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REVIEW ARTICLE

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

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It is puzzling that hydrogen-rich fatty acids are used only poorly as fuel in the brain. The long-standing belief that a slow passage of fatty acids across the blood–brain barrier might be the reason. However, this has been corrected by experimental results. Otherwise, accumulated nonesterified fatty acids or their activated derivatives could exert detrimental activities on mitochondria, which might trigger the mitochondrial route of apoptosis. Here, we draw attention to three particular problems: (1) ATP generation linked to β-oxidation of fatty acids demands more oxygen than glucose, thereby enhancing the risk for neurons to become hypoxic; (2) β-oxidation of fatty acids generates superoxide, which, taken together with the poor anti-oxidative defense in neurons, causes severe oxidative stress; (3) the rate of ATP generation based on adipose tissue-derived fatty acids is slower than that using blood glucose as fuel. Thus, in periods of extended continuous and rapid neuronal firing, fatty acid oxidation cannot guarantee rapid ATP generation in neurons. We conjecture that the disadvantages connected with using fatty acids as fuel have created evolutionary pressure on lowering the expression of the β-oxidation enzyme equipment in brain mitochondria to avoid extensive fatty acid oxidation and to favor glucose oxidation in brain.

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BRAIN ENERGY METABOLISM AT A GLANCE

The minor utilization of the energy-rich long-chain fatty acids in brain energy metabolism has not been well understood. Interestingly, other organs with high energy turnover, such as the heart and kidney, largely oxidize fatty acids. The low fatty acid oxidation in the brain might be explained in terms of (i) a slow passage of fatty acids across the blood–brain barrier (BBB), (ii) a low enzymatic capacity for the fatty acid degradation and, (iii) detrimental side effects of long-chain fatty acids in the nonesterified or activated form on the mitochondrial ATP synthesis and/or on the equilibrium between the generation and the disposal of reactive oxygen species (ROS). Before going into the detailed analysis, a brief summary of the brain energy metabolism will be given.

The high ATP demand in the brain tissue is impressively illustrated by the following numbers: the human brain accounts for ~2% of the body mass, but consumes 20% of the total oxygen consumed by the whole body. Among the neural cells, neurons demand for most of the energy, whereas the energy consumption of astrocytes contributes to only ~5% to 15% of the total energy requirement of the brain.[1]

This fact, together with the analysis of metabolites profiles supports the view that the energy metabolism of neurons is mainly aerobic and that of astrocytes mainly anaerobic glycolysis. Moreover, the largest portion of the ATP turnover occurs in the gray matter of the brain, which has a high density of excitatory glutamatergic synapses.[1,2] For the rodent brain, it has been estimated that ~80% of the ATP is consumed during presynaptic and postsynaptic signaling.[1]

High ATP turnover in the brain is also indicated by the existence of the creatine kinase/phosphocreatine system in this tissue.[3] Phosphocreatine provides a short-term energy reserve and, in addition, connects the sites of major ATP generation, the mitochondria, with the sites of ATP hydrolysis of neurons. In the brain tissue, more than 90% of the ATP is generated in mitochondria using oxidative phosphorylation (oxPhos).

The high energy requirement of brain tissue is matched by multiple interactions between neurons, astrocytes, and cerebral blood vessels to guarantee the supply of neurons with sufficient oxygen and oxidizable substrates (see recent reviews[4,5]). There is a tight coupling between the energy consumption and the energy supply in the brain. A further feature is that the energy need in neural tissue is matched by both oxidative and nonoxidative glucose degradation. Thus, it has been observed that in active neural tissue the ATP production could not any more rely on the oxygen supply.[6]

Glucose is the main energy substrate for neurons and glia cells.[7,8] Nevertheless, neurons and astrocytes have a different metabolic profile.[4,7,8] Contrary to astrocytes, neurons have lower glycolytic capacity, caused by the lack of activity of the glycolysis-promoting enzyme 6-phosphofructo-2-kinase/fructose 2,6-biphosphatase, isoform 3, PFKFB.[8,9] Furthermore, there is an intensive metabolic cooperation between astrocytes and neurons.[4,8] Features of this cooperation are the donation of astrocytically generated lactate to neurons and, otherwise, the stimulation of the glycolysis in astrocytes by neuronal synaptic activity.[4,8] This stimulation operates subsequent to the

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energy-dependent uptake of glutamate, which is released by glutamatergic synapses.[8] The release of lactate by astrocytes and its uptake by neurons is mediated by different monocarboxylate transporters (MCT1, MCT2, and MCT4).[7,8] The uptake of lactate by neurons has shaped the concept of a lactate shuttle from astrocytes to neurons.[4,8] However, there are yet controversial discussions about the astrocyte-to-neuron shuttling of lactate[7,8] and, furthermore *in vivo* evidence still has to be presented. In addition, the use of lactate by neurons requires an active malate/aspartate shuttle for transporting nicotinamide adenine dinucleotide (NADH) from the cytosol into the mitochondrial matrix to maintain the glycolytic degradation of glucose. Alternatively, it has been hypothesized that mitochondria directly take up cytosolic lactate, which is oxidized in the mitochondrial matrix.[10] Lactate, as energy substrate, has the advantage that neurons metabolize more glucose for nicotinamide adenine dinucleotide phosphate (NAPDH) generation by the pentose phosphate pathway, thereby maintaining the anti-oxidative status of neurons.[7–9] In summary, lactate can serve as supplemental, glucose-sparing substrate and as signal molecule, thereby stimulating the blood flow[7] for delivering oxygen and oxidizable substrates to neural cells.

Brain tissue contains ~3 to 12 μmol glycogen per g tissue, which is exclusively stored in astrocytes.[8,11] For comparison, liver content of glycogen is 100 to 500 μmol per g tissue. Besides being a low energy store at hypoglycemia in the brain, glycogen seems to be a dynamic molecule with versatile functions in normal brain function.[11] During neuronal activity, neurons release glutamate and neuromodulators (adenosine, noradrenaline, and vasoactive intestinal peptide).[8] The latter signals trigger the mobilization of glucose from glycogen. Liberated glucose is after glycolysis and conversion to lactate, used for the supply of lactate to neurons.[8,11]

Moreover, liver-derived ketone bodies (β-hydroxybutyrate and acetoacetate) are used as energy substrates by neurons and glia cells extensively during maturation in young age and upon prolonged fasting.[12] Under fasting conditions, the mean total circulating ketone body concentration is in the range of 5.8 to 9.7 mmol/L in humans. Ketone bodies can replace glucose to a high degree. Thus, up to 60% of the human brain energy requirement can be matched by ketone body oxidation at prolonged fasting.[13] Permeation of ketone bodies across the BBB is mostly mediated by the monocarboxylate transporters, MCT1-2.[12] Both, β-hydroxybutyrate and acetoacetate are converted into acetyl-CoA in the cytosol as well as in the mitochondrial matrix. Acetyl-CoA is used in the cytosol for lipid and cholesterol synthesis, whereas the mitochondrial pathway donates acetyl-CoA to the citric acid cycle for oxidation and for anaplerotic purposes, such as the glutamate synthesis.[12]

Finally, it should be also stressed that the use of ketone bodies as energy substrates is not accompanied by side effects, which have been described for long-chain fatty acids. Thus, ketone body oxidation does not generate reduced flavin adenine dinucleotide ($FADH_2$) (except the $FADH_2$ during degradation of acetyl-CoA within the citric acid cycle) and, in addition, enhanced ROS generation due to the oxidation of $FADH_2$ by the electron transfer flavoprotein-ubiquinone oxidoreductase does not exist. Furthermore, ketone bodies have no protonophoric activity. Therefore, they are unable to de-energize mitochondria due to uncoupling.

Finally, it should be taken into consideration that the use of glucose as the main hydrogen source for mitochondrial oxPhos has possible disadvantages. First, the energy content of glucose (17 kJ/g) is only about one-half of that of long-chain saturated fatty acids (39 kJ/g). Second, glucose oxidation imposes an extra demand for thiamine pyrophosphate, the coenzyme for the pyruvate dehydrogenase complex. This enzyme connects the glycolytic pyruvate production with the system of mitochondrial pyruvate oxidation.

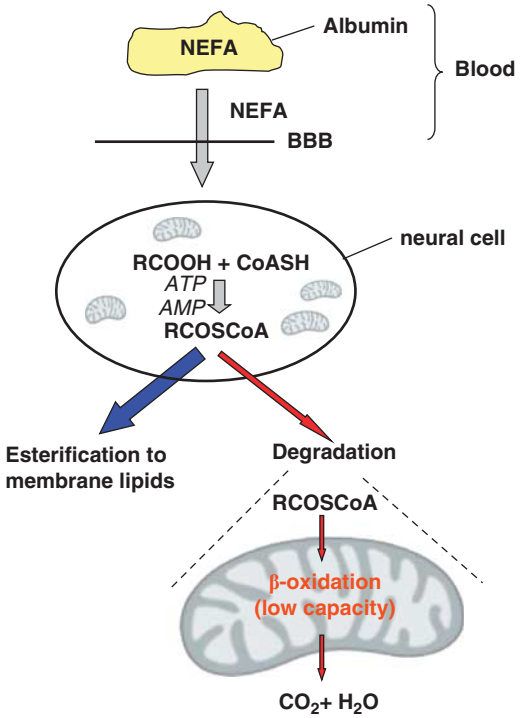

Figure 1. Route of nonesterified fatty acids from blood to the mitochondrial degradation in the brain. After dissociation of albumin-bound nonesterified fatty acids (NEFA) from albumin (indicated in yellow), NEFA migrate across the blood–brain-barrier (BBB). Thereafter, NEFA enter neural cells and are activated to acyl-CoA-derivatives in the cytosolic compartment. In the activated form, NEFA support either the biosynthesis of membrane lipids or the re-acylation of lysophospholipids. Alternatively, β-oxidation of acyl-CoA-derivatives in the mitochondria delivers the reducing equivalents nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$) for fueling the electron transport chain, which generates the electrochemical proton gradient, the driving force for energy-dependent ATP synthesis. CO_2 and H_2O are formed as degradation products of the β-oxidation of acyl-CoA-derivatives.

Keeping these disadvantages in mind, it was an astonishing detection to find that in the brain tissue, the degradation of fatty acids is only poorly used for ATP generation.[14] Nevertheless, fatty acids are taken up during brain development, and it has been reported that fatty acid oxidation could contribute up to 20% of the total brain energy requirement.[15,16] In addition, fatty acid-binding proteins and carnitine have been found in the brain tissue,[17] suggesting that fatty acid metabolism has a role in neurodevelopment, neurotransmission, and repair processes.[18] Moreover, carnitine, widely known for its major role in transport of fatty acids across the inner mitochondrial membrane, is indeed essential for brain functioning.[19] A lack in carnitine causes the development of metabolic encephalopathy, characterized by swollen astrocytes and expanded mitochondria.[20] As astrocytes have some capacity to oxidize fatty acids,[21–23] it is likely that carnitine deficiency causes an increase in the cellular concentration of nonesterified fatty acids (NEFA) reaching toxic levels that are sufficient to stimulate mitochondrial swelling.[24,25]

Figure1 shows the two major steps in the route of fatty acid metabolism in neural cells. Long-chain fatty acids circulate in the nonesterified, albumin-bound form in the blood. After dissociation from albumin, in the first step, NEFA have to migrate across the BBB and, thereafter have to enter neural cells. NEFA become activated to acyl-CoA-derivatives in the cytosol of neural cells. In the activated form, fatty acids are either used for the esterification

to membrane lipids or in the β-oxidation. The latter represents a plentiful source of reducing equivalents NADH and $FADH_2$ inside the mitochondria. Oxidation of both types of reducing equivalents by the electron transport chain (ETC) generates the electrochemical proton gradient, the driving force for ATP synthesis.

As outlined in Figure 1, the reluctant oxidative utilization of fatty acids in the brain tissue raises three important questions: First, does the BBB limit the uptake of NEFA by the brain parenchyma and neural cells and therefore reduce the availability of fatty acids for metabolic consumption in the brain? This question will be discussed in the next chapter. The second question is whether fatty acid uptake would override oxidation. This imbalance results in the accumulation of fatty acids in the free and/or in their esterified forms in the cytosol. Thus, it could be that brain mitochondria are particularly vulnerable against high concentrations of NEFA and those of acylcarnitines and/or acyl-CoA-thioesters. This might explain that mitochondria from individual types of tissue differ considerably in their enzymatic equipment for the oxPhos machinery.[26] The third question is, whether possibly further drawbacks exist. This is indicated by the fact that substantial fatty acid oxidation increases the risk of neural tissue to become hypoxic, which would not be compatible with rapid and sustained neuronal signaling. These latter issues are discussed in the last chapter and lead to a further clue to understand the low usage of fatty acids for brain energy, which has not been considered so far.

BLOOD–BRAIN BARRIER PERMEABILITY AND UPTAKE OF FATTY ACIDS

Both the fact that NEFA are bound mostly to albumin,[27] and the existence of the BBB have led to the previously held view that the passage of long-chain NEFA from blood into the brain through the BBB determines the rate limit. NEFA are tightly bound to plasma proteins, and it seems likely that they are not able to readily flip-flop from one side of the plasma membrane to the other side.[27,28] Consequently, the question was raised: Could the rate-limiting uptake of protein-bound NEFA restrict the utilization of fatty acids for the cerebral ATP generation?

Despite being tightly bound to albumin, however, NEFA have been demonstrated to desorb readily from albumin.[28] However, the passage across the BBB, which consists of tightly connected endothelial cells covering the brain blood capillaries with a continuous layer, could be a second rate-limiting step of the overall transport of NEFA into the brain. Applying the *in situ* perfusion technique to rat brain has clearly allowed demonstrating that saturated and unsaturated long-chain NEFA are rapidly transported through the BBB to a large portion and with similar rates.[29–32]

To illustrate the mechanisms of passage of endothelial cells by NEFA, a brief overview on the lively debate on this topic is added here. After the entry of NEFA into endothelial cells of the BBB, NEFA have to migrate through to the neural cells, thereby crossing cytoplasmic membranes. Two types of transport mechanisms are currently intensely discussed for the passage of NEFA through cytoplasmic membranes, (i) passive transport or alternatively (ii) protein-mediated diffusion (as reviewed in Mitchell *et al.*[17]). According to the first possibility, NEFA cross the cell membranes by a flip-flop mechanism at the luminal and transluminal leaflets of endothelial cells and the cytoplasmic membrane of neural cells.[27,28] The alternative view suggests that the passage of NEFA across the cytoplasmic membrane is assisted by membrane proteins.[33,34] Using nonesterified, radioactively labeled docosahexaenoic acid (22:6 n-3) and eicosapentaenoic acid (20:5 n-3), a rapid passive diffusion across the BBB was found with *in situ* cerebral perfusion.[29] Importantly, the passage of docosahexaenoic acid was not saturable up to the highest concentration (100 μmol/L) applied, suggesting that this NEFA crossed the BBB by simple diffusion. Such diffusion is also consistent with previous reports using palmitic acid (C16:0) or arachidonic acid (C20:4 n-6).[31,32] In addition, a passive diffusion of saturated, long-chain NEFA across the lipid bilayer membranes is also supported from molecular modeling studies.[35] Moreover, using labeled polyunsaturated fatty acids (PUFA) it has been shown that the passage of PUFA across the BBB was not antagonized by their corresponding nonlabeled forms, thereby arguing against a membrane protein-assisted transcellular transport of fatty acids. Otherwise, evidence for the involvement of several fatty acid transport proteins using human brain microvessel endothelial cells was reported recently.[36]

Thus, it can be summarized that the current experimental and theoretical evidence does not support the view that the slow speed of passage of NEFA through the BBB is the cause for the poor fatty acid oxidation by the brain. On the other side, evidence was obtained for the possible damage of the BBB caused by fatty acids. This might happen after chronic ingestion of dietary fat with a high content of saturated fatty acids.[37] In addition, PUFA, such as arachidonic acid, can enhance oxidative stress in endothelial cells and, thereby initiate the dysfunction of the BBB.[38]

FATTY ACID DEGRADATION BY MITOCHONDRIA IN NEURAL CELLS AS COMPARED WITH OTHER TISSUES

Apart from certain hypothalamic neurons, fatty acid oxidation predominantly takes place in astrocytes.[21–23,39,40] After cellular uptake, NEFA are enzymatically activated to acyl-CoA derivatives. In the activated form, fatty acids can be either esterified to membrane lipids or degraded by the mitochondrial β-oxidation to provide cellular energy (Figure1). In both homogenates of neural cells and mitochondria obtained from the brain tissue from rodents, a poor oxidative degradation of long-chain fatty acids (C_{12} to C_{18}) has been detected. In these studies, any restrictions by the BBB are excluded. Rates of the liberation of $^{14}CO_2$ from ^{14}C-labeled fatty acids or the oxygen consumption measured with isolated cells or brain mitochondria reveal poor fatty acid oxidation.[41] For illustration, using palmitoyl-carnitine as a substrate, the oxygen consumption by isolated rat brain mitochondria during ADP phosphorylation was estimated to be 20 nanoatoms of oxygen per min per milligram of protein at 25°C, which is eightfold lower than that of heart mitochondria under comparable conditions.[41] In contrast to long-chain fatty acids, the medium-chain octanoic acid is rapidly oxidized in the brain.[15] However, cerebral octanoate oxidation seems to be a special case for two reasons: First, octanoate oxidation mostly takes place in astrocytes, and, in contrast to long-chain fatty acids, its oxidation by mitochondria is not under the control of the acylcarnitine/carnitine antiporter.

A large number of studies have demonstrated that brain mitochondria have high oxygen consumption with pyruvate or glutamate as hydrogen donors, as compared with heart and skeletal muscle mitochondria. Therefore, it was a surprising observation that brain mitochondria do not use long-chain fatty acids as hydrogen source, in contrast to the mitochondria from heart muscle or kidney, two tissues that exhibit high ATP turnover.

Low-level oxidation of long-chain fatty acids by isolated brain mitochondria has been attributed to (i) the low translocation rate of long-chain fatty acid-CoA esters across inner mitochondrial membrane and (ii) the low enzymatic capacity of the β-oxidation pathway. The first suggestion is supported by the discovery that the brain-specific isoenzyme of the carnitine palmitoyltransferase 1 has a low activity.[42] Moreover, the enzymatic capacity of the β-oxidation in brain mitochondria is remarkably lower than in mitochondria from the other high-energy turnover tissues.[41] Particularly, the 3-ketoacyl-coenzyme A thiolase activity, the terminal enzyme of the four steps of

the β-oxidation pathway, is very low. The activity of this enzyme in the brain has only 0.7% of that in the rat heart mitochondria. The other enzymes of the β-oxidation pathway, such as the acyl-CoA dehydrogenase or the enoyl-CoA-dehydrogenase have $\sim$50% and 19% of that of the heart mitochondria.[41]

In contrast to the low activity of the mitochondrial 3-ketoacyl-coenzyme A thiolase, neurons have a particularly high activity of the cytoplasmic long-chain acyl-CoA thioesterase 7.[43] Acyl-CoA thioesterase 7 is considered to be a regulatory point in the fatty acid metabolism for keeping the acyl-CoA concentration in neurons low, which is beneficial because of the low lipid-storage capacity and the low β-oxidation of fatty acids. It has been suggested that acyl-CoA thioesterase 7 guarantees fatty acid homeostasis in neurons, particularly in fasting-induced alterations of the fatty acid metabolism in neurons.[43] Finally, acyl-CoA thioesterase has been proposed to exert a protective function against detrimental effects caused by excess of fatty acids.

In summary, the slow rate of β-oxidation of fatty acids seems to be a unique intrinsic feature of the brain tissue, particularly of the mitochondria of neurons. For comparison, in the heart and kidney tissue, 60% to 80% of the energy need is provided by fatty acid oxidation.

FATTY ACID FUELING IS A SOURCE OF HARMFUL SIDE EFFECTS IN THE BRAIN MITOCHONDRIA

Generally, from cultured rodent neural cells it is known that in astrocytes and neurons the enzymatic activities of complexes of the ETC are different.[44] Thus, the enzymatic activity of complex I is much higher in astrocytes. A similar difference has been reported for the synaptic and nonsynaptic mitochondria.[45] Such findings indicate that neurons and their mitochondria respond more sensitively to environmental changes and to mitochondrial toxins.[46,47]

A large number of *in vitro* studies using isolated mitochondria have illuminated the potentially dark side of fatty acid oxidation in the tissue. Thus, pathophysiological high concentrations of NEFA impair several processes involved in the oxidative ATP generation.[48,49] Crucially, detrimental effects of NEFA on the mitochondrial physiology are summarized in Figure 2. NEFA depolarize the inner membrane of mitochondria by increasing their conductance to protons, which reduces the electrochemical proton gradient (Δp) (Figure 2, left part). Consequently, the oxPhos of ADP stops and the calcium retention capacity of mitochondria decreases. In addition, binding of NEFA to ETC complexes (Figure 2, middle part) slows down the electron flux and stimulates the generation of superoxide, and thereby the formation of the strong oxidants hydrogen peroxide and the hydroxyl radical. Finally, NEFA sensitize the permeability transition pore to opening, probably by binding to protein components, which assemble the permeability transition pore (Figure 2, right part).

In addition, deleterious effects on mitochondrial functioning under *in situ* conditions have been reported from studies, where glial cells and PC12 cells were exposed to pathologic concentrations of PUFA and saturated NEFA.[50–52] The fatty acids applied in these studies were either the branched-chain phytanic acid (3,7,11,15-tetramethylhexadecanoic acid) or the very long-chain fatty acids docosanoic (C22:0), tetracosanoic (C24:0), and hexacosanoic acid (C26:0). These fatty acids are prominent biochemical hallmarks of specific neurodegenerative diseases, such as Refsum disease or X-linked adrenoleukodystrophy.[53,54] Thus, it has been reported that NEFA increase the cytosolic Ca^{2+} concentration, depolarize mitochondria *in situ,* and enhance the cellular generation of ROS.[50–52] *In situ* depolarization is attributed to the permeabilization of the inner mitochondrial membrane to H^+, whereas ROS generation is associated with partly impaired respiratory chain complexes.[55] Gating of the permeability transition pore (PTP) by micromolar concentrations of free fatty acid is a further harmful activity, which has been found with isolated mitochondria and cells.[48]

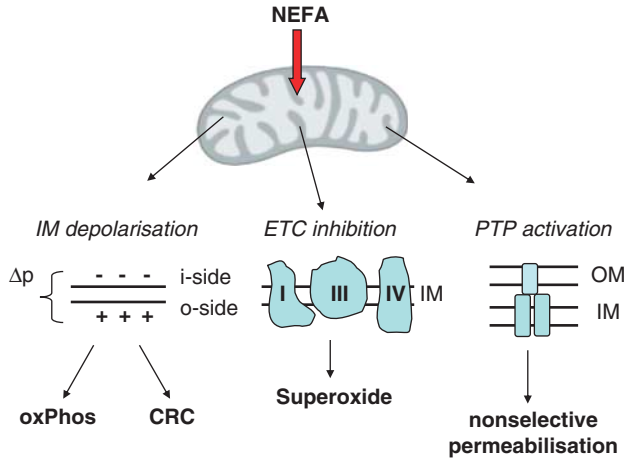

Figure 2. Nonesterified fatty acids (NEFA) impair the mitochondrial physiology. Being natural protonophores, nonesterified fatty acids partly decrease the membrane potential at the inner mitochondrial membrane. This depolarization causes a collapse of the electrochemical proton gradient (Δp), thereby uncoupling the oxidative phosphorylation (oxPhos) and reducing the Ca^{2+} retention capacity (CRC). Binding of NEFA to electron transport chain (ETC) complexes interferes with the electron transport, thereby stimulating the generation of superoxide as by-product of the ETC. Superoxide is the source of other reactive oxygen species, such as hydrogen peroxide, hydroxyl radical, and peroxynitrite. Moreover, both depolarization and binding of NEFA to proteins of the permeability transition pore (PTP) sensitized the opening of the PTP. In the open state, PTP conducts the release of Ca^{2+} from the mitochondrial matrix and of proapoptotic factors (e.g., cytochrome *c*, AIF, Smac-Diablo).[82] OM, outer membrane; IM, inner membrane.

Finally, there is impressive evidence for the beneficial effect of ischemic preconditioning on functional intactness of organs with high-energy metabolism. Considerable attention has been attracted by the mitochondrial K_{ATP} channel as target, which becomes modified by brief sublethal episodes of ischemia.[56,57] Surprisingly, with mitochondria isolated from the perfused rat hearts, it has been found that ischemic preconditioning does not alter the integrity of the inner mitochondrial membrane, but sensitizes mitochondria to fatty acid uncoupling.[58]

Taken together, all the studies carried out with the isolated neural mitochondria or glial cells revealed that NEFA exert harmful effects on mitochondria, comparable to those known from mitochondria of nonneural tissues. In addition, it is worth mentioning that also nonmitochondrial targets, such as the ATP-consuming Na^+/K^+-ATPase, are inhibited by NEFA.[59]

CONSIDERATIONS THAT MAY EXPLAIN THE UNSUITABILITY OF FATTY ACIDS AS FUEL IN BRAIN TISSUE

Oxidation of Fatty Acids Increases the Tendency of Neural Tissue to Become Hypoxic

The generally accepted concept claims that the high ATP requirement makes the brain susceptible to damage associated with anoxia or ischemia. Because of the low capacity of glycolysis to provide ATP to cell metabolism, the adult mammalian brain responds very sensitively to hypoxia, because oxygen limitation restricts the regeneration of ATP by mitochondria.[60,61] The 'critical' oxygen tension, where oxPhos does not supply the full ATP requirement for the brain is indicated by small decreases of pH (caused by lactate enrichment) and decrease of the creatine

phosphate/phosphate ratio (caused by ADP phosphorylation using creatine phosphate). This 'critical' oxygen tension has been estimated to be in the range of 0.8 to 1.2 kPa (6 to 9 mm Hg) in the rat cortex.[62] The oxygen concentration in the brain is low and nonuniform. For example, the oxygen pressure in the cortex gray matter of rats is in the range of 19 to 40 mm Hg, compared with that in the cortex white matter of 6 to 16 mm Hg.[62] Therefore, the difference in oxygen demand for fatty acid- and glucose-based ATP generation by oxPhos is very critical. Complete oxidation of one mol of palmitic acid ($CH_3[CH_2]_{14}COOH$) to CO_2 and H_2O yields 106 moles of ATP, whereas glucose oxidation allows the formation of only 32 moles of ATP per mol of glucose. Hence, in contrast to glucose oxidation, fatty acid degradation liberates a relatively large portion of FAD-bound hydrogen, which during oxidation yields less ATP. With NADH, we obtained 2.5 ATP, but with $FADH_2$ only 1.5 ATP per mol. Consequently, for the generation of a given amount of ATP, the fueling with palmitic acid requires $\sim$15% more oxygen (23 O_2) than fueling with glucose (20 O_2). Thus, the high oxygen consumption associated with the oxidation of fatty acids increases the risk for neurons that their environment in the brain parenchyma becomes hypoxic, where the oxygen pressure is nonuniform and rather low.

It is important to recall that the fetal heart operates under low oxygen tension and in this situation, glucose and lactate are used for ATP generation. In contrast, in the well-oxygenated adult heart, ATP generation mostly comes from fatty acid oxidation.[63] In addition, animal studies revealed that the reduction of fatty acid oxidation protects the heart from ischemia.

The Use of Fatty Acids as Fuel Increases the Risk of enhanced Oxidative Stress

It has been broadly accepted that mitochondrial oxidative stress and dysfunction are factors contributing to neurologic disorders.[64] The mitochondria are considered to be the major source of ROS generation in the central nervous system, which makes them vulnerable to oxidative stress damage.[64] Reactive oxygen species generation as side products during the reduction of molecular oxygen by the ETC has been well known for more than five decades. Superoxide is formed by a one-electron transfer from certain sites within the ETC to molecular oxygen, as described in a comprehensive review.[65] Moreover, a further source of "electron leakage" is β-oxidation, probably at the levels of the acyl-CoA dehydrogenase, the electron transfer flavoprotein, the electron transfer flavoprotein-ubiquinone oxidoreductase, and complex III (for reviews, see[55] and for references[66–69]). Besides the mitochondrial sources, additional ROS production has been described in the brain and other tissues to derive from the peroxisomal β-oxidation and the plasma membrane-associated NADPH oxidase.[70] Indeed, inflammation-activated microglia cells specifically generate superoxide via the NADPH oxidase.[71]

In addition to the enhanced ROS generation associated with the β-oxidation pathway of fatty acids, there is a second cause of the stimulation of ROS generation by NEFA. Binding of NEFA to complexes of the ETC interferes with the electron transport from NADH to oxygen. This impairment of the electron transport reduces not only the oxidative ATP generation, but also stimulates the superoxide generation by complexes I and III. Harmful consequences of oxidative stress associated with the enrichment of fatty acids in the nervous system have been observed in X-linked adrenoleukodystrophy. This inherited neurodegenerative disease became broadly popular from the movie entitled *Lorenzo's oil*. X-linked adrenoleukodystrophy results from the defective degradation of very long-chain fatty acids by peroxisomes. Thereby, very long-chain fatty acids accumulate in the tissue and plasma.[54,53] There is accumulating evidence that during development of the pathology enhanced oxidative stress induces axonal demyelination.[72,73] Thus, X-linked adrenoleukodystrophy is a striking example for the increased susceptibility of the brain tissue to oxidative stress.

The high risk of oxidative stress for the brain tissue has been attributed to the high content of peroxidizable PUFA in membranes, oxygen-sensitive catecholamines, and the relatively low capacity of antioxidant enzymes in brain as compared with other organs.[73] Therefore, it seems likely that evolution has developed a strategy to minimize ROS generation in neuronal cells, which moreover have a very poor regenerative potential. According to a recent hypothesis, the ROS prevention strategy consists in the abolishment of the β-oxidation in neurons.[74] This should be substantiated by the following quantitative analysis: during complete degradation of one glucose molecule, two molecules $FADH_2$ and 10 molecules of NADH are formed, which corresponds to a $FADH_2$/NADH ratio of 0.2. In contrast, β-oxidation of palmitic acid generates 15 molecules of $FADH_2$ and 31 molecules of NADH, with an $FADH_2$/NADH ratio of $\approx$0.5. Consequently, during β-oxidation there is competition of 'NADH and $FADH_2$ electrons' for oxidized ubiquinone as electron acceptor. This situation would most likely enhance oxidative stress in neurons for two reasons. Thus, slow NADH oxidation maintained the redox state of the electron carriers upstream of complex III in a highly reduced state, a situation similar to rotenone inhibition of complex I. Such situation enhances the superoxide generations by ETC. Moreover, at a high $FADH_2$/NADH ratio, more $FADH_2$ becomes oxidized by the electron transfer flavoprotein-ubiquinone oxidoreductase, a reaction known to be a potent source for superoxide generation.[66,68]

Fatty Acid Oxidation is too Slow for Matching the ATP Requirements During Rapid Sustained Neuronal Electrical Activity

There is good reason to ask the question whether the rate of ATP regeneration by burning of fatty acids would be rapid enough to fulfill the ATP requirements of the brain during periods of rapid sustained neuronal firing. There are two arguments, which evoke this question. First, it has been demonstrated that in the activated brain areas, the local increase of the oxygen consumption is smaller than that of the glucose consumption.[6,75] Second, the elevated lactate concentration observed during high neuronal activity is indicative for an uncoupling of the glucose use from oxygen consumption.[76] Obviously, during periods of high sustained neuronal activity the oxidative ATP supply is at its maximal capacity. To match the ATP requirement, in addition the anaerobic glycolytic ATP regeneration becomes stimulated.

Furthermore, a detailed analysis of the energy budget for gray matter of rodents has shown that at a firing rate of 4 Hz, 30 μmol ATP per g wet weight per min is consumed by neurons.[1] This ATP turnover is comparable with that in the human leg muscle during marathon run,[77] where glycogen plus fatty acids are burnt. Generally, the ATP usage of the gray matter is considered to be in the range of 33 to 50 μmol ATP per g wet weight per minute, which is significantly higher than that of the whole brain on average ($\sim$21 μmol ATP per g wet weight per minute). In addition, at a firing rate of 18 Hz, the ATP consumption amounts to 120 μmol ATP per g wet weight per minute. Assuming an ATP consumption by the gray matter of 50 μmol ATP per g wet weight per minute or even higher, it is likely that the fatty acid fueling limits the oxidative ATP generation, as the pathway of liberation of reducing equivalents from stored triglycerides of the white fat tissue is long and complex. When considering only the degradation of fatty acids by the mitochondria, the control of this pathway depends on (1) the uptake of fatty acids by neurons from the blood, (2) the entry of activated fatty acids into the mitochondria, and (3) the oxidative cleavage of the hydrocarbon chain by the enzymes of the β-oxidation. The latter process is under a complex regulation, as three ratios participate in the control of β-oxidation. These ratios are the redox states of

NAD^+/NADH, FAD/$FADH_2$, and the ratio of [acetyl- or acyl-CoA]/[CoASH].[78] The acyl-CoA dehydrogenase and the 3-hydroxy-acyl-CoA dehydrogenase respond to the ratios of FAD/$FADH_2$ and of NAD^+/NADH, whereas the activity of the 3-ketoacyl-CoA thiolase is sensitive to the acetyl-CoA/CoASH ratio.

In contrast to the low rate of oxidation of long-chain fatty acids, neural cells can oxidize ketone bodies with a seven- to ninefold greater rate than glucose, an observation derived from the measurement of the liberation of CO_2 from ^{14}C-labeled glucose or ^{14}C-labeled acetoacetate/β-hydroxybutyrate.[22] In addition, fueling the energy of brain tissue with ketone bodies has the advantage that the permeation of NEFA across the BBB and their β-oxidation are bypassed.

Taken these facts together, there is reason to speculate that the rate of the ATP generation based on the utilization of adipose tissue-delivered fatty acids is too slow to match the ATP consumption in cerebral tissue during periods of rapid sustained neuronal firing. It might be argued that such conclusion contrasts with the situation in the heart, where in spite of a highly flexible beating rate, most of the energy comes from the mitochondrial fatty acid oxidation.[79] In the heart tissue, NEFA are supplied to cardiomyocytes in the albumin-bound form and from triacylglycerol-rich serum lipoproteins. From the latter, NEFA are liberated by a lipoprotein lipase, which is bound to the coronary vascular endothelium. Moreover, triacylglycerides are also stored in cardiomyocytes as lipid droplets in the proximity of the mitochondria.[80,81] In addition, it has been reported that the oxidation of lipid droplet-derived fatty acids essentially contributes to the ATP generation in cardiomyocytes. Thus, it is not surprising that impaired heart function has been associated with a deranged metabolism of triacylglycerides (see[80] and references therein).

In conclusion, contrary to neural cells, the concentration of cytosolic fatty acids available for mitochondrial oxidation is buffered in cardiomyocytes because of existing lipid droplets. Therefore, the heart activity does not strictly depend on the supply of fatty acids derived from serum lipids.

CONCLUSIONS

Neural cells have very high, but fluctuating ATP requirements. Despite the fact that fatty acids are the substrates richest in hydrogen for supplying redox energy to the mitochondrial ETC, they are not used significantly as fuel in neural cells. High susceptibility of brain tissue to oxidative stress is generally claimed to be the underlying reason. However, evolution has eliminated the enzymatic activity of fatty acid oxidation in brain mitochondria. We hypothesize that this helps to protect neural cells against enhanced oxidative stress. Considering the low anti-oxidative defense capacity in neural cells and the fact that β-oxidation is a most prominent source of ROS generation, this provides a convincing explanation for the finding of low enzymatic capacity for fatty acid degradation in neural cells. In addition, we speculate that the situation of the energy metabolism in neural cells of the gray matter could be compared with that of fast-twitching muscle fibers. To support fast-twitching skeletal muscle tissue rapidly with ATP, the ATP regeneration has to be based on glucose degradation. ATP regeneration based on the oxidative fatty acid degradation is too slow. Quantitatively, it has been estimated that the rate of ATP generation based on the carbohydrate oxidation is in the range of 0.51 to 0.68 mmol per second per kg body mass.[81] In comparison, the rate of ATP generation based on triacylglycerol fueling is approximately two- to threefold lower (0.24 mmol per second per kg body mass). We argue that, as a consequence, the overall process starting from the liberation of NEFA in the white adipose tissue to their oxidation in the matrix compartment of neural mitochondria is much too slow for supplying enough ATP to neurons with rapid sustained firing.

DISCLOSURE/CONFLICT OF INTEREST

The authors declare no conflict of interest.

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