albany NY)*, *6*(8), 675–689.

Kim, D. Y., Vallejo, J., & Rho, J. M. (2010). Ketones prevent synaptic dysfunction induced by mitochondrial respiratory complex inhibitors. *Journal of Neurochemistry*, *114*(1), 130–141. https://doi.org/10.1111/j.1471-4159.2010.06728.x

Kim, K. Y., Shin, K. Y., & Chang, K.-A. (2023). GFAP as a Potential Biomarker for Alzheimer's Disease: A Systematic Review and Meta-Analysis. *Cells*, *12*(9), 1309. https://doi.org/10.3390/cells12091309

Kim, Y., Dube, S. E., & Park, C. B. (2025). Brain energy homeostasis: The evolution of the astrocyte-neuron lactate shuttle hypothesis. *The Korean Journal of Physiology & Pharmacology : Official Journal of the Korean Physiological*

*Society and the Korean Society of Pharmacology*, *29*(1), 1–8. https://doi.org/10.4196/kjpp.24.388

Kraus, W. E., Bhapkar, M., Huffman, K. M., Pieper, C. F., Krupa Das, S., Redman, L. M., Villareal, D. T., Rochon, J., Roberts, S. B., Ravussin, E., Holloszy, J. O., & Fontana, L. (2019). 2 years of calorie restriction and cardiometabolic risk (CALERIE): Exploratory outcomes of a multicentre, phase 2, randomised controlled trial. *The Lancet Diabetes & Endocrinology*, *7*(9), 673–683. https://doi.org/10.1016/S2213-8587(19)30151-2

Kyriazis, I. D., Vassi, E., Alvanou, M., Angelakis, C., Skaperda, Z., Tekos, F., Garikipati, V. N. S., Spandidos, D. A., & Kouretas, D. (2022). The impact of diet upon mitochondrial physiology (Review). *International Journal of Molecular Medicine*, *50*(5), 1–26. https://doi.org/10.3892/ijmm.2022.5191

Lappano, R., Sebastiani, A., Cirillo, F., Rigiracciolo, D. C., Galli, G. R., Curcio, R., Malaguarnera, R., Belfiore, A., Cappello, A. R., & Maggiolini, M. (2017). The lauric acid-activated signaling prompts apoptosis in cancer cells. *Cell Death Discovery*, *3*(1), 1–9. https://doi.org/10.1038/cddiscovery.2017.63

Lettieri Barbato, D., Baldelli, S., Pagliei, B., Aquilano, K., & Ciriolo, M. R. (2012). Caloric Restriction and the Nutrient-Sensing PGC-1α in Mitochondrial Homeostasis: New Perspectives in Neurodegeneration. *International Journal of Cell Biology*, *2012*, 759583. https://doi.org/10.1155/2012/759583

Li, B., Matter, E., Hoppert, H., Seeley, R., & Sandoval, D. (2014). Identification of optimal reference genes for RT-qPCR in the rat hypothalamus and intestine for the study of obesity. *International Journal of Obesity (2005)*, *38*(2), 192–197. https://doi.org/10.1038/ijo.2013.86

Liberato, M. V., Nascimento, A. S., Ayers, S. D., Lin, J. Z., Cvoro, A., Silveira, R. L., Martínez, L., Souza, P. C. T., Saidemberg, D., Deng, T., Amato, A. A., Togashi, M., Hsueh, W. A., Phillips, K., Palma, M. S., Neves, F. A. R., Skaf, M. S., Webb, P., & Polikarpov, I. (2012). Medium Chain Fatty Acids Are Selective Peroxisome Proliferator Activated Receptor (PPAR) γ Activators and Pan-PPAR Partial Agonists. *PLOS ONE*, *7*(5), e36297. https://doi.org/10.1371/journal.pone.0036297

Lin, A.-L., Zhang, W., Gao, X., & Watts, L. (2015). Caloric restriction increases ketone bodies metabolism and preserves blood flow in aging brain. *Neurobiology of Aging*, *36*(7), 2296–2303. https://doi.org/10.1016/j.neurobiolaging.2015.03.012

Lin, D.-T., Kao, N.-J., Cross, T.-W. L., Lee, W.-J., & Lin, S.-H. (2022). Effects of ketogenic diet on cognitive functions of mice fed high-fat-high-cholesterol diet. *The Journal of Nutritional Biochemistry*, *104*, 108974. https://doi.org/10.1016/j.jnutbio.2022.108974

Liu, R. T., Walsh, R. F. L., & Sheehan, A. E. (2019). Prebiotics and probiotics for depression and anxiety: A systematic review and meta-analysis of controlled clinical trials. *Neuroscience & Biobehavioral Reviews*, *102*, 13–23. https://doi.org/10.1016/j.neubiorev.2019.03.023

Lobo, F., Haase, J., & Brandhorst, S. (2022). The Effects of Dietary Interventions on Brain Aging and Neurological Diseases. *Nutrients*, *14*(23), 5086. https://doi.org/10.3390/nu14235086

Longo, V. D., & Panda, S. (2016). Fasting, circadian rhythms, and time restricted feeding in healthy lifespan. *Cell Metabolism*, *23*(6), 1048–1059. https://doi.org/10.1016/j.cmet.2016.06.001

Malapaka, R. R. V., Khoo, S., Zhang, J., Choi, J. H., Zhou, X. E., Xu, Y., Gong, Y., Li, J., Yong, E.-L., Chalmers, M. J., Chang, L., Resau, J. H., Griffin, P. R., Chen, Y. E., & Xu, H. E. (2012). Identification and Mechanism of 10-Carbon Fatty Acid as Modulating Ligand of Peroxisome Proliferator-activated Receptors. *Journal of Biological Chemistry*, *287*(1), 183–195. https://doi.org/10.1074/jbc.M111.294785

Mehrabani, S., Bagherniya, M., Askari, G., Read, M. I., & Sahebkar, A. (2020). The effect of fasting or calorie restriction on mitophagy induction: A literature review. *Journal of Cachexia, Sarcopenia and Muscle*, *11*(6), 1447–1458. https://doi.org/10.1002/jcsm.12611

Mezhnina, V., Pearce, R., Poe, A., Velingkaar, N., Astafev, A., Ebeigbe, O. P., Makwana, K., Sandlers, Y., & Kondratov, R. V. (2020). CR reprograms acetyl-CoA metabolism and induces long-chain acyl-CoA dehydrogenase and CrAT expression. *Aging Cell*, *19*(11), e13266. https://doi.org/10.1111/acel.13266

Mitchell, R. W., & Hatch, G. M. (2011). Fatty acid transport into the brain: Of fatty acid fables and lipid tails. *Prostaglandins, Leukotrienes and Essential Fatty Acids (PLEFA)*, *85*(5), 293–302. https://doi.org/10.1016/j.plefa.2011.04.007

Mitchell, R. W., On, N. H., Del Bigio, M. R., Miller, D. W., & Hatch, G. M. (2011). Fatty acid transport protein expression in human brain and potential role in fatty acid transport across human brain microvessel endothelial cells. *Journal of Neurochemistry*, *117*(4), 735–746. https://doi.org/10.1111/j.1471-4159.2011.07245.x

Nath, A., Li, I., Roberts, L. R., & Chan, C. (2015). Elevated free fatty acid uptake via CD36 promotes epithelial-mesenchymal transition in hepatocellular carcinoma. *Scientific Reports*, *5*(1), 14752. https://doi.org/10.1038/srep14752

Ngo, J., Choi, D. W., Stanley, I. A., Stiles, L., Molina, A. J. A., Chen, P., Lako, A., Sung, I. C. H., Goswami, R., Kim, M., Miller, N., Baghdasarian, S., Kim-

Vasquez, D., Jones, A. E., Roach, B., Gutierrez, V., Erion, K., Divakaruni, A. S., Liesa, M., … Shirihai, O. S. (2023). Mitochondrial morphology controls fatty acid utilization by changing CPT1 sensitivity to malonyl-CoA. *The EMBO Journal*, *42*(11). https://doi.org/10.15252/embj.2022111901

Osellame, L. D., Blacker, T. S., & Duchen, M. R. (2012). Cellular and molecular mechanisms of mitochondrial function. *Best Practice & Research Clinical Endocrinology & Metabolism*, *26*(6), 711–723. https://doi.org/10.1016/j.beem.2012.05.003

Papamandjaris, A. A., Macdougall, D. E., & Jones, P. J. H. (1998). Medium chain fatty acid metabolism and energy expenditure: Obesity treatment implications. *Life Sciences*, *62*(14), 1203–1215. https://doi.org/10.1016/S0024-3205(97)01143-0

Patel, A. B., Lai, J. C. K., Chowdhury, G. M. I., Hyder, F., Rothman, D. L., Shulman, R. G., & Behar, K. L. (2014). Direct evidence for activity-dependent glucose phosphorylation in neurons with implications for the astrocyte-to-neuron lactate shuttle. *Proceedings of the National Academy of Sciences*, *111*(14), 5385–5390. https://doi.org/10.1073/pnas.1403576111

Phillips, M. C. L., Murtagh, D. K. J., Gilbertson, L. J., Asztely, F. J. S., & Lynch, C. D. P. (2018). Low-fat versus ketogenic diet in Parkinson's disease: A pilot randomized controlled trial. *Movement Disorders*, *33*(8), 1306–1314. https://doi.org/10.1002/mds.27390

PubChem. (n.d.). *RPL4—Ribosomal protein L4 (human)*. Retrieved 31 December 2024, from https://pubchem.ncbi.nlm.nih.gov/gene/RPL4/human

Raichle, M. E. (2010). Two views of brain function. *Trends in Cognitive Sciences*, *14*(4), 180–190. https://doi.org/10.1016/j.tics.2010.01.008

Rappeneau, V., Koti, P., Wilmes, L., Widner-Andrae, R., Busch, K., & Touma, C. (2023). Molecular changes in hippocampal energy metabolism in mice selectively bred for extremes in stress reactivity: Relevance of mitochondrial dysfunction for affective disorders. *European Journal of Neuroscience*, *58*(2), 2579–2602. https://doi.org/10.1111/ejn.16044

Rho, J. M. (2017). How does the ketogenic diet induce anti-seizure effects? *Neuroscience Letters*, *637*, 4–10. https://doi.org/10.1016/j.neulet.2015.07.034

Robbins, J. P., & Solito, E. (2022). Does Neuroinflammation Underlie the Cognitive Changes Observed With Dietary Interventions? *Frontiers in Neuroscience*, *16*. https://doi.org/10.3389/fnins.2022.854050

Roth, I., Campbell, H., Rubio, C., Vennin, C., Wilson, M., Wiles, A., Williams, G., Woolley, A., Timpson, P., Berridge, M. V., Fleming, N., Baird, M., & Braithwaite, A. W. (2016). The Δ133p53 isoform and its mouse analogue

Δ122p53 promote invasion and metastasis involving pro-inflammatory molecules interleukin-6 and CCL2. *Oncogene*, *35*(38), 4981–4989. https://doi.org/10.1038/onc.2016.45

Röhrig, F., & Schulze, A. (2016). The multifaceted roles of fatty acid synthesis in cancer. *Nature Reviews Cancer*, *16*(11), 732–749. https://doi.org/10.1038/nrc.2016.89

Sampaio, L. P. de B. (2016). Ketogenic diet for epilepsy treatment. *Arquivos de Neuro-Psiquiatria*, *74*, 842–848. https://doi.org/10.1590/0004-282X20160116

Sasi, M., Vignoli, B., Canossa, M., & Blum, R. (2017). Neurobiology of local and intercellular BDNF signaling. *Pflugers Archiv*, *469*(5), 593–610. https://doi.org/10.1007/s00424-017-1964-4

Schönfeld, P., & Reiser, G. (2013). Why does brain metabolism not favor burning of fatty acids to provide energy? - Reflections on disadvantages of the use of free fatty acids as fuel for brain. *Journal of Cerebral Blood Flow & Metabolism*, *33*(10), 1493–1499. https://doi.org/10.1038/jcbfm.2013.128

Seifert, E. L., & Hajnóczky, G. (2023). Fuel oxidation under the control of mitochondrial shape and dynamics. *The EMBO Journal*, *42*(11), e114129. https://doi.org/10.15252/embj.2023114129

Seiler, S. E., Martin, O. J., Noland, R. C., Slentz, D. H., DeBalsi, K. L., Ilkayeva, O. R., An, J., Newgard, C. B., Koves, T. R., & Muoio, D. M. (2014). Obesity and lipid stress inhibit carnitine acetyltransferase activity. *Journal of Lipid Research*, *55*(4), 635–644. https://doi.org/10.1194/jlr.M043448

Sekine, M., Fujiwara, M., Okamoto, K., Ichida, K., Nagata, K., Hille, R., & Nishino, T. (2024). Significance and amplification methods of the purine salvage pathway in human brain cells. *Journal of Biological Chemistry*, *300*(8), 107524. https://doi.org/10.1016/j.jbc.2024.107524

Sethi, S., Wakeham, D., Ketter, T., Hooshmand, F., Bjornstad, J., Richards, B., Westman, E., Krauss, R. M., & Saslow, L. (2024). Ketogenic Diet Intervention on Metabolic and Psychiatric Health in Bipolar and Schizophrenia: A Pilot Trial. *Psychiatry Research*, *335*, 115866. https://doi.org/10.1016/j.psychres.2024.115866

Seyfried, B. T. N., Kiebish, M., Marsh, J., & Mukherjee, P. (2009). Targeting energy metabolism in brain cancer through calorie restriction and the ketogenic diet. *Journal of Cancer Research and Therapeutics*, *5*(Suppl 1), S7. https://doi.org/10.4103/0973-1482.55134

Shaafi, S., Najmi, S., Aliasgharpour, H., Mahmoudi, J., Sadigh-Etemad, S., Farhoudi, M., & Baniasadi, N. (2016). The efficacy of the ketogenic diet on motor functions in Parkinson's disease: A rat model. *Iranian Journal of*

*Neurology*, *15*(2), 63–69.

Sharma, A., Ahmad, S., Ahmad, T., Ali, S., & Syed, M. A. (2021). Mitochondrial dynamics and mitophagy in lung disorders. *Life Sciences*, *284*, 119876. https://doi.org/10.1016/j.lfs.2021.119876

Shcherbakova, K., Schwarz, A., Apryatin, S., Karpenko, M., & Trofimov, A. (2022). Supplementation of Regular Diet With Medium-Chain Triglycerides for Procognitive Effects: A Narrative Review. *Frontiers in Nutrition*, *9*. https://doi.org/10.3389/fnut.2022.934497

Shin, H., Morty, R. E., Sucre, J. M., Negretti, N. M., Markmann, M., Hossain, H., Krauss-Etschmann, S., Dehmel, S., & Hilgendorff, A. (2022). Reference genes for the developing mouse lung under consideration of biological, technical and experimental confounders. *Scientific Reports*, *12*(1), 17679. https://doi.org/10.1038/s41598-022-19071-1

Skvortsova, A., Veldhuijzen, D. S., Kloosterman, I. E. M., Pacheco-López, G., & Evers, A. W. M. (2021). Food anticipatory hormonal responses: A systematic review of animal and human studies. *Neuroscience & Biobehavioral Reviews*, *126*, 447–464. https://doi.org/10.1016/j.neubiorev.2021.03.030

Slatter, T. L., Hung, N., Bowie, S., Campbell, H., Rubio, C., Speidel, D., Wilson, M., Baird, M., Royds, J. A., & Braithwaite, A. W. (2015). Δ122p53, a mouse model of Δ133p53α, enhances the tumor-suppressor activities of an attenuated p53 mutant. *Cell Death & Disease*, *6*(6), e1783. https://doi.org/10.1038/cddis.2015.149

Steinke, I., Govindarajulu, M., Pinky, P. D., Bloemer, J., Yoo, S., Ward, T., Schaedig, T., Young, T., Wibowo, F. S., Suppiramaniam, V., & Amin, R. H. (2023). Selective PPAR-Delta/PPAR-Gamma Activation Improves Cognition in a Model of Alzheimer's Disease. *Cells*, *12*(8), 1116. https://doi.org/10.3390/cells12081116

Sternberg, F., Sternberg, C., Dunkel, A., Beikbaghban, T., Gregor, A., Szarzynski, A., Somoza, V., Walter, I., Duszka, K., Kofler, B., & Pohl, E. E. (2023). Ketogenic diets composed of long-chain and medium-chain fatty acids induce cardiac fibrosis in mice. *Molecular Metabolism*, *72*, 101711. https://doi.org/10.1016/j.molmet.2023.101711

Sun, Y., Zhou, D., Wang, Y., Wang, Z., Zhang, D., Qian, Z., Yan, J., Li, Z., Huang, G., & Li, W. (2024). Medium-chain triglycerides combined with DHA improve cognitive function by inhibiting neurocyte apoptosis of the brain in SAMP8 mice. *Experimental Gerontology*, *194*, 112520. https://doi.org/10.1016/j.exger.2024.112520

Tanic, N., Perovic, M., Mladenovic, A., Ruzdijic, S., & Kanazir, S. (2007). Effects of

aging, dietary restriction and glucocorticoid treatment on housekeeping gene expression in rat cortex and hippocampus—Evaluation by real time RT-PCR. *Journal of Molecular Neuroscience*, *32*(1), 38–46. https://doi.org/10.1007/s12031-007-0006-7

Tilokani, L., Nagashima, S., Paupe, V., & Prudent, J. (2018). Mitochondrial dynamics: Overview of molecular mechanisms. *Essays in Biochemistry*, *62*(3), 341–360. https://doi.org/10.1042/EBC20170104

Tisdale, M. J., & Brennan, R. A. (1988). A comparison of long-chain triglycerides and medium-chain triglycerides on weight loss and tumour size in a cachexia model. *British Journal of Cancer*, *58*(5), 580–583.

Treuting, P. M., Dintzis, S. M., & Montine, K. S. (2012). 1—Introduction. In P. M. Treuting & S. M. Dintzis (Eds.), *Comparative Anatomy and Histology* (pp. 1–6). Academic Press. https://doi.org/10.1016/B978-0-12-381361-9.00001-9

Twig, G., Elorza, A., Molina, A. J. A., Mohamed, H., Wikstrom, J. D., Walzer, G., Stiles, L., Haigh, S. E., Katz, S., Las, G., Alroy, J., Wu, M., Py, B. F., Yuan, J., Deeney, J. T., Corkey, B. E., & Shirihai, O. S. (2008). Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. *The EMBO Journal*, *27*(2), 433–446. https://doi.org/10.1038/sj.emboj.7601963

Vasim, I., Majeed, C. N., & DeBoer, M. D. (2022). Intermittent Fasting and Metabolic Health. *Nutrients*, *14*(3), 631. https://doi.org/10.3390/nu14030631

Ventura-Antunes, L., Mota, B., & Herculano-Houzel, S. (2013). Different scaling of white matter volume, cortical connectivity, and gyrification across rodent and primate brains. *Frontiers in Neuroanatomy*, *7*. https://doi.org/10.3389/fnana.2013.00003

Voss, M., Wagner, M., von Mettenheim, N., Harter, P. N., Wenger, K. J., Franz, K., Bojunga, J., Vetter, M., Gerlach, R., Glatzel, M., Paulsen, F., Hattingen, E., Baehr, O., Ronellenfitsch, M. W., Fokas, E., Imhoff, D., Steinbach, J. P., Rödel, C., & Rieger, J. (2020). ERGO2: A Prospective, Randomized Trial of Calorie-Restricted Ketogenic Diet and Fasting in Addition to Reirradiation for Malignant Glioma. *International Journal of Radiation Oncology*Biology*Physics*, *108*(4), 987–995. https://doi.org/10.1016/j.ijrobp.2020.06.021

Wang, Y., Yu, W., Li, S., Guo, D., He, J., & Wang, Y. (2022). Acetyl-CoA Carboxylases and Diseases. *Frontiers in Oncology*, *12*, 836058. https://doi.org/10.3389/fonc.2022.836058

Waniewski, R. A., & Martin, D. L. (1998). Preferential Utilization of Acetate by Astrocytes Is Attributable to Transport. *Journal of Neuroscience*, *18*(14), 5225–5233. https://doi.org/10.1523/JNEUROSCI.18-14-05225.1998

Wei, S.-J., Schell, J. R., Chocron, E. S., Varmazyad, M., Xu, G., Chen, W. H., Martinez, G. M., Dong, F. F., Sreenivas, P., Trevino, R., Jiang, H., Du, Y., Saliba, A., Qian, W., Lorenzana, B., Nazarullah, A., Chang, J., Sharma, K., Munkácsy, E., … Gius, D. (2024). Ketogenic diet induces p53-dependent cellular senescence in multiple organs. *Science Advances*, *10*(20), eado1463. https://doi.org/10.1126/sciadv.ado1463

Weindruch, R., Walford, R. L., Fligiel, S., & Guthrie, D. (1986). The Retardation of Aging in Mice by Dietary Restriction: Longevity, Cancer, Immunity and Lifetime Energy Intake. *The Journal of Nutrition*, *116*(4), 641–654. https://doi.org/10.1093/jn/116.4.641

Wheless, J. W. (2008). History of the ketogenic diet. *Epilepsia*, *49*(s8), 3–5. https://doi.org/10.1111/j.1528-1167.2008.01821.x

Xu, N., LaGrow, T. J., Anumba, N., Lee, A., Zhang, X., Yousefi, B., Bassil, Y., Clavijo, G. P., Khalilzad Sharghi, V., Maltbie, E., Meyer-Baese, L., Nezafati, M., Pan, W.-J., & Keilholz, S. (2022). Functional Connectivity of the Brain Across Rodents and Humans. *Frontiers in Neuroscience*, *16*. https://doi.org/10.3389/fnins.2022.816331

Xu, S., Tao, H., Cao, W., Cao, L., Lin, Y., Zhao, S.-M., Xu, W., Cao, J., & Zhao, J.-Y. (2021). Ketogenic diets inhibit mitochondrial biogenesis and induce cardiac fibrosis. *Signal Transduction and Targeted Therapy*, *6*, 54. https://doi.org/10.1038/s41392-020-00411-4

Yang, Z., Mi, J., Wang, Y., Xue, L., Liu, J., Fan, M., Zhang, D., Wang, L., Qian, H., & Li, Y. (2021). Effects of low-carbohydrate diet and ketogenic diet on glucose and lipid metabolism in type 2 diabetic mice. *Nutrition*, *89*, 111230. https://doi.org/10.1016/j.nut.2021.111230

Yudkoff, M., Daikhin, Y., Nissim, I., Horyn, O., Lazarow, A., Luhovyy, B., Wehrli, S., & Nissim, I. (2005). Response of brain amino acid metabolism to ketosis. *Neurochemistry International*, *47*(1), 119–128. https://doi.org/10.1016/j.neuint.2005.04.014

Zhang, W., Chen, S., Huang, X., Tong, H., Niu, H., & Lu, L. (2023). Neuroprotective effect of a medium-chain triglyceride ketogenic diet on MPTP-induced Parkinson's disease mice: A combination of transcriptomics and metabolomics in the substantia nigra and fecal microbiome. *Cell Death Discovery*, *9*, 251. https://doi.org/10.1038/s41420-023-01549-0

Zheng, F., & Cai, Y. (2019). Concurrent exercise improves insulin resistance and nonalcoholic fatty liver disease by upregulating PPAR-γ and genes involved in the β-oxidation of fatty acids in ApoE-KO mice fed a high-fat diet. *Lipids in Health and Disease*, *18*, 6. https://doi.org/10.1186/s12944-018-0933-z

Zhou, B., Zuo, Y., & Jiang, R. (2019). Astrocyte morphology: Diversity, plasticity,

and role in neurological diseases. *CNS Neuroscience & Therapeutics*, *25*(6), 665–673. https://doi.org/10.1111/cns.13123

Zhu, J. J., Luo, J., Wang, W., Yu, K., Wang, H. B., Shi, H. B., Sun, Y. T., Lin, X. Z., & Li, J. (2014). Inhibition of FASN reduces the synthesis of medium-chain fatty acids in goat mammary gland. *Animal*, *8*(9), 1469–1478. https://doi.org/10.1017/S1751731114001323

Zupec-Kania, B., & Zupanc, M. L. (2008). Long-term management of the ketogenic diet: Seizure monitoring, nutrition, and supplementation. *Epilepsia*, *49*(s8), 23–26. https://doi.org/10.1111/j.1528-1167.2008.01827.x

# 10 Supplement

| Ingredients | | LCT | SD | LCT/MCT |
|---|---|---|---|---|
| Product_N | | S9139-E025 | S9139-E028 | S9139-E032 |
| Casein | % | 9.000 | 18.200 | 9.000 |
| Corn starch | % | —— | 53.400 | —— |
| Sucrose | % | —— | 5.000 | —— |
| Cellulose | % | 10.000 | 10.000 | 10.000 |
| L-Cystine | % | 0.200 | 0.200 | 0.200 |
| Vitamin premixture | % | 1.000 | 1.000 | 1.000 |
| Mineral premix | % | 5.000 | 5.000 | 5.000 |
| Choline Cl | % | 0.200 | 0.200 | 0.200 |
| C 8 | % | —— | —— | 15.00 |
| C10 | % | —— | —— | 10.00 |
| Butter fat | % | 8.750 | 0.800 | 5.80 |
| Pork lard | % | 65.850 | 6.200 | 43.80 |
| *Proximate contents* | | | | |
| Crude Protein | % | 8.1 | 16.1 | 8.1 |
| Crude fat | % | 74.6 | 7.1 | 74.6 |
| Crude fiber | % | 9.9 | 10.0 | 9.9 |
| Crude ash | % | 4.4 | 4.5 | 4.4 |
| Sugar | % | 1.0 | 6.0 | 1.0 |
| Starch | % | —— | 51.2 | —— |
| Lysine | % | 0.66 | 1.33 | 0.66 |
| Methionine | % | 0.35 | 0.57 | 0.35 |
| Cystine | % | 0.23 | 0.27 | 0.23 |
| Threonine | % | 0.35 | 0.71 | 0.35 |
| Tryptophan | % | 0.10 | 0.21 | 0.10 |
| Arginine | % | 0.31 | 0.63 | 0.31 |
| Histidine | % | 0.24 | 0.48 | 0.24 |
| Valine | % | 0.55 | 1.12 | 0.55 |
| Isoleucine | % | 0.45 | 0.91 | 0.45 |
| Leucine | % | 0.79 | 1.59 | 0.79 |
| Phenylalanine | % | 0.41 | 0.83 | 0.41 |
| Tyrosine | % | 0.42 | 0.85 | 0.42 |
| Glycine | % | 0.16 | 0.32 | 0.16 |
| Glutamic acid | % | 1.79 | 3.62 | 1.79 |
| Aspartic acid | % | 0.59 | 1.19 | 0.59 |
| Proline | % | 0.91 | 1.84 | 0.91 |
| Serine | % | 0.48 | 0.96 | 0.48 |
| Alanine | % | 0.24 | 0.48 | 0.24 |

| Ingredients | | LCT | SD | LCT/MCT |
|---|---|---|---|---|
| Product No. | | S9139-E025 | S9139-E028 | S9139-E032 |
| ME – Atwater [1)] | MJ/kg | 29.7 | 15.1 | 29.7 |
| Protein | kcal% | ~4.5 | 18 | ~ 4.5 |
| Fat | kcal% | ~94.6 | 18 | ~ 94.6 |
| CHO | kcal% | ~0.9 | 64 | ~ 0.9 |
| *Fatty acids, % in the diet* | | | | |
| C 4:0 | | 0.33 | 0.03 | 0.22 |
| C 6:0 | | 0.22 | 0.02 | 0.17 |
| C 8:0 | | 0.12 | 0.01 | 15.08 |
| C 10:0 | | 0.33 | 0.03 | 10.22 |
| C 12:0 | | 0.43 | 0.04 | 0.29 |
| C 14:0 | | 1.81 | 0.18 | 1.21 |
| C 16:0 | | 18.12 | 1.73 | 1.20 |
| C 17:0 | | 0.05 | 0.01 | 0.04 |
| C 18:0 | | 9.74 | 0.92 | 6.47 |
| C 20:0 | | 0.21 | 0.02 | 0.14 |
| C 16:1 | | 2.11 | 0.20 | 1.40 |
| C 18:1 | | 29.43 | 2.79 | 19.58 |
| C 18:2 | | 6.41 | 0.60 | 4.26 |
| C 18:3 | | 0.71 | 0.07 | 0.47 |
| C 20:4 | | 1.19 | 0.10 | 0.74 |

[1)] *Physiological fuel value*

***Suppl. table 1 : Detailed dietary composition of SD, LCT and LCT/MCT*** *from the company ssniff.*

# 11 List of Figures and Tables

## 11.1 Figures

## 11.2 Tables