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Current Opinion in
Endocrine and Metabolic Research

Reviews

Fasting-regulated mechanisms in inter-organ crosstalk

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The adaptation to changing environmental cues represents a key prerequisite for the survival of an organism. Mammals, including humans, have evolved intricate endocrine signals to convey information about the nutritional status to individual organs, cells, and eventually the cell nucleus, to trigger appropriate molecular-metabolic responses. To this end, mounting a proper fasting response is determined by not only intra-organ adaptations but also inter-tissue crosstalk mechanisms that orchestrate whole-body energy homeostasis under nutrient-deprived conditions. Here, we shortly summarize recent advances in our current understanding of the key processes driving the adaptive response to fasting with a focus on the crosstalk between the adipose tissue and liver ketogenesis.

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Current Opinion in Endocrine and Metabolic Research 2024, **36:**100540

This review comes from a themed issue on **Adipose (2024)**

Edited by **Carolina Hagberg** and **Zoi Michailidou**

For complete overview of the section, please refer the article collection - Adipose (2024)

Available online 17 July 2024

https://doi.org/10.1016/j.coemr.2024.100540

Introduction

Higher organisms have evolved a complex inter-organ network of nutrient sensing and signal transduction pathways to maintain energy homeostasis during fluctuating periods of nutrient availability. Physiological cues such as fasting and feeding signals are sensed in multiple organs and translated into cooperative metabolic responses to adapt not only the behavior, but also the storage and production of energy metabolites.

During fasting, a radical change in cellular physiology and metabolism is exerted in order to maintain energy homeostasis. The overall metabolic response to fasting has been studied for decades and is relatively well characterized.

In the early fasting stages, 4–12 h after food ingestion, the glycogen stores in the liver are utilized to sustain blood glucose levels. As fasting persists, the liver undergoes a shift toward *de novo* glucose production, primarily utilizing amino acids from muscle protein breakdown and glycerol released from adipose tissue as substrates. Beyond 16 h of fasting, there is a progressive switch in metabolic fuel utilization from carbohydrate to fat and fat-derived products. At this stage, insulin-sensitive tissues such as muscles reduce their glucose consumption and instead prioritize the oxidation of free fatty acids (FFAs) released from adipose tissue. This increase in circulating FFAs promotes the synthesis of ketone bodies, which become vital alternative fuels for various tissues, particularly the brain [1,2].

All these processes are orchestrated by an array of bioactive molecules such as hormones, cytokines, and neurotransmitters. Key metabolic hormones such as pancreatic insulin and glucagon were discovered already in the 1920s, and their history is reviewed in Refs. [3,4]. Thanks to technological advances, the identification of metabolic messengers has increased exponentially during recent years. Signaling molecules from the liver (hepatokines), white adipose tissue (WAT; adipokines), brown adipose tissue (BAT; batokines), and skeletal muscle (myokines) have been identified and are reviewed in Refs. [5–8].

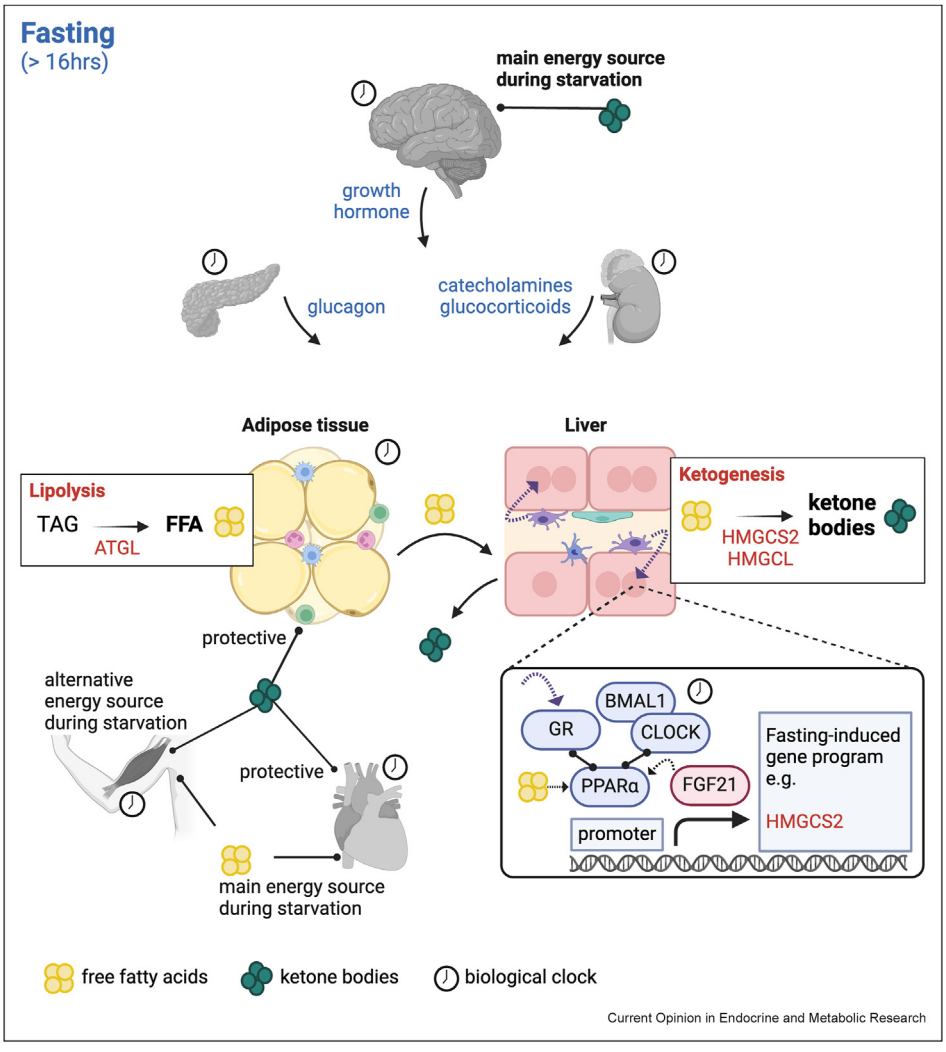
The imbalance of energy consumption and/or dysregulation of this signaling network leads to pathologic conditions. However, entrainment of the system through fasting regimes has proven to be an effective treatment for a broad number of diseases [9,10]. In addition, ketone bodies and ketogenic diets have gained attention in the clinical setting, particularly in areas such as neurological disorders and metabolic complications [11,12]. Therefore, understanding the crosstalk between adipose tissue and liver ketogenesis during fasting may contribute to the development of approaches to improve health in the future.

Metabolic adaptations of the adipose tissue during fasting

Throughout evolution, organisms had to cope with periods of little to no food. In line, humans have evolved a complex mechanism to deal with the energy demands of the body. The adipose tissue is the main energy storage depot in the body and thus is crucial for the adaptive response to fasting. There are two adipose tissue depots in humans: WAT and BAT, named after their color and developmental origin. This review will focus on the fasting-induced response in WAT.

WAT is a complex organ composed of multiple cell types, including adipocytes, adipose stem/stromal cells, and immune cells such as macrophages and lymphocytes [13]. Furthermore, the adipose tissue is heavily innervated by the sympathetic nervous system [14]. Adipocytes store most of the body's energy in the form of triglycerides (TAGs). During fasting, the circulating levels of insulin decline, while the levels of glucagon, growth hormone, glucocorticoids, and catecholamines increase. This combinatory signal stimulates the intracellular hydrolysis of TAGs into glycerol and FFAs (Figure 1). This process, known as lipolysis, is catalyzed by three enzymes: adipose triglyceride lipase (ATGL) catalyzes the hydrolysis of TAGs to diacylglycerol; hormone-sensitive lipase converts diacylglycerol into monoacylglycerol; and monoglyceride lipase converts monoacylglycerol into glycerol and FFA [15]. Glycerol and FFAs are then released into circulation to be utilized as substrates for glucose and ketone bodies synthesis in the liver. FFAs can also be directly utilized by multiple organs such as the heart, kidneys, and muscles as an energy source.

Figure 1



Crosstalk between white adipose tissue and liver ketogenesis during prolonged fasting. Upon fasting, the plasma levels of various hormones and signaling molecules change. This hormonal profile results in the activation of lipolysis in the white adipose tissue and ketogenesis in the liver. Free fatty acids (FFAs) and ketone bodies serve as an alternative energy source when the carbohydrate storage is depleted. FFA and ketone bodies also act as signaling molecules mediating tissue remodeling, inflammation, and gene expression. All these metabolic processes are intrinsically regulated by the biological clock.

Although it was a concept already well accepted in the field, Fougerat et al. recently demonstrated that adipocyte ATGL-dependent lipolysis is indeed required for the fasting-induced response in the liver [16]**. In this study, the authors generated an adipocyte-specific ATGL knockout mouse ($Atgl^{adipo-/-}$) and showed that ketone production in the liver is impaired in mice lacking ATGL in adipocytes. Interestingly, the liver transcriptome of fasted ($Atgl^{adipo-/-}$) mice was drastically affected. The fasting-induced activation of hepatic genes involved in fatty acid metabolism was hampered in $Atgl^{adipo-/-}$ mice. Further analysis revealed that the set of ATGL-dependent genes contained many targets of peroxisome proliferator—activated receptor alpha (PPARα), a fasting-induced transcriptional regulator of fatty acid transport and oxidation as well as ketone biosynthesis. These results suggest that adipose-derived FFAs directly regulate PPARα to activate the fasting/ketogenic transcriptional program.

In addition to its role in nutrient handling, the adipose tissue also acts as an endocrine organ secreting a variety of messenger molecules collectively known as adipokines. The first adipokine, leptin, was discovered in 1994. Leptin is known to act in the brain and contribute to appetite regulation, reviewed in Ref. [17]. Besides leptin, the adipose tissue secretes many other active biomolecules that regulate inflammation, angiogenesis, cardiac function, and muscle metabolism. The role in health and disease of the key adipokines has been reviewed previously [6].

Collectively, these studies support a crucial role of the adipose tissue in generating not only energy metabolites but also signaling molecules that orchestrate a crosstalk with multiple organs in order to modulate an adequate metabolic response to changes in energy states.

Ketogenesis and inter-organ crosstalk

In mammals, severe food deprivation as well as caloric restriction results in a decrease in the size of most organs, with the exception of the brain and the testicles [18]. This suggests that maintaining a strong cognitive function in times of food scarcity is a crucial priority.

In 1967, Owen et al identified that oxidation of β-hydroxybutyrate (BHB) and acetoacetate (AcAc), also known as ketone bodies, replace glucose as the brain's primary fuel during starvation, thereby sparing *de novo* glucose production and, in turn, sparing protein breakdown [19]. This metabolic switch from glucose to ketone body utilization would allow a healthy man to survive periods of starvation to about two months [2].

The hepatic production and levels of ketone bodies in the blood are highly dependent on the nutritional status. In the postprandial state, the blood concentrations of BHB and AcAc in healthy individuals are around 0.03 mM and 0.01 mM, respectively [1,20]. After an overnight fasting period longer than 16 h, the blood concentration of BHB and AcAc increases to around 0.14 mM and 0.04 mM, respectively [20—26]. After 3 days of fasting, the blood concentration of BHB and AcAc is further increased to levels around 3.2 mM and 0.6 mM, respectively [24—27]. At this point, the influx of ketone bodies into the brain is increased by more than 10 times [26]. After one week of starvation, the blood concentration of BHB and AcAc is around 4 mM and 1 mM, respectively [24]. From this point onward, ketone bodies constitute the major source of fuel for the brain.

Ketone bodies are produced in the liver by an enzymatic cascade known as ketogenesis. In brief, acetyl-CoA generated from fatty acids via β-oxidation is further condensed to acetoacetyl-CoA by the enzyme thiolase. Acetoacetyl-CoA is then combined with another acetyl-CoA molecule by HMG-CoA synthase 2 (HMGCS2) to form HMG-CoA. This reaction is the rate-limiting step, and it is regulated by the nutritional status of the body, as explained below. The enzyme HMG-CoA lyase (HMGCL) finally removes an acetyl-CoA from HMG-CoA to produce AcAc, BHB, and acetone which are then secreted into circulation [28].

As most metabolic processes, ketogenesis is driven in a cooperative manner by multiple organs. Low insulin levels in combination with elevated levels of glucagon secreted by the pancreas together with high rates of FFAs released from the adipose tissue are the main drivers of ketogenesis during fasting (Figure 1).

As mentioned above, HMGCS2 acts as a major control site of ketogenesis, serving as the rate-limiting enzyme. The activity of HMGCS2 is regulated by succinylation, a post-translational modification that inhibits its function; this process is counteracted by glucagon [29]. At the transcriptional level, the expression of HMGCS2 is upregulated by PPARα, a ligand-activated transcription factor induced upon fasting [30,31]. The ligand-dependent activation of gene transcription by PPARα can be triggered by direct binding of FFAs [32—34]. Conversely, in the transition from fasting to re-feeding states, ketogenesis is tightly regulated by insulin [35]. Insulin inhibits lipolysis in the adipose tissue, thereby diminishing the pool of substrates available for ketogenesis [15]. Moreover, insulin has been shown to inhibit the expression of hepatic HMGCS2 *in vitro* [36].

In line with the fasting and re-feeding cycles, the hormonal profile and the production of ketone bodies present a diurnal rhythm in mammals. A connection between ketogenesis and the circadian rhythm was established already in 1996 when the expression of PPARα was shown to follow a diurnal rhythm, which parallels the circulating levels of corticosterone *in vitro* [37]. A recent study performed by Mezhnina et al.

showed that ketogenesis is regulated by the molecular clock [38]**. First, they observed that caloric restriction and periodic fasting induced daily rhythms in the circulating levels of BHB, independently of light to dark transition and uncoupled from blood glucose levels. In line with this, the expression of hepatic PPARα as well as several direct target genes, including fibroblast growth factor 21 (FGF21), were rhythmic with a maximum expression just before the induction of ketogenesis. Further investigation revealed that the expression of FGF21 is regulated by key components of the molecular clock, i.e. CLOCK and BMAL1 promoted FGF21 expression, while CRY1 inhibited it through a promoter interference mechanism.

FGF21 has been implicated in the regulation of hepatic lipid metabolism upon fasting in mice [39]. Although the direct mechanisms remain to be elucidated, FGF21 has been shown to promote the production of ketone bodies in response to fasting [40]. These studies showcase the crucial, but sometimes neglected, circadian regulation of metabolic pathways.

The production of BHB is not 100% confined to hepatocytes; BHB is also produced by the small intestine stem cells, renal epithelial cells, and immune cells [41–44]. However, Goldberg et al. recently demonstrated that despite the presence of non-hepatic ketogenesis, the liver is the only organ that can support the production of enough ketone bodies to induce a full adaptive response to fasting and low-carbohydrate diets [45]**. The authors generated tissue-specific Hmgcl knockout mice to deplete HMGCL from hepatocytes ($Hmgcl^{Alb}$), neutrophils ($Hmgcl^{S100a8}$), and myeloid cells ($Hmgcl^{LysM}$) and demonstrated that hepatic ketogenesis is the main driver of metabolic adaptation and inflammation. Thus, the role of ketone synthesis pathways in innate immune cells remains to be clarified.

Interestingly, Nishitani et al. recently showed that HMGCS2 is expressed in WAT and is upregulated in response to fasting [46]*. Using an *in vitro* system, they demonstrated that expression of HMGCS2 in differentiated white adipocyte cells results in BHB synthesis and secretion. Utilizing a whole-body Hmgcs2 knockout mouse ($Hmgcs2^{-/-}$), the authors demonstrated that impaired ketogenesis inhibits the expression of antioxidative genes in WAT. On the other hand, treatment with BHB in wild-type mice enhanced the expression of antioxidative stress and lipogenic factors in WAT. Despite these insights, the precise implications of WAT ketogenesis remain elusive and require further study. Importantly, the absence of detectable HMGCS2 expression in human WAT raises intriguing questions about species-specific metabolic pathways and the importance of future investigation into the translational relevance of these findings.

The studies described above showcase the essential interconnection of the adipose tissue with liver ketogenesis during the adaptive response to fasting.

Ketogenesis and intra-organ crosstalk

Similar to the adipose tissue, the liver is composed of multiple cell types. Hepatocytes constitute approximately 70% of the liver cell population, while non-parenchymal cells, including hepatic stellate cells, liver macrophages, and liver endothelial cells, make up the rest. As showcased above, the liver is a central hub for the synthesis of energy metabolites and has been studied for decades. Yet, the interplay between hepatic cell types remains incompletely characterized.

Charlotte et al. presented a novel approach to unravel the function, spatial organization and crosstalk of hepatic cells [47]*. They utilized cellular indexing of transcriptomes and epitopes by sequencing, which relies on oligonucleotide-labeled antibodies to identify surface proteins, using single-cell sequencing as a readout [48] and combined it with 10x Visium Spatial Gene Expression analysis. This enabled the identification of a new subset of hepatic macrophages termed lipid-associated macrophages (LAMs) around the bile ducts. Applying this approach to human liver biopsies from healthy and steatotic patients, they found that LAMs accumulate peri-centrally in zones with steatosis, suggesting that LAMs are induced by local lipid exposure. Furthermore, they predicted fibroblast-derived ligands to be key mediators of the recruitment of LAMs into steatotic regions. These findings highlight the need for further investigation into the function of multiple hepatic cell types in metabolism and disease progression.

In our own study, we employed a cell-type-resolved genomics approach to unravel how liver macrophages contribute to the fasting response of hepatocytes [49]. Using the 'isolation of nuclei tagged in specific cell types' method, we selectively tagged, isolated, and analyzed nuclei from both hepatocytes and liver macrophages in mice fasted throughout their active phase (dark phase). We identified the glucocorticoid receptor (GR) as a key mediator of the fasting response in liver macrophages. During early fasting, GR induces the expression of multiple macrophage-secreted factors that subsequently influence PPARα activation in hepatocytes. Importantly, we demonstrated that this crosstalk mechanism is dependent on hepatocyte GR as well. Consistently, the deletion of either macrophage-specific GR (GR^{LysM}) or hepatocyte-specific GR ($GR^{Hep(Cre)}$) impaired the transcriptional activity of hepatocyte PPARα and reduced the circulating levels of BHB to a comparable degree.

By understanding how hepatic cells interact and contribute to metabolic pathways such as ketogenesis,

key molecular mechanisms driving disease progression can be identified, paving the way for more effective therapeutic interventions.

Implications for health and disease

Fasting has been practiced for millennia by multiple societies for religious, ethical, or health reasons [50]. Accumulating evidence has demonstrated the beneficial effects of fasting interventions on prolonging lifespan and promoting health by delaying the onset and slowing the progression of various diseases and conditions such as cancer, inflammation, and metabolic complications [51–55]. Recent studies showed that patients suffering from type 2 diabetes on a dietary protocol designed to mimic the effects of prolonged fasting, known as fasting-mimicking diet, required significantly less glucose-lowering medication and that fasting-mimicking diets improved renal function in patients with chronic kidney disease [56–59]. Whether the beneficial effects of fasting or fasting-mimicking diets rely solely on their impact on body weight loss remains an open question. However, the shift from glucose metabolism to the metabolism of fatty acids and ketone bodies observed during fasting has been promoted as a therapeutic intervention for decades. In the 1920s, a low-carbohydrate high-fat diet was first proposed as a treatment to control seizures in epilepsy patients. This diet, which mimicked fasting by promoting fatty acid utilization, was termed the 'ketogenic diet' as it correlates with increased BHB and AcAc levels in the circulation, reviewed in Ref. [60].

In recent years, the therapeutic application of ketone bodies has broadened to multiple areas including overweight, diabetes and related cardiovascular complications, as well as neurodegenerative and infectious diseases [61–64]. A comprehensive review of the therapeutic application of ketogenic diets can be found here [12]. Therefore, extensive research has been conducted to identify the underlying mechanisms behind the beneficial effects of ketogenic diets and to assess whether these effects can be directly attributed to ketone bodies. Emerging evidence suggests that the role of ketone bodies indeed extends beyond that of mere energy metabolites. Multiple studies have shown that ketone bodies also act as signaling molecules playing a key role in the regulation of metabolism and immunity. BHB is an endogenous ligand of G-protein-coupled receptors and has been shown to suppress sympathetic nervous system activity, mediate a negative feedback loop on adipocyte lipolysis, enhance reverse cholesterol transport in macrophages, inhibit the NLRP3 inflammasome, and regulate epigenetic modifications as well as gene expression by inhibiting histone deacetylase I, reviewed in Refs. [65,66]. In addition, Karagiannis et al. recently showed that nutritional ketone bodies, in particular BHB, enhanced the antiviral immune response upon COVID-19 infection by restoring the metabolism and function of human and mouse $CD4^+$ T cells [67]*. Today, ketogenic diets are being tested as a therapeutic approach for the management and prevention of COVID-19 [68,69].

It is not surprising that ketogenesis is impaired by high carbohydrate intake, as contained in the western diet. Studies performed in patients with diabetes or metabolic-associated fatty liver disease show that ketogenesis is blunted in response to persistent *de novo* lipid synthesis during fasting, a process that attenuates ketone body synthesis via substrate competition for acetyl-CoA; this effect was stronger in patients with insulin resistance [70,71]. Although the mechanisms linking peripheral insulin resistance with increased fasting *de novo* lipid synthesis and impaired ketogenesis are not completely understood, studies in mice suggest that this process could be attributed to sustained mTORC1 signaling [72].

Another pathological state linked to an aberrant regulation of ketogenesis is cancer cachexia, a condition characterized by involuntary weight loss. It has been recently demonstrated that tumor-induced interleukin-6 (IL-6) suppressed ketogenesis in two mouse models of cancer cachexia by blocking the activation of PPARα; the exact mechanism remains to be elucidated, but it was shown to be independent of decreased fat mass [73]. This effect on hepatic ketogenesis subtracts a crucial energy source that is required to compensate for the decrease in caloric intake upon cancer cachexia development.

As exemplified above, the dysregulation of metabolic circuits is a prime driver of disease progression. Nevertheless, entrainment of these metabolic networks through fasting regimes and low-carbohydrate diets represents a key approach to prevent and treat metabolic complications [9,10,74]. The recent progress in the field of circadian rhythms has led to the idea that the time of day when food is ingested affects multiple metabolic parameters such as glucose and lipid metabolism. This new field, known as chronobiology, proposes that our fasting window should comprise the rest phase (night) while our feeding window should be restricted to the active phase (day). The relevance of timely food ingestion is further supported by the fact that night eating has been associated not only with reduced beneficial effects from caloric restriction programs, but also with increased cardiometabolic risk factors [75]. In line, feeding rodents a ketogenic diet *ad libitum* not only blunted the expected beneficial effects but also resulted in body weight gain, glucose intolerance, as well as increased inflammatory markers within

the adipose tissue and cardiac fibrosis [76,77]. These results further support the notion that adopting a diurnal temporal pattern of caloric intake that aligns with the body's natural rhythms could be a feasible and effective therapeutic approach. While more research is needed to refine specific guidelines, the existing evidence strongly supports the benefits of this strategy in promoting overall health and well-being.

Conclusions and future perspectives

A suitable adaptive response to fasting seems imperative for health. In this review, we have highlighted the complex and cooperative network of adipose tissue and liver ketogenesis crosstalk. Additionally, we provide an overview of recent clinical outcomes associated with inducing ketosis through various strategies, including fasting and reducing dietary carbohydrates.

Although the metabolic adaptation upon fasting of the adipocytes and hepatocytes is relatively well characterized, a deeper understanding on the fasting response in other cell types within the adipose tissue and the liver is required to elucidate the molecular mechanisms behind these processes. New cell culturing technologies such as organ-on-a-chip could present new opportunities to address these questions utilizing human material.

In addition, it would be pertinent to design our metabolic studies to be performed in concordance with the circadian rhythm, meaning that fasting and re-feeding analysis should be performed in the rest and active phase, respectively.

Finally, while numerous clinical studies have demonstrated the potential of fasting protocols to ameliorate metabolic dysfunction in diabetes, obesity, and their complications, recent studies suggested that the beneficial impact of fasting on metabolism might not hold true for all age groups [78]. This clearly validates further and more stratified preclinical and clinical studies on fasting pathways and their therapeutic benefit for distinct metabolic complications and/or subcategories of patients.

CRediT authorship contribution statement

Ana Jimena Alfaro: Conceptualization, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Stephan Herzig:** Conceptualization, Investigation, Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data were used for the research described in the article.

Acknowledgements

Figure 1 was created with Biorender.com.
This work was supported by the Else-Kröner-Fresenius-Stiftung (2020 EKSE.23) and the Edith-Haberland-Wagner Stiftung (SH).

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** of outstanding interest

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