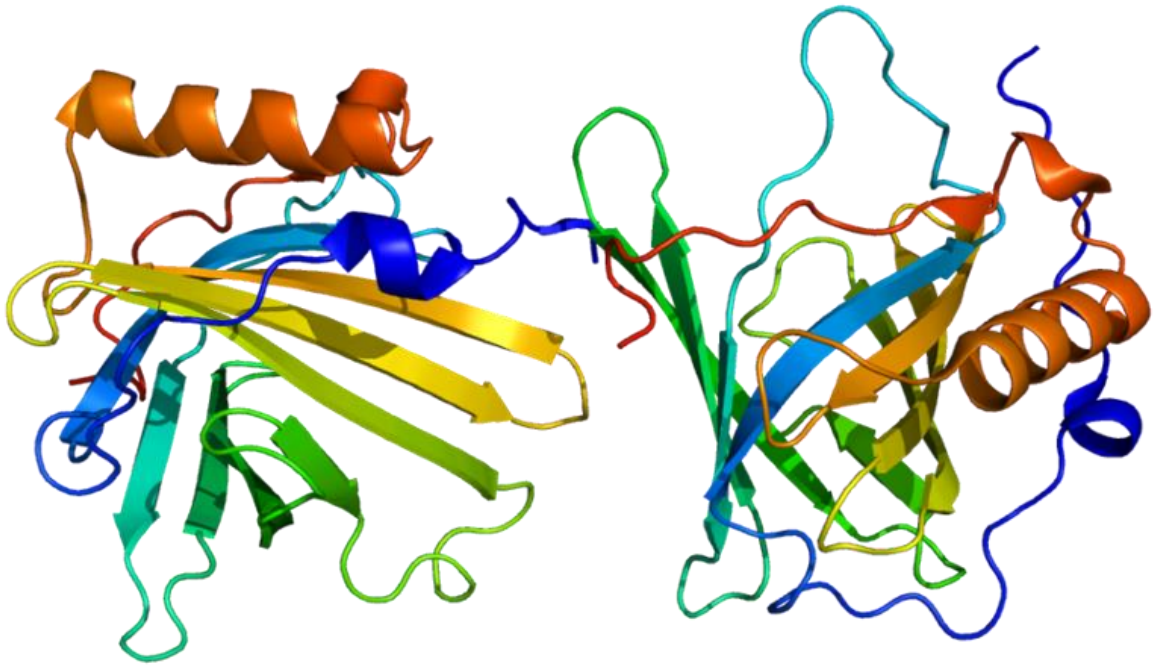
Unraveling Lipocalin-2: Friend or foe?



S. van Heijningen – s1868934
University of Groningen – 26-11-2015
Supervisors: U.L.M. Eisel & S.A. Nelemans

Introduction

In mice, Lipocalin-2 (LCN-2) (or 24p3) was first identified by an overexpressed gene in simian-virus-infected kidney cells (Hraba-Renevey et al. 1989). Later, the human analog of LCN-2 was discovered by Kjeldsen et al in 1993 (Kjeldsen et al. 1993). Polyclonal antibodies raised against human neutrophil gelatinase were used to classify this 25kDa protein (Kjeldsen et al. 1993). For this reason LCN-2 is also known as neutrophil gelatinase associated lipocalin (NGAL), at least in humans. For the remainder of this essay, LCN-2 will be used to refer to all analogs of this protein, while a discrimination will be made between rodent and human functions. Liu & Nilsen-Hamilton found LCN-2 to be upregulated in the liver during the acute-phase response (Liu and Nilsen-Hamilton 1995). Indeed in rodents, LCN-2 has been further described to be an important mediator in the acute-phase response (Sultan et al. 2012). LCN-2 is furthermore believed to be a bacteriostatic agent (Flo et al. 2004), acting as an iron-depleting factor in the innate immune response (Berger et al. 2006). The examples above lie at the basis of the goal of this current study, which is to describe the extensive and complex functions of LCN-2. The molecular functions and related biological processes will be exposed to try and understand the primary function of LCN-2.

LCN-2 belongs to the Lipocalin superfamily of proteins, which were first considered to function as binding proteins for small hydrophobic molecules (Flower, North, and Attwood 1993). However more functions became clear later on, such as retinol transport, olfaction, pheromone transport, and the enzymatic synthesis of prostaglandins (Flower 1996). Also the implications on the regulation of the immune response and mediation of cell homeostasis became apparent for the Lipocalin protein family (Flower 1996). Although LCN-2 belongs to this superfamily of proteins, sequence homology and functions within this family varies greatly. The characterization of a lipocalin is based on structure similarity. In humans, LCN-2 is covalently attached to neutrophil gelatinase, closely associated with matrix metalloproteinase-9 (MMP-9) (Kjeldsen et al. 1993; Triebel et al. 1992). The finding that LCN-2 is a positive acute-phase protein indicates that this protein may possess immunosuppressive or anti-inflammatory properties (Triebel et al. 1992; Liu and Nilsen-Hamilton 1995), or at least regulatory functions. The direct relationship between neutrophil activation and LCN-2 concentrations becomes increasingly clear by the action of granulocyte macrophage colony-stimulating factor (GM-CSF) on human neutrophils. Induction with GM-CSF shows significant synthesis and secretion of LCN-2 (Axelsson, Bergenfeldt, and Ohlsson 1995). GM-CSF is a haemapoietic growth factor and immune modulator that is known to sustain the viability and activation of function of neutrophils (Katano et al. 2009; Shi et al. 2006). Upon receiving immune stimuli, GM-CSF is primarily produced by a variety of immune-cells including T cells, macrophages, endothelial cells and fibroblasts, although also produced locally (Shi et al. 2006). Mice injected with GM-CSF also developed an increase in blood neutrophils (Metcalf et al. 1987). Normal human plasma and serum levels of LCN-2 seem to differ between several papers. Table 1 contains an overview of several known papers that measured plasma and serum levels of LCN-2 in control subjects.

paper analyzed (#)	Serum/plasma LCN-2	Sampling method	Study
1	**72 ng/mL**	Not described	Axelsson et al 1995
2	**132 ng/mL**	Not described	Naude et al 2012
3	**163 ng/mL**	Fasting	Choi et al 2011
4	**163 ng/mL**	Overnight fasting	Magnusson et al 2012
5	**31,1 ng/mL**	Overnight fasting	Ni et al 2013
6	**93 ng/mL**	Not described	Sung et al 2012
7	**50,3 ng/mL**	Morning samples	Kafkas et al 2012

Table 1. Plasma/Serum LCN-2 levels in humans.

Table 1 seems to show a large spread of plasma levels of LCN-2 (100 ± 53,2 ng/mL). Although there are obvious differences between studies in methodology, it seems that a large part of the studies take samples directly after fasting. Even within these studies there is still a large spread of the plasma LCN-2 levels. Clearly there has to be a modulatory effect on these levels. Perhaps the time of day can play a role in this variability, or an unknown inflammatory upregulation at that point in time. Scheer et al have studied a variation in plasma LCN-2 levels concerning day/night rhythm and fed/fasted state in humans (Scheer et al. 2010). They found that in the fed-state the mean concentration of plasma LCN-2 is 29.7 ± 10.1 ng/mL, while in the fasted state this concentration was higher at 63.1 ± 15.4 ng/mL. Day/night rhythm showed the highest peak of plasma LCN-2 around 16:00-17:00 pm, while the lowest levels were measured around 04:00-05:00 am (Scheer et al. 2010). This suggests there might be a strong circadian regulation in plasma LCN-2 levels. Whether this is specifically regulated by an entrainable oscillator or because of other time-dependent factors, remains to be revealed. A direct link between the innate immune system, of which LCN-2 is thought to be a part of, and circadian rhythmicity has been established (Gibbs et al. 2012; Scheiermann et al. 2012). Macrophages possess an efficient clock machinery, thus show tight circadian regulation (Hayashi, Shimba, and Tezuka 2007; Keller et al. 2009). For LCN-2 however it is not clear whether its expression is regulated under circadian conditions. It has been shown that a host's response to a Salmonella infection can greatly differ depending on the phase of induction, whether the infection is induced in the active or rest phase of mice (Bellet et al. 2013).

It was shown that in the brain LCN-2 is produced in the choroid plexus (CP) (Marques et al. 2008), also responsible for the main production of CSF. CSF production shows circadian rhythmicity showing peak production around 02:00 am and lowest production around 6:00 pm in humans (Skipor and Thiery 2008). In mice there is less evidence on circadian rhythmicity and the production of CSF. There is however a study on transportin1 (Tnpo1), which is a transport receptor that transports substrates between the nucleus and the cytoplasm (Sato et al. 2011). Aside from this well described transporter function, suggested physiological roles of Tnpo1 include sensing and production of CSF under the regulation of

the suprachiasmatic nucleus (SCN), the major circadian clock regulator. Sato et al found high Tnpo1 signals in the CSF, SCN and in the subventricular zone of the lateral ventricle (Sato et al. 2011). There is no connection between Tnpo1 and LCN-2 yet, however this study does raise the possibility of a strong circadian regulation of CSF production in rodents, and possibly LCN-2 as well. It would be beneficial to study the possible circadian rhythmicity of LCN-2, both circulatory and in the brain, since this would yield greater accuracy when performing assays for this protein.

Iron transport and apoptosis

As mentioned before, LCN-2 is believed to be a bacteriostatic agent (Flo et al. 2004), acting as an iron-depleting factor in the innate immune response (Berger et al. 2006). By sequestering iron, LCN-2 can reduce the growth of susceptible bacteria (Flo et al. 2004). Iron is an essential part of sustaining life, since it is needed to form the functionality of red blood cells by making the oxygen-carrying protein hemoglobin. Since iron is essential for viral and bacterial cell synthesis, iron concentration and availability has to be tightly regulated. Excessive iron can favor viral and bacterial infections and increase oxidative stress (Weinberg 1984, 1996). Because of the low bioavailability of ferric iron, bacteria can produce iron chelators-siderophores that can remove iron from the host by high-affinity binding to iron (Ratledge and Dover 2000). Bacteria use these siderophores to acquire iron (Raymond, Müller, and Matzanke 1984). An iron-limiting response is therefore considered an active anti-bacterial response in mammals. LCN-2 seems to be a potent iron-limiting agent by binding to bacterial catecholate-type ferric siderophores with great affinity (Goetz et al. 2002). LCN-2 responds in this way to the activation of the innate immune Toll-like receptor 4 (TLR4), which detects lipopolysaccharide (LPS) from Gram-negative bacteria like *Escherichia coli* (E. coli) and *Salmonella enterica* (Flo et al. 2004; Roach et al. 2005). Essentially, LCN-2 starves bacteria from iron (Fischbach et al. 2006). Studies done in LCN-2-deficient mice show a profound increase in sensitivity and susceptibility to E.coli (Flo et al. 2004; Berger et al. 2006). An infection with Salmonella in macrophages and dendritic cells can lead to the production of cytokines. This release of cytokines can lead to the recruitment of neutrophils, which is an important component of the antibacterial response (Raffatellu et al. 2009), possibly enhanced by the action of LCN-2.

LCN-2 does not interact with bound iron; instead it acts as a transporter to deliver iron to cells (Yang et al. 2002; Yang et al. 2003; Goetz et al. 2002). LCN-2 delivers iron to the cytoplasm, either activating or repressing iron-responsive genes (Yang et al. 2002). Interestingly, LCN-2 seems to play a role in apoptosis considering this response. Apoptosis is regulated by various transcription factors, however recently also withdrawal of interleukin-3 (IL-3) is shown to induce apoptosis in haemapoietic cells (Devireddy et al. 2001). LCN-2 seems to be implicated in this response, either stimulating or inhibiting apoptosis, depending on whether LCN-2 is loaded with iron or not. Iron-loaded LCN-2 can increase

intracellular iron concentration, thereby inhibiting apoptosis by decreasing the proapoptic protein *Bim* (Bcl-2-interacting mediator of cell death) (Devireddy et al. 2005; Richardson 2005). Conversely, iron-free LCN-2 decreases intracellular iron by its high-affinity binding, this in turn induces the expression of protein *Bim*, resulting in the stimulation of apoptosis (Devireddy et al. 2005; Richardson 2005). An overview of this response can be seen in figure 1.

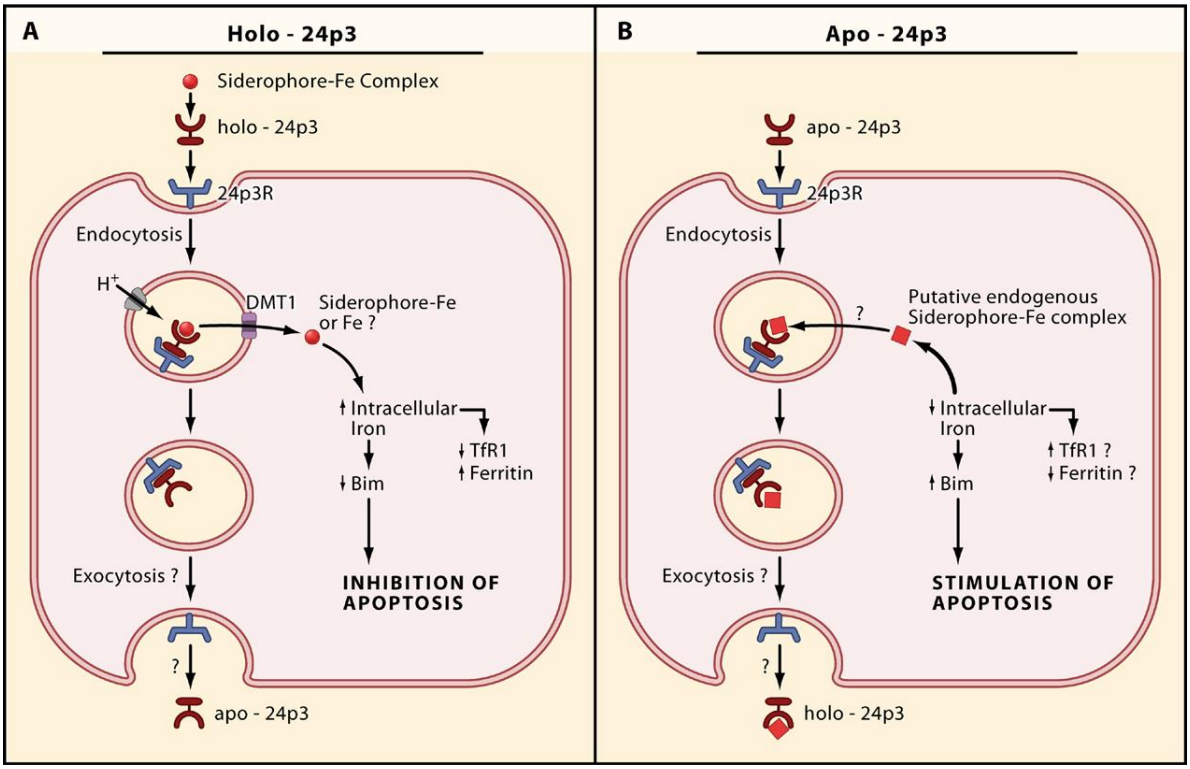


Figure 1. Intracellular iron transport and the route to apoptosis. **A:** Iron-bound LCN-2 can increase intracellular iron concentration, thereby inhibiting pro-apoptotic protein *Bim*, inhibiting apoptosis. **B:** Iron-free LCN-2 can decrease intracellular iron concentration, thereby increasing pro-apoptotic protein *Bim*, stimulating apoptosis. Source: Richardson 2006.

Further involvement in the apoptotic pathway becomes apparent when considering that a potent inducer of LCN-2, MK886, is also a strong pro-apoptotic agent (Tong, Wu, and Kehrer 2003; Correnti et al. 2012). However it also seems that LCN-2 might play a role in cell survival. This was made clear in a study om human cell lines by Tong et al, who found that purified recombinant LCN-2 was non-toxic, antibodies against LCN-2 were highly toxic, and the overexpression of LCN-2 significantly reduced apoptosis-induced cell death (Tong et al. 2005).

What has become clear from this section is that LCN-2 plays an important role in the anti-bacterial response by limiting iron-availability for bacterial proliferation. However what happens after this response raises more questions about the effects of LCN-2. Iron bound to LCN-2 is suggested to have an anti-apoptotic effect, while iron-free LCN-2 is hypothesized to

stimulate apoptosis. The discussion whether LCN-2 is either pro- or anti-apoptotic is an ongoing one, however theories can be built upon them. For example from an evolutionary standpoint, if there is an initial induction of LCN-2 directly upon bacterial infection, one can think LCN-2 mediates an acute anti-bacterial response. Perhaps an overshoot in LCN-2 production, due to immune system hypersensitivity, results in side-effects. Or perhaps this same hypersensitivity induces an immune response, while there is no immediate foreign threat. Perhaps oxidative stress, as a co-morbidity of infection, induces this response, since LCN-2 also seems to possess antioxidant properties by induction of heme-oxygenase-1 and super-oxide dismutase (Bahmani et al. 2010). For these reasons, LCN-2 does seem to have a modulatory effect of the inflammatory response.

Renal & hepatic injury

The LCN-2 gene was first discovered in mice, which were infected with a simian-virus in the kidneys (Hraba-Renevey et al. 1989). Furthermore during the acute-phase response, specifically LCN-2 was found to be upregulated in the liver (Liu and Nilsen-Hamilton 1995). This was a relatively new insight compared to an earlier wide molecular characterization of LCN-2 tissue expression in humans. This showed high LCN-2 expression in bone marrow, uterus, prostate, salivary glands, stomach, appendix, colon, trachea and lungs (Cowland and Borregaard 1997). A study that used a transcriptome-wide strategy to identify genes upregulated after renal ischemia, identified LCN-2, which was previously unrecognized in renal injury (Mishra et al. 2003). This study showed a very rapid response, with LCN-2 appearing in urine (first urine output after intervention) 2 hours after ischemic injury (Mishra et al. 2003). Renal re-epithelization is crucial for renal repair. LCN-2 enhances the epithelial phenotype by acting as an iron-transporter during nephrogenesis (Mishra et al. 2003; Yang et al. 2002). This is also supported by research done on cultured epithelial cells, in which is shown that LCN-2 regulates epithelial morphogenesis (Gwira et al. 2005). A follow up by Mishra et al also show a striking increase in LCN-2 concentration in both the serum and urine after acute renal failure (Mishra et al. 2005). And recently Shum et al also correlated plasma LCN-2 levels with the severity of acute kidney injury (Shum et al. 2015). Kjeldsen et al find that synthesis of LCN-2 is induced in epithelial cells during inflammation (Kjeldsen, Cowland, and Borregaard 2000). And LCN-2 expression also seems to be induced in injured epithelia in sputum of asthma patients and in bronchial fluid from emphysematous lungs (Xu and Venge 2000). Bengatta et al provide information that MMP-9 protects mice from apoptosis in acute kidney injury (Bengatta et al. 2009). LCN-2 is able to bind to MMP-9, protecting it from degradation (Ni et al. 2013). This LCN-2/MMP-9 complex has a high molecular weight, which makes it detectable in urine. These studies are all exploring the possibility of using LCN-2 as a biomarker for kidney disease, since it can be monitored using non-invasive techniques in a clinical setting. This provides great insights in the status of the injured kidneys (Devarajan 2008).

Besides the fact that LCN-2 is expressed in renal tubule cells, it is also expressed in immune hepatic cells and adipose tissue (Mori and Nakao 2007; Schmidt-Ott et al. 2007; Roth et al. 2013). The study by Roth et al shows that LCN-2 is upregulated in the serum of acute and chronic liver failure patients (Roth et al. 2013). A pro-inflammatory response, involving the release of chemokines and cytokines, is a hallmark of hepatic failure (Roth et al. 2013; Leifeld et al. 2003). Pro-inflammatory mediators like tumor necrosis (TNF)-α, interleukin (IL)-1β, IL-6 and IL-18 further contribute to a systemic inflammatory cascade, which can eventually cause further hepatic injury (Roth et al. 2013). LCN-2 expression can be upregulated by IL-1β and oxidative stress in epithelial cells and hepatocytes (Roudkenar et al. 2007; Jayaraman et al. 2005). Perhaps LCN-2 protects hepatic cells from oxidative stress by this immune hyper activation, due to its antioxidant properties (Bahmani et al. 2010).

Another mechanism could be that energy needed for tissue repair in the liver, which is provided by glucose (Singh et al. 1998), is initiated by an inflammatory response concerning LCN-2. Insulin growth factor 1 (IGF-1), which is produced by the liver under influence of growth hormone (GH) from the pituitary (Dunaiski and Belford 2002), stimulates glucose uptake into injured tissue (Rajpathak et al. 2009). LCN-2 plays a synergistic as well as an individual role in this response by its induction by IGF-1, acting mainly as an anti-bacterial agent in the process of wound healing (Sorensen et al. 2003). This insight would definitely broaden the perspective on the role of the inflammatory response on wound healing. A synergy with anti-bacterial peptides like LCN-2 greatly increases efficacy and decreases risk of further infection. In any case, inflammation seems to play a great role in the processes named above. The next section of this essay will further focus on the topic of inflammation and the role of LCN-2

Inflammation

The role of LCN-2 in the immune response is apparent, since it is rapidly upregulated in an extensive list of bacterial infections. However its exact role in the molecular processes underlying inflammation have yet to be elucidated. As already mentioned the pro-inflammatory cytokines TNF-α, IL-1β, IL-6 and IL-18 play an important role in the inflammatory cascade concerning hepatic injury. Firstly the global topic of inflammation needs to be further discussed. The reaction of the human body to harmful stimuli, like tissue damage or invasion of the host by harmful pathogens, lies for one part in an inflammatory response; inflammation. To limit ongoing infection and initiate tissue repair, various cell types (platelets, neutrophils, macrophages, fibroblasts, endothelial cells, nerve cells and lymphocytes) are recruited and interact to clear out any harmful pathogens (Nathan 2002). However, if inflammation is not properly phased it can lead to persistent tissue damage (Nathan 2002). Macrophages are considered to be the first responders in the inflammatory response. The TLR's on macrophages recognize pathogens, thereby generating an immune response (Takeuchi and Akira 2010). TNF-α is suggested to be one of the most important

mediators of inflammation, mainly released from macrophages (Gustafson 2010). This response underlies the acute phase reaction (Kmiec 2001). TNF-α can locally either promote cell death, through apoptosis, or stimulate a cell survival pathway through nuclear factor kappa B (NF-κB) (Rath and Aggarwal 1999; Gupta et al. 2005).

Other inflammatory cytokines like interleukins all have their specific function in immune regulation, either pro- or anti-inflammatory depending on its receptor and pathway (Akdis et al. 2011). IL-1β, IL-6 and IL-18 are all pro-inflammatory cytokines mainly released by activated macrophages (Akdis et al. 2011). LCN-2 can be upregulated by a number of these inflammatory mediators, including IL-1β (Cowland et al. 2003). However, upregulation of LCN-2 via IL-1β seems greatly dependent of a transcription factor upstream of NF-κB, namely IkappaB-zeta (IκB-ζ) (Cowland, Muta, and Borregaard 2006). IκB-ζ is essential for the induction of various inflammatory genes, represented by IL-6, and inhibits TNF-α expression (Matsuo et al. 2007). LCN-2 also seems to be regulated at a transcriptional level by IL-17, which is highly dependent on NF-κB, plays a role in the defense against bacteria and fungi, and seems to play a role in developing an adaptive immune response (Li and Chan 2011; Shen et al. 2005). This last statement is made clear by the fact that IL-17 can also be produced by T-cells, which are attracted by chemokines generated by macrophages (Godinez et al. 2008), this in turn amplifies LCN-2 expression. Figure 2 shows a suggested mechanism for this induction of LCN-2 expression. This figure also shows that after innate immune activation TNFα and IL-1β are released, which then leads to activation of the IL-1β and NF-κB pathway and subsequently LCN-2 induction (Li and Chan 2011).

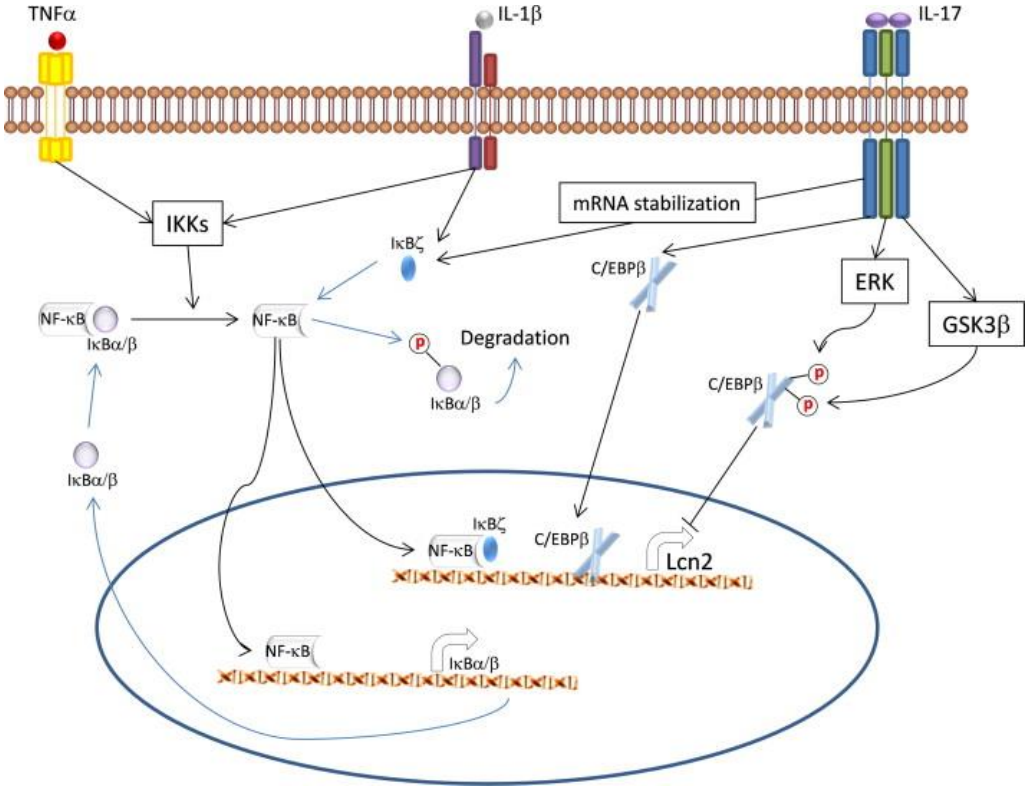


Figure 2. Receptor activation and negative regulation of Lipocalin-2. This model shows how TNFα, IL-1β, and IL-17 could acutely upregulate lipocalin-2 expression. As inflammation progresses, LCN-2 can be repressed as well. Source: Li and Chan 2011.

Figure 2 in fact proposes that the inflammatory stimuli, leading to the upregulated expression of LCN-2, can also repress LCN-2 as inflammation progresses (Li and Chan 2011). The figure shows that binding of IL-17 to its ligand can subsequently activate glycogen synthase kinase 3β (GSK-3β). TNF-α can lead to downregulation of GSK-3β, which can lead to apoptosis (Takada et al. 2004). In figure 3, GSK-3β is upregulated via IL-17, therefore thought of as a cell-survival mechanism. GSK-3β leads to phosphorylation of C/EBPβ (which is a transcription factor), in turn suppressing LCN-2 (Li and Chan 2011). In summary, there seems to be a complex interaction with LCN-2 and the inflammatory mediators named above.

An inflammatory response that is generated in the periphery can also coincide with the appearance of a similar inflammatory response in the brain (Riazi et al. 2008). Inflammation in the brain, neuroinflammation, is characterized by activated microglia (the resident macrophages of the brain), and increases in pro-inflammatory cytokines like TNF-α, IL-1β, IL-6 (Riazi et al. 2008; Dantzer et al. 2008). Excessive neuroinflammation can result in neurotoxicity and is also thought to accelerate the progression of numerous neurodegenerative diseases like Alzheimer's diseases (AD) and Parkinson's disease (PD) (Cunningham et al. 2005; Dilger and Johnson 2008). Aged individuals are particularly at risk for this phenomenon, since they are more infection-prone, and most neurodegenerative diseases are commonly driven by age (Dilger and Johnson 2008). There are several proposed mechanisms of how peripheral inflammation "migrates" to the brain. This so-called cross-talk between the immune system and the brain is believed to be primarily mediated by the blood-brain-barrier (BBB) (Dilger and Johnson 2008). The permeability of the BBB is highly selective to protect the brain from blood-borne compounds and to maintain a strict homeostasis for optimal brain functioning (de Vries et al. 1997). The BBB is known to be permeable to mainly water and lipid soluble substances and other molecules which is dependent of their physicochemical properties such as molecular weight, electrical charge and extent of ionization (de Vries et al. 1997). There are also specific active transport systems present in the BBB. One example is GLUT1, a selective stereospecific glucose carrier system (de Vries et al. 1997). The permeability of the BBB may be affected however under inflammatory stress, for example by the opening of tight junctions (de Vries et al. 1997). Studies have shown that administration of TNF-α, IL-1β and IL-6 can also lead to increased BBB permeability (de Vries et al. 1996). There are several brain regions that do not possess a BBB; the circumventricular organs. These regions lie in close proximity to the hypothalamus, and it is suggested that the lacking of a BBB in these regions is to maintain homeostatic functions like thermoregulation, neuroendocrine secretions, and behavior (Dilger and Johnson 2008). Cytokines, like IL-1β, can pass into the brain via these regions (Komaki, Arimura, and Koves 1992). Evidence even suggests that pro-inflammatory cytokines like TNF-α, IL-1β and IL-6 can pass over the BBB by an active carrier-mediated transport system (Banks and Kastin 1991; Banks, Kastin, and Broadwell 1995; Gutierrez, Banks, and Kastin 1993). However it remains undefined whether cytokines transported via this pathway are sufficient to mediate any behavioral responses. It is further theorized that endothelial cells that make up the BBB itself are able to produce specific immune-related molecules, like NO,

prostaglandins, IL-1 and IL-6, under the influence of a peripheral immune challenge (Dilger and Johnson 2008; Fabry et al. 1993). This would suggest an increase in pro-inflammatory cytokines in the brain, without any physical entry of blood-born molecules.

As opposed from blood-derived stimuli for "migration" of a peripheral immune response to the brain (and CNS), there can also be neuroimmune communication by the autonomic nervous system. Vagal afferents have been shown to possess IL-1 receptors on vagal paraganglia (Goehler et al. 1999). This would suggest rapid communication as opposed to a slower humoral response concerning cytokine signaling and overall neuroimmune communication.

A recent study by Louveau et al describes a unique new insight in the interaction between peripheral and brain (CNS) immunity (Louveau et al. 2015). Namely the fact that the CNS seems to possess a lymphatic system that governs the entrance and exit of immune cells (Louveau et al. 2015). Functional lymphatic vessels, lying within the dural sinuses (in the meningeal compartment), carry fluids and immune cells from the CSF, and are connected to cervical lymph nodes (Louveau et al. 2015). This discovery gains new insights into theories on immune system dysfunction and how this affects neuroinflammatory-induced neurodegeneration.

The interaction between the periphery and the brain, concerning the classical inflammation pathways, has been described in great extent. The same cannot be said considering LCN-2. Clearly LCN-2 is not a classical marker of inflammation yet. However recent work by Ferreira et al describe the broad spectrum of functions of LCN-2 and the interaction between the periphery and the brain (Ferreira et al. 2015). The involvement of LCN-2 in innate and adaptive immunity, and its IL-1β and NF-κB dependent upregulation (Bu et al. 2006) confirms its role in immunomodulation and inflammation. What this role is specifically remains to be discovered. The next section will focus on these classical inflammatory pathways in the brain and the interaction with LCN-2.

Neuroinflammation

As discussed in the previous section, the BBB forms a firm barrier for any blood-born molecules to pass over from the periphery to the brain. LCN-2 seems to play a role in protecting the brain from infection in case pathogens do enter the brain. As mentioned before, in the brain LCN-2 is produced in the CP (Marques et al. 2008), but also in astrocytes (Mesquita et al. 2014). The CP is responsible for most of the production of the CSF, showing peak production during nighttime (around 02:00 am) and minimum production at night (around 06.00 pm) (Skipor and Thiery 2008). The CP also mediates secretion of various proteins and processes that are necessary for the clearance of substances from the CSF and

blood, including amyloid-β (Gonzalez-Marrero et al. 2015). Dysfunction of this clearance is one of the most prominent pathological constituents for the development and progression of AD (Gonzalez-Marrero et al. 2015). Amyloid-β concentrations in the CSF have been widely implicated as a biomarker of later clinical stages of AD. Namely, CSF amyloid-β seems to be significantly reduced in patients with AD (Prvulovic and Hampel 2011). Patients with MCI, who later develop AD, also show lower CSF amyloid-β concentrations compared to patients that do not develop AD (Diniz, Pinto Junior, and Forlenza 2008). It has been suggested that an impaired clearance of amyloid-β, reflected as lower levels in the CSF, lies as the basis of neuronal toxicity and the progression of AD. This is because modification of CSF turnover might favor transformation of amyloid-β and tauopathy (Serot, Bene, and Faure 2003). Naudé et al describe a similar interaction with CSF LCN-2, being significantly lowered in individuals with MCI and AD (Naude et al. 2012). Mesquita et al describe LCN-2 to be highly upregulated by CP epithelial cells and astrocytes after incubation with amyloid-β (Mesquita et al. 2014). In LCN-2 (+/+) astrocytes from mice, induction with amyloid-β showed reduced survival of astrocytes that produce LCN-2 by reduced dehydrogenase activity (Mesquita et al. 2014). This effect was avoided in LCN (-/-) astrocytes from mice, and induced again by addition of exogenous LCN-2 onto these cells (Mesquita et al. 2014). Underlying this effect might lie in the mediation of the pro-apoptotic protein *Bim*.

Taken together, the findings by Mesquita et al could relate to the findings by Naudé et al. If higher amyloid-β leads to higher levels of LCN-2, lower levels of amyloid-β could in turn result in the decrease of CSF LCN-2. This decrease of CSF LCN-2 could be the result of increased expression of LCN-2 in the entorhinal cortex and the hippocampus (Naude et al. 2012). These regions are crucial in the pathology of AD, and indeed show an accumulation of amyloid-β in AD brains (Small et al. 2006). Also TNF-α expression in the same brain regions is increased, possibly upregulating LCN-2 in the early clinical stages of AD.

Possibly mediating the activity of LCN-2, and the clearance of amyloid-β in the CP is megalin. Megalin is a member of the low-density lipoprotein receptor family and has also been demonstrated to act as a cellular receptor for LCN-2 (Hvidberg et al. 2005). This study also demonstrates that there does not seem to be a difference in affinity for megalin between iron-bound and iron free LCN-2 (Hvidberg et al. 2005). The affinity of megalin for LCN-2 seems to be determinant on a number of unique positive charges identified on LCN-2's crystalline structure (Goetz et al. 2002). During inflammation, LCN-2 is highly expressed in the instestinal epithelia, during which megalin is also highly expressed (Nielsen et al. 1996; Moestrup and Verroust 2001). In the brain, megalin is also expressed in brain capillaries and in the CP (Dietrich et al. 2014; Chun et al. 1999). In the CP, megalin serves as a clearance transporter for amyloid-β (Zlokovic et al. 1996; Carro et al. 2005). Spuch et al demonstrate that soluble megalin binding to amyloid-β is decreased in the CSF of AD patients (Spuch et al. 2015). This response seems highly similar to the decreased levels of LCN-2 in the CSF in AD patients that was described earlier. Megalin also contains binding sites for apolipoprotein-E, leptin, insulin and IGF-1 (Orlando et al. 1997; Orlando et al. 1998; Dietrich et al. 2008; Carro

et al. 2005). Leptin is considered to be a neuroprotective hormone because of its involvement in memory processing in the hippocampus (Farr, Banks, and Morley 2006). Insulin and IGF-1 can also mediate neuroprotection, since deficiency can lead to the appearance of hyperphosphorylated tau and loss of cognition (Schubert et al. 2003). IGF-1 can also protect against amyloid-β toxicity and can even promote hippocampal neurogenesis (Niikura et al. 2001; Trejo, Carro, and Torres-Aleman 2001). In any case, endocrine-brain communication, characterized by transport over the blood-CSF barrier, seems highly dependent on megalin (Dietrich et al. 2008). With these new insights, LCN-2 expression in the CSF can also be related to megalin acitivity. Lower LCN-2 expression in the CSF now not only serves as an indicator for impaired clearance of amyloid-β, but might also indicate impaired endocrine-brain communication.

Circulatory levels of LCN-2 seem to be upregulated as a form of pro-inflammation. Further studies by Naudé et al describe circulatory LCN-2 concentrations to be significantly associated with increasing age, as well as depression (Naude et al. 2013). A further follow-up by Naudé et al describe the comorbidity of LCN-2 in heart failure patients and cognitive decline (Naude et al. 2014), which reflects on the interaction between periphery and the brain. Choi et al indicate increased plasma LCN-2 levels in patients with mild cognitive impairment (MCI), preceding clinical AD (Choi, Lee, and Suk 2011). These findings are crucial for establishing LCN-2 as a biomarker for the neurodegenerative processes underlying AD.

The clinical implementations considering circulatory and CSF LCN-2 could be very significant. CSF samples are relatively easy to obtain and analyze in both human and animal models. However, CSF LCN-2 concentrations do seem to be 10-fold lower than circulatory LCN-2 (Naude et al. 2012). Nonetheless, the CSF does seem to provide more diagnostic value for neurodegenerative diseases like AD than circulatory samples do, since it can be related to a direct neuropathological effect in the brain underlying AD.

Mesquita et al also describe the production of LCN-2 in astrocytes, the most abundant glial cell type in the brain, and its role in astrocytosis (Mesquita et al. 2014). Astrocytosis is the changing of astrocyte morphology and molecular functioning in response to CNS injury, which can increase the pathogenesis of AD and other neurodegenerative diseases (Jain, Wadhwa, and Jadhav 2015). LCN-2 is believed to be an autocrine mediator of reactive astrocytosis, since in cultured astrocytes its expression and secretion is increased after inflammatory stimulation (Lee et al. 2009). Treatment with LCN-2 increased the sensitivity of astrocytes to cytotoxic stimuli involving iron and the *Bim* protein (Lee et al. 2009). Recently it has been shown that LCN-2 can also promote M1 macrophage polarization, which is the activated state of macrophages (Cheng et al. 2015). A similar study, however on the morphological changes of microglia, show that LCN-2 is involved in the deramification (thus activation) of microglia. LCN-2 seems to have a feedback loop to microglia, making them more sensitive to apoptotic signals (Lee et al. 2007). This proposed dual role of LCN is illustrated in figure 3.

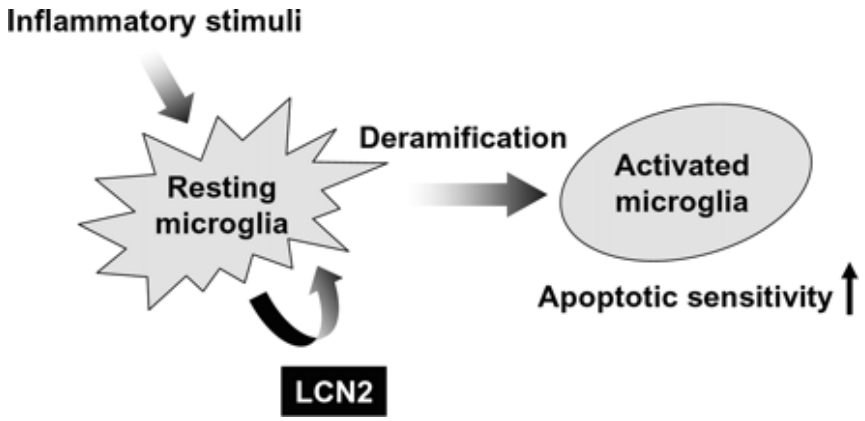


Figure 3. LCN-2 seems to feed back to the microglia, causing deramification to the activated state of the microglia. This also increases the apoptotic sensitivity (Lee et al 2007).

TNF-α can also be considered as a key regulator of LCN-2 in neurodegenerative diseases like AD, since it may serve as a stimulating factor of LCN-2 in astrocytes (Lee et al. 2009; Naude et al. 2012). Evidence for this was found in a study on patients with Crohn's disease. Treatment with infliximab, a monoclonal antibody preventing TNF-α from binding to its receptors, showed significantly lower levels of peripheral LCN-2 (Bolignano et al. 2010). What was already previously known is that TNF-α is upregulated in the brain of patients with AD, particularly in the vicinity of crucial brain regions affected in AD (Naude et al. 2012; Rao, Rapoport, and Kim 2011; Zhao et al. 2003). TNF-α mediates its signals via two receptors: TNF receptor 1 (TNFR1) and TNF receptor 2 (TNFR2) (Zettlitz et al. 2010). One receptor, TNFR1 is predominantly associated with pro-inflammatory effects, actuated to exert axonal and neuronal damage upon binding of TNF-α (Fischer et al. 2011). While the other, TNFR2, is particularly associated with neuroprotective effects like cell survival and neuronal protection by activation of the NF-κB pathway (Marchetti et al. 2004; Wajant, Pfizenmaier, and Scheurich 2003; Fontaine et al. 2002). In the AD brain, Cheng et al found increased expression of TNFR1 and decreased expression of TNFR2 (Cheng et al. 2010). This confirms the importance of these two different TNF signaling pathways in neurodegenerative diseases like AD and (neuro)inflammation as an underlying mechanism. LCN-2 production can be triggered in neurons, astrocytes and microglia by TNF-α, solely via the TNFR1 pathway (Naude et al. 2012). These three cell type all express TNFR1 and TNFR2 (Viel et al. 2001; Fernandes et al. 2011; Veroni et al. 2010), however the cells in this study were treated with receptor-specific agonistic antibodies to determine the specific signaling pathway (Naude et al. 2012). TNFR2 seems to be silenced by LCN-2, which shifts the neuroprotective effects downstream of this receptor to a more pro-apoptotic response (Naude et al. 2012). This TNF-α induced upregulation of LCN-2, was confirmed to be dependent of NF-κB signaling, which is illustrated in figure 4.

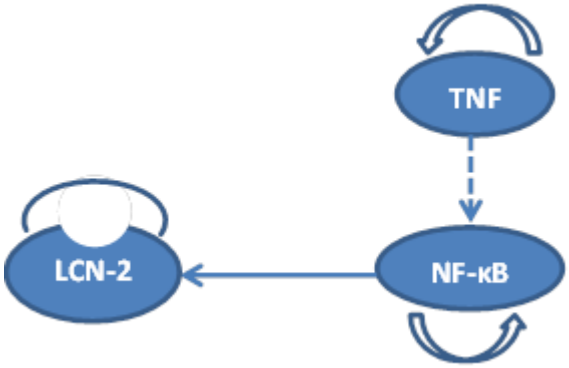


Figure 4. TNF-α-induced LCN-2 upregulation is dependent of NF-κB signaling. Adapted from: Naude et al 2012.

The release of TNF-α from microglia and other neuroimmune cells evidently contributes to chronic inflammation in the brain, which is a key element in the pathogenesis of AD (Eikelenboom et al. 2010). AD is driven by advanced age. Microglia are also affected by age, showing specific changes like enhanced sensitivity to pro-inflammatory stimuli (Raj et al. 2014). This phenomenon is called microglial "priming". A peripheral LPS challenge can lead to a hyperactive microglial response, which seems to be exaggerated in the aged brain. The reason for this is a higher induction of the pro-inflammatory cytokines TNF-α, IL-1β, IL-6 and anti-inflammatory cytokines IL-10 and TGFβ1 (Henry et al. 2009; Sierra et al. 2007). To our knowledge, no research is done on this phenomenon in association with LCN-2, either peripheral or in the brain. Possibly, microglial priming can have an upregulating effect on LCN-2 as well, further contributing to the neuroinflammatory-induced neurodegeneration driven by age.

Overall, these findings suggest the implications of LCN-2 in the pro-(neuro)inflammatory processes are prominent. Hopefully, LCN-2 can answer some unsolved questions or might even be considered as a viable biomarker for early detecting of AD before any clinical symptoms.

Metabolic consequences

Diabetes, obesity and other metabolic disorders can greatly contribute to inflammation, as well as homeostatic and other regulatory functions in the body. Evidence for this is based on loss of function of adipose tissue, which can lead to abnormal production of adipokines and cytokines from lipid-filled adipocytes, preadipocytes, fibroblasts, endothelial cells and immune cells like macrophages and T cells, which constitute adipose tissue (Guo et al. 2013). Furthermore, in mice fed a high fat diet (HFD), LCN-2 might have a role in adipose tissue remodeling and in regulating adipogenesis (Guo et al. 2013). TNF-α can also be produced by adipose tissue, even by adipocytes, in obese and hyperinsulemic mice and humans (Moller

2000). The expression of LCN-2 seems altered in patients with obesity, insulin resistance or metabolic syndrome in general. Evidence for this is based on observations of increased expression of LCN-2 in adipose tissue of human obese subjects (Catalan et al. 2009), as well as in mice (Wang et al. 2007). Furthermore LCN-2 is shown to promote insulin resistance (Yan et al. 2007), further strengthened by the fact that LCN-2 deficiency improves age- and obesity-induced insulin resistance (Law et al. 2010). Administration of an insulin-sensitizing drug, rosiglitazone, which is used as an antidiabetic drug in patients with type 2 diabetes mellitus, also shows significant decreases in LCN-2 concentrations (Wang et al. 2007).

The fact that metabolism and inflammation can also contribute to the formation of atherogenic plaques, implicates LCN-2 in this process as well. Indeed increased serum LCN-2 levels are positively correlated with the development of coronary artery disease in patients with atherosclerosis (Ni et al. 2013). As mentioned in the introduction, LCN-2 is covalently bound to neutrophils in humans, which associates LCN-2 with the gelatinase MMP-9, also secreted by neutrophils (Kjeldsen et al. 1993). MMP-9 is involved in the degradation and remodeling of extracellular matrix, which is a pathogenic hallmark of vascular remodeling (Galis and Khatri 2002). LCN-2 can form complexes with MMP-9, a so called LCN-2/MMP-9 complex, which can destabilize artery plaques (Galis and Khatri 2002; Ni et al. 2013). MMP-9 on its own can promote infiltration of white blood cells and cytokines into the intima by degradation of the vascular basement membrane, which increases endothelial permeability (Ni et al. 2013). LCN-2 serves as a stabilizing agent for MMP-9, protecting it from degradation (Yan et al. 2001). As mentioned before, the LCN-2/MMP-9 complex has a high molecular weight, which makes it readily detectable in urine. This might possibly serve as a diagnostic marker for coronary artery disease and associated pathogenic indicators. In most tissues however, MMP-9 expression is very low (Labrie and St-Pierre 2013). Particularly Il-1β and TNF-α, are potent inducers of MMP-9 gene activation (Labrie and St-Pierre 2013). Essential for MMP-9 gene-expression, are NF-κB binding sites, further implicating MMP-9 and LCN-2 in the characteristic inflammatory responses underlying metabolic and brain diseases (Esteve et al 2002) .

Overall, metabolic alterations can lead to an increased inflammatory state characterized by an increase in inflammatory cytokines like TNF-α, IL-1β and IL-6. Alterations in hormone concentrations like leptin, adiponectin and ghrelin are also considered hallmarks of inflammation (Saucedo et al. 2011). In particular, reduced levels of ghrelin is considered to be a hallmark of inflammation derived from impaired metabolism (Saucedo et al. 2011). Ghrelin is an important regulator of food intake; being up-regulated during fasting, and directly downregulated after ingestion of a meal (Schwartz et al. 2000; Ott et al. 2012). A recent study by De la Chesnaye et al found a reduction in ghrelin, similar to one in plasmatic LCN-2 levels in patients suffering from long-term type 2 diabetes (De la Chesnaye et al. 2015). A reduction in LCN-2 levels however would suggest a lower state of inflammation, since most literature associate LCN-2 with pro-inflammation. The outcome of the study by De la Chesnaye et al therefore seems counter intuitive. The same is true for the outcome of

a study by Guo et al, which find that LCN-2 deficient mice gain more body weight when fed a HFD (Guo et al. 2010). Furthermore they find that LCN-2 deficient mice seem to have a dysregulated thermogenic response to thermal stress (Guo et al. 2010), which is normally regulated by brown adipose tissue (BAT). LCN-2 deficient mice, fed a HFD, also demonstrate increased sensitivity for insulin resistance, hyperglycemia, hyperinsulemia and hypoadiponectinemia (Guo et al. 2010). The specific effects of LCN-2 on regulating metabolism after food intake seem unclear. Multiple studies suggest a minor role for LCN-2 in mediating age- or obesity-related metabolic consequences (Jun, Siddall, and Rosen 2011; Friedlander et al. 2014). Perhaps there is an overall regulatory/homeostatic role for LCN-2, since deficiency seems to lead to disorganization of metabolism.

Discussion

The iron-binding properties, thereby bacteriostatic functions, of LCN-2 greatly involve this protein in the innate immune response, as described by Flo *et al* and Berger *et al* (Flo et al. 2004; Berger et al. 2006). The innate immune response has been a crucial determinant for the evolutionary advantage of human beings and other mammals alike. Should we not possess an innate immune response, life span would be much shorter. Being part of the innate immune response, LCN-2's response is immediate. Its siderophoric properties are not specific to a particular pathogen, which is advantageous. This non-specific action is made clear by the necessity of iron by all bacteria and viruses, and LCN-2's ability to limit this iron-availability. Apoptosis is another process which is crucial for mammalian development and healthy life. Apoptosis is a highly regulated and programmed cell death to ensure healthy cell turnover. When iron binds to LCN-2, this bound protein can get transported intracellularly, where it is hypothesized to inhibit apoptosis. Dysregulation of apoptosis can lead to different forms of cancer. One study showed that reduced levels of LCN-2 are in accordance with increased cancerous tumor progression (Candido et al. 2014). This would suggest that LCN-2 plays a protective role in metastatic development. On the other side, as mentioned before, MK886 is a potent inducer of LCN-2, as well as a strong pro-apoptotic agent (Tong, Wu, and Kehrer 2003; Correnti et al. 2012). Thus the role of LCN-2, whether involved in the pro- or anti- apoptotic response is still unclear. Perhaps there is no specific role for LCN-2 in apoptosis, but is merely coincidental because of its great versatility or its presence as a "rest-product" from its role in the innate immune response.

Inflammation is an interesting topic considering LCN-2, since it connects the periphery to the brain. Numerous mechanisms seem to be involved in the peripheral inflammatory response considering LCN-2. In the first section of this essay, this involvement first became clear from LCN-2's effects in renal and hepatic injury. During the acute-phase response, LCN-2 was specifically and acutely upregulated in the liver. In renal injury LCN-2 was rapidly upregulated as well, which could be readily detected in urine output, implicating its role as a possible biomarker. This also led to the discovery that LCN-2 regulates epithelial

morphogenesis in the kidneys. The release of LCN-2 by induction of classical inflammatory markers like TNF-α, Il-1β and IL-6 in epithelial cells and hepatocytes during hepatic failure further implicates LCN-2 in inflammation. The same is true for the synergistic response of LCN-2 and IGF-1 during tissue repair in the liver. "Migration" of inflammation from the periphery to the brain is known to occur, however via multiple suggestive routes. An overview of LCN-2's involvement in (neuro)inflammation, originating at its bacteriostatic properties, can be seen in figure 5. This image also shows the production of LCN-2 in the brain itself. Dong *et al* showed this in their study on intracerebral hemorrhage (Dong et al. 2013). However, as mentioned before, Mesquita *et al* and Marques *et al* also found LCN-2 to be produced in the CP (Mesquita et al. 2014; Marques et al. 2008). The CP produced the majority of CSF, and is also an important mediator in clearance of amyloid-β from the CSF. Impaired clearance of amyloid-β is considered to be a pathological hallmark of amyloid-β accumulation in crucial brain regions affected in AD. This sparks the idea of using CSF for diagnostic purposes. The fact that a similar decrease in CSF LCN-2 is seen in patients preceding MCI and AD is a clinically relevant finding. This might serve as a basis for classifying LCN-2 as a biomarker for AD. Higher circulatory and brain levels of LCN-2 might give an indication about the inflammatory status, while lower CSF LCN-2 levels could indicate AD-like pathologies. For these reasons it would be beneficial to determine a precise sampling regime for (brain) tissue, plasma and CSF to determine LCN-2 expression. The suggested circadian rhythmicity of LCN-2 in humans, which is not yet confirmed in rodent models, could yield greater accuracy within this sampling regime.

Figure 5 also shows an interesting involvement of megalin in the brain. Megalin was mentioned earlier as a possible mediator for the interaction between the CSF, brain and metabolism, considering LCN-2 and neuroinflammation. This is suggested because megalin not only serves as cellular receptor for LCN-2, but also contains binding sites for apolipoprotein-E, leptin, insulin and IGF-1 (Orlando et al. 1997; Orlando et al. 1998; Dietrich et al. 2008; Carro et al. 2005), and seems to be a functional receptor for MMP-9 (Van den Steen et al. 2006; Ghosh et al. 2015). This is interesting because diabetes and obesity are commonly associated with leptin and insulin resistance. These and other metabolic alterations can lead to an increased inflammatory state. LCN-2 only seems to play minor roles in these homeostatically regulated processes. It does seem however that LCN-2 is involved in the processes when the systems governing homeostasis are disorganized, perhaps by (neuro)inflammation. Physiological states like diabetes and obesity might present a higher circulatory availability of neuroprotective hormones like insulin and leptin. However, if the function of the CP is hampered by (neuro)inflammation, these physiological states might not be protected towards a predisposition of dementia as suggested by Qizilbash *et al* (Qizilbash et al. 2015). Furthermore, dementia and neurodegenerative diseases like AD, are commonly driven by age. Megalin actitivity is also known to be affected by ageing (Carro et al. 2005; Dietrich et al. 2008). MMP-9 fits into this picture by means of Leptin, which enhances the expression of MMP-9 in vitro (Park et al. 2001). Furthermore, in a study on epilepsy, MMP-9 seems to have a dual role (Stawarski, Stefaniuk, and Wlodarczyk

2014). Firstly, MMP-9 seems to have a modulating effect on synaptic plasticity; mediating structural plasticity (Stawarski, Stefaniuk, and Wlodarczyk 2014). However on the other hand MMP-9 seems to play a role in the pathogenesis of epileptic seizures (Michaluk and Kaczmarek 2007). The latter might involve excessive activity of MMP-9 leading to glutamate excitotoxicity, which is detrimental to brain tissue (Michaluk and Kaczmarek 2007). The interaction between endocrine hormones (like leptin and insulin), MMP-9 and LCN-2 all seem to be connected to megalin, and is also illustrated in figure 5. If LCN-2 indeed plays a role in metabolism, an important link to (neuro)inflammation is further strengthened by the fact that metabolism and (neuro)inflammation have been commonly linked as reviewed by van Dijk et al (van Dijk et al. 2015).

Conclusion

Answering the main question of this essay still remains difficult; what is the primary biological/evolutionary function of LCN-2? From the information obtained in this essay, it is possible to make a literature-supported approximation of its most prominent functions and possible side-effects. LCN-2 clearly possesses a wide range of biological functions. First described as an acute-phase protein, later also as bacteriostatic agent, mediator of apoptosis, regulator of epithelial morphogenesis, mediator of (neuro)inflammation, adipokine and even as a possible biomarker for neurodegenerative diseases like AD.

LCN-2's primary functions seem (immuno)protective in the first case. Its role in the innate immune response therefore seems very prominent. Its capability of efficiently binding iron grants LCN-2 these immunomodulatory properties. This also grants LCN-2 its great versatility in signaling and transport capabilities, which probably results in the range of other processes in which LCN-2 is also involved. These connections, like its role in (neuro)inflammation and neurodegeneration, are not as clear and confirmed as LCN-2's immunomodulatory effects. Precise investigation is therefore wanted to fully understand LCN-2's complex interactions and its diagnostic properties.

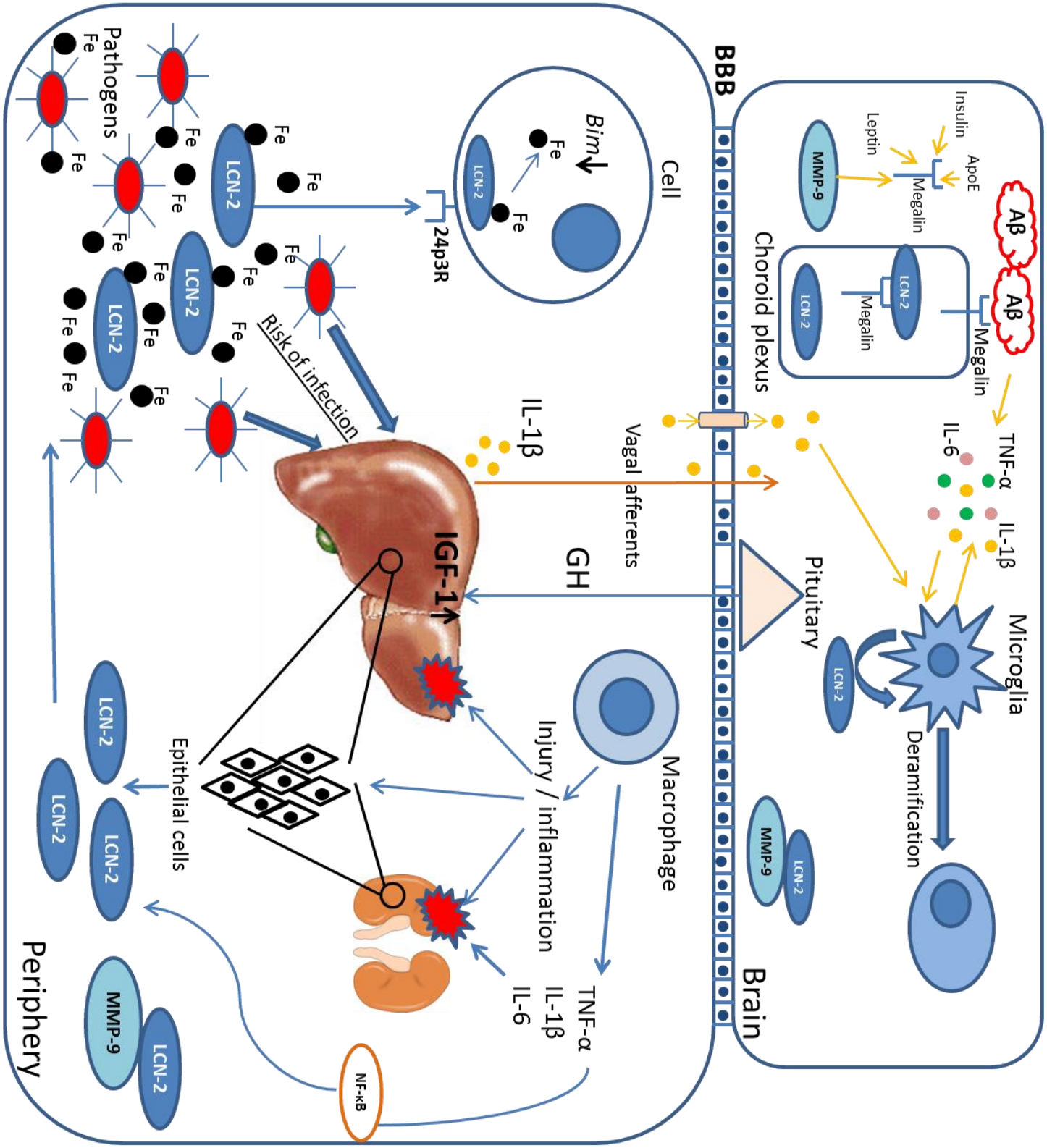


Figure 5. Overview of LCN-2's response to an immune challenge in the periphery and in the brain. When pathogens enter the body, LCN-2 responds by chelating Iron. Iron is then transported intracellularly which increases iron concentration. This results in downregulation of the pro-apoptotic protein *Bim*, thus inhibiting apoptosis. Injury, inflammation and risk of infection on the liver and kidneys can also trigger an immune response in its respective epithelia. Characterized by an upregulation of pro-inflammatory cytokines, this response is mediated via NF-κB. Under influence of pituitary GH, the liver is able to produce IGF-1. IGF-1 and LCN-2 work synergistically to repair damaged tissue. IL-1β produced from the liver can transport via vagal afferents, via "gaps" or via active transporters, through the BBB to the brain. This upregulation of IL-1β, together with LCN-2, can also result in the deramification (activation) of microglia, and release of more pro-inflammatory cytokines like IL-6 and TNF-α. In the brain, LCN-2 is produced in the choroid plexus. LCN-2 can be transported over the blood-CSF barrier by megalin, an important receptor mediating the clearance of Aβ through the choroid plexus. LCN-2 is also able to form a complex with MMP-9, which is also bound by megalin, stabilizing its degradation.

References

Akdis, M., S. Burgler, R. Crameri, T. Eiwegger, H. Fujita, E. Gomez, S. Klunker, N. Meyer, L. O'Mahony, O. Palomares, C. Rhyner, N. Ouaked, A. Schaffartzik, W. Van De Veen, S. Zeller, M. Zimmermann, and C. A. Akdis. 2011. 'Interleukins, from 1 to 37, and interferon-gamma: receptors, functions, and roles in diseases', *J Allergy Clin Immunol*, 127: 701-21 e1-70.

Axelsson, L., M. Bergenfeldt, and K. Ohlsson. 1995. 'Studies of the release and turnover of a human neutrophil lipocalin', *Scand J Clin Lab Invest*, 55: 577-88.

Bahmani, P., R. Halabian, M. Rouhbakhsh, A. M. Roushandeh, N. Masroori, M. Ebrahimi, A. Samadikuchaksaraei, M. A. Shokrgozar, and M. H. Roudkenar. 2010. 'Neutrophil gelatinase-associated lipocalin induces the expression of heme oxygenase-1 and superoxide dismutase 1, 2', *Cell Stress Chaperones*, 15: 395-403.

Banks, W. A., and A. J. Kastin. 1991. 'Blood to brain transport of interleukin links the immune and central nervous systems', *Life Sci*, 48: PL117-21.

Banks, W. A., A. J. Kastin, and R. D. Broadwell. 1995. 'Passage of cytokines across the blood-brain barrier', *Neuroimmunomodulation*, 2: 241-8.

Bellet, M. M., E. Deriu, J. Z. Liu, B. Grimaldi, C. Blaschitz, M. Zeller, R. A. Edwards, S. Sahar, S. Dandekar, P. Baldi, M. D. George, M. Raffatellu, and P. Sassone-Corsi. 2013. 'Circadian clock regulates the host response to Salmonella', *Proc Natl Acad Sci U S A*, 110: 9897-902.

Bengatta, S., C. Arnould, E. Letavernier, M. Monge, H. M. de Preneuf, Z. Werb, P. Ronco, and B. Lelongt. 2009. 'MMP9 and SCF protect from apoptosis in acute kidney injury', *J Am Soc Nephrol*, 20: 787-97.

Berger, T., A. Togawa, G. S. Duncan, A. J. Elia, A. You-Ten, A. Wakeham, H. E. Fong, C. C. Cheung, and T. W. Mak. 2006. 'Lipocalin 2-deficient mice exhibit increased sensitivity to Escherichia coli infection but not to ischemia-reperfusion injury', *Proc Natl Acad Sci U S A*, 103: 1834-9.

Bolignano, D., A. Della Torre, A. Lacquaniti, G. Costantino, W. Fries, and M. Buemi. 2010. 'Neutrophil gelatinase-associated lipocalin levels in patients with crohn disease undergoing treatment with infliximab', *J Investig Med*, 58: 569-71.

Bu, D. X., A. L. Hemdahl, A. Gabrielsen, J. Fuxe, C. Zhu, P. Eriksson, and Z. Q. Yan. 2006. 'Induction of neutrophil gelatinase-associated lipocalin in vascular injury via activation of nuclear factor-kappaB', *Am J Pathol*, 169: 2245-53.

Candido, S., R. Maestro, J. Polesel, A. Catania, F. Maira, S. S. Signorelli, J. A. McCubrey, and M. Libra. 2014. 'Roles of neutrophil gelatinase-associated lipocalin (NGAL) in human cancer', *Oncotarget*, 5: 1576-94.

Carro, E., C. Spuch, J. L. Trejo, D. Antequera, and I. Torres-Aleman. 2005. 'Choroid plexus megalin is involved in neuroprotection by serum insulin-like growth factor I', *J Neurosci*, 25: 10884-93.

Catalan, V., J. Gomez-Ambrosi, A. Rodriguez, B. Ramirez, C. Silva, F. Rotellar, M. J. Gil, J. A. Cienfuegos, J. Salvador, and G. Fruhbeck. 2009. 'Increased adipose tissue expression of lipocalin-2 in obesity is related to inflammation and matrix metalloproteinase-2 and metalloproteinase-9 activities in humans', *J Mol Med (Berl)*, 87: 803-13.

Cheng, L., H. Xing, X. Mao, L. Li, X. Li, and Q. Li. 2015. 'Lipocalin-2 promotes m1 macrophages polarization in a mouse cardiac ischaemia-reperfusion injury model', *Scand J Immunol*, 81: 31-8.

Cheng, X., L. Yang, P. He, R. Li, and Y. Shen. 2010. 'Differential activation of tumor necrosis factor receptors distinguishes between brains from Alzheimer's disease and non-demented patients', *J Alzheimers Dis*, 19: 621-30.

Choi, J., H. W. Lee, and K. Suk. 2011. 'Increased plasma levels of lipocalin 2 in mild cognitive impairment', *J Neurol Sci*, 305: 28-33.

Chun, J. T., L. Wang, G. M. Pasinetti, C. E. Finch, and B. V. Zlokovic. 1999. 'Glycoprotein 330/megalin (LRP-2) has low prevalence as mRNA and protein in brain microvessels and choroid plexus', *Exp Neurol*, 157: 194-201.

Correnti, C., V. Richardson, A. K. Sia, A. D. Bandaranayake, M. Ruiz, Y. Suryo Rahmanto, Z. Kovacevic, M. C. Clifton, M. A. Holmes, B. K. Kaiser, J. Barasch, K. N. Raymond, D. R. Richardson, and R. K. Strong. 2012. 'Siderocalin/Lcn2/NGAL/24p3 does not drive apoptosis through gentisic acid mediated iron withdrawal in hematopoietic cell lines', *PLoS One*, 7: e43696.

Cowland, J. B., and N. Borregaard. 1997. 'Molecular characterization and pattern of tissue expression of the gene for neutrophil gelatinase-associated lipocalin from humans', *Genomics*, 45: 17-23.

Cowland, J. B., T. Muta, and N. Borregaard. 2006. 'IL-1beta-specific up-regulation of neutrophil gelatinase-associated lipocalin is controlled by IkappaB-zeta', *J Immunol*, 176: 5559-66.

Cowland, J. B., O. E. Sorensen, M. Sehested, and N. Borregaard. 2003. 'Neutrophil gelatinase-associated lipocalin is up-regulated in human epithelial cells by IL-1 beta, but not by TNF-alpha', *J Immunol*, 171: 6630-9.

Crichton, R. R., D. T. Dexter, and R. J. Ward. 2011. 'Brain iron metabolism and its perturbation in neurological diseases', *J Neural Transm*, 118: 301-14.

Cunningham, C., D. C. Wilcockson, S. Campion, K. Lunnon, and V. H. Perry. 2005. 'Central and systemic endotoxin challenges exacerbate the local inflammatory response and increase neuronal death during chronic neurodegeneration', *J Neurosci*, 25: 9275-84.

Dantzer, R., J. C. O'Connor, G. G. Freund, R. W. Johnson, and K. W. Kelley. 2008. 'From inflammation to sickness and depression: when the immune system subjugates the brain', *Nat Rev Neurosci*, 9: 46-56.

De la Chesnaye, E., L. Manuel-Apolinar, A. Zarate, L. Damasio, N. Espino, M. C. Revilla-Monsalve, and S. Islas-Andrade. 2015. 'Lipocalin-2 plasmatic levels are reduced in patients with long-term type 2 diabetes mellitus', *Int J Clin Exp Med*, 8: 2853-9.

de Vries, H. E., M. C. Blom-Roosemalen, M. van Oosten, A. G. de Boer, T. J. van Berkel, D. D. Breimer, and J. Kuiper. 1996. 'The influence of cytokines on the integrity of the blood-brain barrier in vitro', *J Neuroimmunol*, 64: 37-43.

de Vries, H. E., J. Kuiper, A. G. de Boer, T. J. Van Berkel, and D. D. Breimer. 1997. 'The blood-brain barrier in neuroinflammatory diseases', *Pharmacol Rev*, 49: 143-55.

Devarajan, P. 2008. 'Neutrophil gelatinase-associated lipocalin (NGAL): a new marker of kidney disease', *Scand J Clin Lab Invest Suppl*, 241: 89-94.

Devireddy, L. R., C. Gazin, X. Zhu, and M. R. Green. 2005. 'A cell-surface receptor for lipocalin 24p3 selectively mediates apoptosis and iron uptake', *Cell*, 123: 1293-305.

Devireddy, L. R., J. G. Teodoro, F. A. Richard, and M. R. Green. 2001. 'Induction of apoptosis by a secreted lipocalin that is transcriptionally regulated by IL-3 deprivation', *Science*, 293: 829-34.

Dietrich, M., D. Antequera, C. Pascual, N. Castro, M. Bolos, and E. Carro. 2014. 'Alzheimer's disease-like impaired cognition in endothelial-specific megalin-null mice', *J Alzheimers Dis*, 39: 711-7.

Dietrich, M. O., C. Spuch, D. Antequera, I. Rodal, J. G. de Yebenes, J. A. Molina, F. Bermejo, and E. Carro. 2008. 'Megalin mediates the transport of leptin across the blood-CSF barrier', *Neurobiol Aging*, 29: 902-12.

Dilger, R. N., and R. W. Johnson. 2008. 'Aging, microglial cell priming, and the discordant central inflammatory response to signals from the peripheral immune system', *J Leukoc Biol*, 84: 932-9.

Diniz, B. S., J. A. Pinto Junior, and O. V. Forlenza. 2008. 'Do CSF total tau, phosphorylated tau, and beta-amyloid 42 help to predict progression of mild cognitive impairment to Alzheimer's disease? A systematic review and meta-analysis of the literature', *World J Biol Psychiatry*, 9: 172-82.

Dong, M., G. Xi, R. F. Keep, and Y. Hua. 2013. 'Role of iron in brain lipocalin 2 upregulation after intracerebral hemorrhage in rats', *Brain Res*, 1505: 86-92.

Dunaiski, V., and D. A. Belford. 2002. 'Contribution of circulating IGF-I to wound repair in GH-treated rats', *Growth Horm IGF Res*, 12: 381-7.

Eikelenboom, P., E. van Exel, J. J. Hoozemans, R. Veerhuis, A. J. Rozemuller, and W. A. van Gool. 2010. 'Neuroinflammation - an early event in both the history and pathogenesis of Alzheimer's disease', *Neurodegener Dis*, 7: 38-41.

Fabry, Z., K. M. Fitzsimmons, J. A. Herlein, T. O. Moninger, M. B. Dobbs, and M. N. Hart. 1993. 'Production of the cytokines interleukin 1 and 6 by murine brain microvessel endothelium and smooth muscle pericytes', *J Neuroimmunol*, 47: 23-34.

Farr, S. A., W. A. Banks, and J. E. Morley. 2006. 'Effects of leptin on memory processing', *Peptides*, 27: 1420-5.

Fernandes, A., A. Barateiro, A. S. Falcao, S. L. Silva, A. R. Vaz, M. A. Brito, R. F. Silva, and D. Brites. 2011. 'Astrocyte reactivity to unconjugated bilirubin requires TNF-alpha and IL-1beta receptor signaling pathways', *Glia*, 59: 14-25.

Ferreira, A. C., S. Da Mesquita, J. C. Sousa, M. Correia-Neves, N. Sousa, J. A. Palha, and F. Marques. 2015. 'From the periphery to the brain: Lipocalin-2, a friend or foe?', *Prog Neurobiol*, 131: 120-36.

Fischbach, M. A., H. Lin, L. Zhou, Y. Yu, R. J. Abergel, D. R. Liu, K. N. Raymond, B. L. Wanner, R. K. Strong, C. T. Walsh, A. Aderem, and K. D. Smith. 2006. 'The pathogen-associated iroA gene cluster mediates bacterial evasion of lipocalin 2', *Proc Natl Acad Sci U S A*, 103: 16502-7.

Fischer, R., O. Maier, M. Siegemund, H. Wajant, P. Scheurich, and K. Pfizenmaier. 2011. 'A TNF receptor 2 selective agonist rescues human neurons from oxidative stress-induced cell death', *PLoS One*, 6: e27621.

Flo, T. H., K. D. Smith, S. Sato, D. J. Rodriguez, M. A. Holmes, R. K. Strong, S. Akira, and A. Aderem. 2004. 'Lipocalin 2 mediates an innate immune response to bacterial infection by sequestrating iron', *Nature*, 432: 917-21.

Flower, D. R. 1996. 'The lipocalin protein family: structure and function', *Biochem J*, 318 (Pt 1): 1-14.

Flower, D. R., A. C. North, and T. K. Attwood. 1993. 'Structure and sequence relationships in the lipocalins and related proteins', *Protein Sci*, 2: 753-61.

Fontaine, V., S. Mohand-Said, N. Hanoteau, C. Fuchs, K. Pfizenmaier, and U. Eisel. 2002. 'Neurodegenerative and neuroprotective effects of tumor Necrosis factor (TNF) in retinal ischemia: opposite roles of TNF receptor 1 and TNF receptor 2', *J Neurosci*, 22: RC216.

Friedlander, N. J., M. S. Burhans, L. Ade, L. M. O'Neill, X. Chen, and J. M. Ntambi. 2014. 'Global deletion of lipocalin 2 does not reverse high-fat diet-induced obesity resistance in stearoyl-CoA desaturase-1 skin-specific knockout mice', *Biochem Biophys Res Commun*, 445: 578-83.

Galis, Z. S., and J. J. Khatri. 2002. 'Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly', *Circ Res*, 90: 251-62.

Ghosh, M., S. S. Sodhi, J. H. Kim, N. E. Kim, R. K. Mongre, N. Sharma, S. W. Kim, S. J. Oh, K. K. Pulicherla, and D. K. Jeong. 2015. 'An Integrated In Silico Approach for the Structural and Functional Exploration of Lipocalin 2 and its Functional Insights with Metalloproteinase 9 and Lipoprotein Receptor-Related Protein 2', *Appl Biochem Biotechnol*, 176: 712-29.

Gibbs, J. E., J. Blaikley, S. Beesley, L. Matthews, K. D. Simpson, S. H. Boyce, S. N. Farrow, K. J. Else, D. Singh, D. W. Ray, and A. S. Loudon. 2012. 'The nuclear receptor REV-ERBalpha mediates circadian regulation of innate immunity through selective regulation of inflammatory cytokines', *Proc Natl Acad Sci U S A*, 109: 582-7.

Godinez, I., T. Haneda, M. Raffatellu, M. D. George, T. A. Paixao, H. G. Rolan, R. L. Santos, S. Dandekar, R. M. Tsolis, and A. J. Baumler. 2008. 'T cells help to amplify inflammatory responses induced by Salmonella enterica serotype Typhimurium in the intestinal mucosa', *Infect Immun*, 76: 2008-17.

Goehler, L. E., R. P. Gaykema, K. T. Nguyen, J. E. Lee, F. J. Tilders, S. F. Maier, and L. R. Watkins. 1999. 'Interleukin-1beta in immune cells of the abdominal vagus nerve: a link between the immune and nervous systems?', *J Neurosci*, 19: 2799-806.

Goetz, D. H., M. A. Holmes, N. Borregaard, M. E. Bluhm, K. N. Raymond, and R. K. Strong. 2002. 'The neutrophil lipocalin NGAL is a bacteriostatic agent that interferes with siderophore-mediated iron acquisition', *Mol Cell*, 10: 1033-43.

Gonzalez-Marrero, I., L. Gimenez-Llort, C. E. Johanson, E. M. Carmona-Calero, L. Castaneyra-Ruiz, J. M. Brito-Armas, A. Castaneyra-Perdomo, and R. Castro-Fuentes. 2015. 'Choroid plexus dysfunction impairs beta-amyloid clearance in a triple transgenic mouse model of Alzheimer's disease', *Front Cell Neurosci*, 9: 17.

Guo, H., M. Bazuine, D. Jin, M. M. Huang, S. W. Cushman, and X. Chen. 2013. 'Evidence for the regulatory role of lipocalin 2 in high-fat diet-induced adipose tissue remodeling in male mice', *Endocrinology*, 154: 3525-38.

Guo, H., D. Jin, Y. Zhang, W. Wright, M. Bazuine, D. A. Brockman, D. A. Bernlohr, and X. Chen. 2010. 'Lipocalin-2 deficiency impairs thermogenesis and potentiates diet-induced insulin resistance in mice', *Diabetes*, 59: 1376-85.

Gupta, S., R. Bi, C. Kim, S. Chiplunkar, L. Yel, and S. Gollapudi. 2005. 'Role of NF-kappaB signaling pathway in increased tumor necrosis factor-alpha-induced apoptosis of lymphocytes in aged humans', *Cell Death Differ*, 12: 177-83.

Gustafson, B. 2010. 'Adipose tissue, inflammation and atherosclerosis', *J Atheroscler Thromb*, 17: 332-41.

Gutierrez, E. G., W. A. Banks, and A. J. Kastin. 1993. 'Murine tumor necrosis factor alpha is transported from blood to brain in the mouse', *J Neuroimmunol*, 47: 169-76.

Gwira, J. A., F. Wei, S. Ishibe, J. M. Ueland, J. Barasch, and L. G. Cantley. 2005. 'Expression of neutrophil gelatinase-associated lipocalin regulates epithelial morphogenesis in vitro', *J Biol Chem*, 280: 7875-82.

Hayashi, M., S. Shimba, and M. Tezuka. 2007. 'Characterization of the molecular clock in mouse peritoneal macrophages', *Biol Pharm Bull*, 30: 621-6.

Henry, C. J., Y. Huang, A. M. Wynne, and J. P. Godbout. 2009. 'Peripheral lipopolysaccharide (LPS) challenge promotes microglial hyperactivity in aged mice that is associated with exaggerated induction of both pro-inflammatory IL-1beta and anti-inflammatory IL-10 cytokines', *Brain Behav Immun*, 23: 309-17.

Hraba-Renevey, S., H. Turler, M. Kress, C. Salomon, and R. Weil. 1989. 'SV40-induced expression of mouse gene 24p3 involves a post-transcriptional mechanism', *Oncogene*, 4: 601-8.

Hvidberg, V., C. Jacobsen, R. K. Strong, J. B. Cowland, S. K. Moestrup, and N. Borregaard. 2005. 'The endocytic receptor megalin binds the iron transporting neutrophil-gelatinase-associated lipocalin with high affinity and mediates its cellular uptake', *FEBS Lett*, 579: 773-7.

Jain, P., P. K. Wadhwa, and H. R. Jadhav. 2015. 'Reactive Astrogliosis: Role in Alzheimer's Disease', *CNS Neurol Disord Drug Targets*, 14: 872-9.

Jayaraman, A., K. A. Roberts, J. Yoon, D. M. Yarmush, X. Duan, K. Lee, and M. L. Yarmush. 2005. 'Identification of neutrophil gelatinase-associated lipocalin (NGAL) as a discriminatory marker of the hepatocyte-secreted protein response to IL-1beta: a proteomic analysis', *Biotechnol Bioeng*, 91: 502-15.

Jun, L. S., C. P. Siddall, and E. D. Rosen. 2011. 'A minor role for lipocalin 2 in high-fat diet-induced glucose intolerance', *Am J Physiol Endocrinol Metab*, 301: E825-35.

Katano, M., K. Okamoto, M. Arito, Y. Kawakami, M. S. Kurokawa, N. Suematsu, S. Shimada, H. Nakamura, Y. Xiang, K. Masuko, K. Nishioka, K. Yudoh, and T. Kato. 2009. 'Implication of granulocyte-macrophage colony-stimulating factor induced neutrophil gelatinase-associated lipocalin in pathogenesis of rheumatoid arthritis revealed by proteome analysis', *Arthritis Res Ther*, 11: R3.

Keller, M., J. Mazuch, U. Abraham, G. D. Eom, E. D. Herzog, H. D. Volk, A. Kramer, and B. Maier. 2009. 'A circadian clock in macrophages controls inflammatory immune responses', *Proc Natl Acad Sci U S A*, 106: 21407-12.

Kjeldsen, L., J. B. Cowland, and N. Borregaard. 2000. 'Human neutrophil gelatinase-associated lipocalin and homologous proteins in rat and mouse', *Biochim Biophys Acta*, 1482: 272-83.

Kjeldsen, L., A. H. Johnsen, H. Sengelov, and N. Borregaard. 1993. 'Isolation and primary structure of NGAL, a novel protein associated with human neutrophil gelatinase', *J Biol Chem*, 268: 10425-32.

Kmiec, Z. 2001. 'Cooperation of liver cells in health and disease', *Adv Anat Embryol Cell Biol*, 161: III-XIII, 1-151.

Komaki, G., A. Arimura, and K. Koves. 1992. 'Effect of intravenous injection of IL-1 beta on PGE2 levels in several brain areas as determined by microdialysis', *Am J Physiol*, 262: E246-51.

Law, I. K., A. Xu, K. S. Lam, T. Berger, T. W. Mak, P. M. Vanhoutte, J. T. Liu, G. Sweeney, M. Zhou, B. Yang, and Y. Wang. 2010. 'Lipocalin-2 deficiency attenuates insulin resistance associated with aging and obesity', *Diabetes*, 59: 872-82.

Lee, S., J. Lee, S. Kim, J. Y. Park, W. H. Lee, K. Mori, S. H. Kim, I. K. Kim, and K. Suk. 2007. 'A dual role of lipocalin 2 in the apoptosis and deramification of activated microglia', *J Immunol*, 179: 3231-41.

Lee, S., J. Y. Park, W. H. Lee, H. Kim, H. C. Park, K. Mori, and K. Suk. 2009. 'Lipocalin-2 is an autocrine mediator of reactive astrocytosis', *J Neurosci*, 29: 234-49.

Leifeld, L., F. L. Dumoulin, I. Purr, K. Janberg, C. Trautwein, M. Wolff, M. P. Manns, T. Sauerbruch, and U. Spengler. 2003. 'Early up-regulation of chemokine expression in fulminant hepatic failure', *J Pathol*, 199: 335-44.

Leskovjan, A. C., A. Kretlow, A. Lanzirotti, R. Barrea, S. Vogt, and L. M. Miller. 2011. 'Increased brain iron coincides with early plaque formation in a mouse model of Alzheimer's disease', *Neuroimage*, 55: 32-8.

Li, C., and Y. R. Chan. 2011. 'Lipocalin 2 regulation and its complex role in inflammation and cancer', *Cytokine*, 56: 435-41.

Liu, Q., and M. Nilsen-Hamilton. 1995. 'Identification of a new acute phase protein', *J Biol Chem*, 270: 22565-70.

Louveau, A., I. Smirnov, T. J. Keyes, J. D. Eccles, S. J. Rouhani, J. D. Peske, N. C. Derecki, D. Castle, J. W. Mandell, K. S. Lee, T. H. Harris, and J. Kipnis. 2015. 'Structural and functional features of central nervous system lymphatic vessels', *Nature*, 523: 337-41.

Malbora, B., Z. Avci, M. Gulsan, B. Orhan, and N. Ozbek. 2013. 'Low serum lipocalin levels in patients with iron deficiency anemia', *J Pediatr Hematol Oncol*, 35: 218-20.

Marchetti, L., M. Klein, K. Schlett, K. Pfizenmaier, and U. L. Eisel. 2004. 'Tumor necrosis factor (TNF)-mediated neuroprotection against glutamate-induced excitotoxicity is enhanced by N-methyl-D-aspartate receptor activation. Essential role of a TNF receptor 2-mediated phosphatidylinositol 3-kinase-dependent NF-kappa B pathway', *J Biol Chem*, 279: 32869-81.

Marques, F., A. J. Rodrigues, J. C. Sousa, G. Coppola, D. H. Geschwind, N. Sousa, M. Correia-Neves, and J. A. Palha. 2008. 'Lipocalin 2 is a choroid plexus acute-phase protein', *J Cereb Blood Flow Metab*, 28: 450-5.

Matsuo, S., S. Yamazaki, K. Takeshige, and T. Muta. 2007. 'Crucial roles of binding sites for NF-kappaB and C/EBPs in IkappaB-zeta-mediated transcriptional activation', *Biochem J*, 405: 605-15.

Mesquita, S. D., A. C. Ferreira, A. M. Falcao, J. C. Sousa, T. G. Oliveira, M. Correia-Neves, N. Sousa, F. Marques, and J. A. Palha. 2014. 'Lipocalin 2 modulates the cellular response to amyloid beta', *Cell Death Differ*, 21: 1588-99.

Metcalf, D., C. G. Begley, D. J. Williamson, E. C. Nice, J. De Lamarter, J. J. Mermod, D. Thatcher, and A. Schmidt. 1987. 'Hemopoietic responses in mice injected with purified recombinant murine GM-CSF', *Exp Hematol*, 15: 1-9.

Michaluk, P., and L. Kaczmarek. 2007. 'Matrix metalloproteinase-9 in glutamate-dependent adult brain function and dysfunction', *Cell Death Differ*, 14: 1255-8.

Mishra, J., C. Dent, R. Tarabishi, M. M. Mitsnefes, Q. Ma, C. Kelly, S. M. Ruff, K. Zahedi, M. Shao, J. Bean, K. Mori, J. Barasch, and P. Devarajan. 2005. 'Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery', *Lancet*, 365: 1231-8.

Mishra, J., Q. Ma, A. Prada, M. Mitsnefes, K. Zahedi, J. Yang, J. Barasch, and P. Devarajan. 2003. 'Identification of neutrophil gelatinase-associated lipocalin as a novel early urinary biomarker for ischemic renal injury', *J Am Soc Nephrol*, 14: 2534-43.

Miyajima, H., Y. Takahashi, T. Kamata, H. Shimizu, N. Sakai, and J. D. Gitlin. 1997. 'Use of desferrioxamine in the treatment of aceruloplasminemia', *Ann Neurol*, 41: 404-7.

Moestrup, S. K., and P. J. Verroust. 2001. 'Megalin- and cubilin-mediated endocytosis of protein-bound vitamins, lipids, and hormones in polarized epithelia', *Annu Rev Nutr*, 21: 407-28.

Moller, D. E. 2000. 'Potential role of TNF-alpha in the pathogenesis of insulin resistance and type 2 diabetes', *Trends Endocrinol Metab*, 11: 212-7.

Mori, K., and K. Nakao. 2007. 'Neutrophil gelatinase-associated lipocalin as the real-time indicator of active kidney damage', *Kidney Int*, 71: 967-70.

Nathan, C. 2002. 'Points of control in inflammation', *Nature*, 420: 846-52.

Naude, P. J., U. L. Eisel, H. C. Comijs, N. A. Groenewold, P. P. De Deyn, F. J. Bosker, P. G. Luiten, J. A. den Boer, and R. C. Oude Voshaar. 2013. 'Neutrophil gelatinase-associated lipocalin: a novel inflammatory marker associated with late-life depression', *J Psychosom Res*, 75: 444-50.

Naude, P. J., P. M. Mommersteeg, W. P. Zijlstra, L. Gouweleeuw, N. Kupper, U. L. Eisel, W. J. Kop, and R. G. Schoemaker. 2014. 'Neutrophil Gelatinase-Associated Lipocalin and depression in patients with chronic heart failure', *Brain Behav Immun*, 38: 59-65.

Naude, P. J., C. Nyakas, L. E. Eiden, D. Ait-Ali, R. van der Heide, S. Engelborghs, P. G. Luiten, P. P. De Deyn, J. A. den Boer, and U. L. Eisel. 2012. 'Lipocalin 2: novel component of proinflammatory signaling in Alzheimer's disease', *FASEB J*, 26: 2811-23.

Ni, J., X. Ma, M. Zhou, X. Pan, J. Tang, Y. Hao, Z. Lu, M. Gao, Y. Bao, and W. Jia. 2013. 'Serum lipocalin-2 levels positively correlate with coronary artery disease and metabolic syndrome', *Cardiovasc Diabetol*, 12: 176.

Nielsen, B. S., N. Borregaard, J. R. Bundgaard, S. Timshel, M. Sehested, and L. Kjeldsen. 1996. 'Induction of NGAL synthesis in epithelial cells of human colorectal neoplasia and inflammatory bowel diseases', *Gut*, 38: 414-20.

Niikura, T., Y. Hashimoto, T. Okamoto, Y. Abe, T. Yasukawa, M. Kawasumi, T. Hiraki, Y. Kita, K. Terashita, K. Kouyama, and I. Nishimoto. 2001. 'Insulin-like growth factor I (IGF-I) protects cells from apoptosis by Alzheimer's V642I mutant amyloid precursor protein through IGF-I receptor in an IGF-binding protein-sensitive manner', *J Neurosci*, 21: 1902-10.

Orlando, R. A., M. Exner, R. P. Czekay, H. Yamazaki, A. Saito, R. Ullrich, D. Kerjaschki, and M. G. Farquhar. 1997. 'Identification of the second cluster of ligand-binding repeats in megalin as a site for receptor-ligand interactions', *Proc Natl Acad Sci U S A*, 94: 2368-73.

Orlando, R. A., K. Rader, F. Authier, H. Yamazaki, B. I. Posner, J. J. Bergeron, and M. G. Farquhar. 1998. 'Megalin is an endocytic receptor for insulin', *J Am Soc Nephrol*, 9: 1759-66.

Ott, V., M. Friedrich, J. Zemlin, H. Lehnert, B. Schultes, J. Born, and M. Hallschmid. 2012. 'Meal anticipation potentiates postprandial ghrelin suppression in humans', *Psychoneuroendocrinology*, 37: 1096-100.

Park, H. Y., H. M. Kwon, H. J. Lim, B. K. Hong, J. Y. Lee, B. E. Park, Y. Jang, S. Y. Cho, and H. S. Kim. 2001. 'Potential role of leptin in angiogenesis: leptin induces endothelial cell proliferation and expression of matrix metalloproteinases in vivo and in vitro', *Exp Mol Med*, 33: 95-102.

Prvulovic, D., and H. Hampel. 2011. 'Amyloid beta (Abeta) and phospho-tau (p-tau) as diagnostic biomarkers in Alzheimer's disease', *Clin Chem Lab Med*, 49: 367-74.

Qizilbash, N., J. Gregson, M. E. Johnson, N. Pearce, I. Douglas, K. Wing, S. J. Evans, and S. J. Pocock. 2015. 'BMI and risk of dementia in two million people over two decades: a retrospective cohort study', *Lancet Diabetes Endocrinol*, 3: 431-6.

Raffatellu, M., M. D. George, Y. Akiyama, M. J. Hornsby, S. P. Nuccio, T. A. Paixao, B. P. Butler, H. Chu, R. L. Santos, T. Berger, T. W. Mak, R. M. Tsolis, C. L. Bevins, J. V. Solnick, S. Dandekar, and A. J. Baumler. 2009. 'Lipocalin-2 resistance confers an advantage to Salmonella enterica serotype Typhimurium for growth and survival in the inflamed intestine', *Cell Host Microbe*, 5: 476-86.

Raj, D. D., D. Jaarsma, I. R. Holtman, M. Olah, F. M. Ferreira, W. Schaafsma, N. Brouwer, M. M. Meijer, M. C. de Waard, I. van der Pluijm, R. Brandt, K. L. Kreft, J. D. Laman, G. de Haan, K. P. Biber, J. H. Hoeijmakers, B. J. Eggen, and H. W. Boddeke. 2014. 'Priming of microglia in a DNA-repair deficient model of accelerated aging', *Neurobiol Aging*, 35: 2147-60.

Rajpathak, S. N., M. J. Gunter, J. Wylie-Rosett, G. Y. Ho, R. C. Kaplan, R. Muzumdar, T. E. Rohan, and H. D. Strickler. 2009. 'The role of insulin-like growth factor-I and its binding proteins in glucose homeostasis and type 2 diabetes', *Diabetes Metab Res Rev*, 25: 3-12.

Rao, J. S., S. I. Rapoport, and H. W. Kim. 2011. 'Altered neuroinflammatory, arachidonic acid cascade and synaptic markers in postmortem Alzheimer's disease brain', *Transl Psychiatry*, 1: e31.

Rath, P. C., and B. B. Aggarwal. 1999. 'TNF-induced signaling in apoptosis', *J Clin Immunol*, 19: 350-64.

Ratledge, C., and L. G. Dover. 2000. 'Iron metabolism in pathogenic bacteria', *Annu Rev Microbiol*, 54: 881-941.

Raymond, KennethN, Gertraud Müller, and BertholdF Matzanke. 1984. 'Complexation of iron by siderophores a review of their solution and structural chemistry and biological function.' in, *Structural Chemistry* (Springer Berlin Heidelberg).

Riazi, K., M. A. Galic, J. B. Kuzmiski, W. Ho, K. A. Sharkey, and Q. J. Pittman. 2008. 'Microglial activation and TNFalpha production mediate altered CNS excitability following peripheral inflammation', *Proc Natl Acad Sci U S A*, 105: 17151-6.

Richardson, D. R. 2005. '24p3 and its receptor: dawn of a new iron age?', *Cell*, 123: 1175-7.

Roach, J. C., G. Glusman, L. Rowen, A. Kaur, M. K. Purcell, K. D. Smith, L. E. Hood, and A. Aderem. 2005. 'The evolution of vertebrate Toll-like receptors', *Proc Natl Acad Sci U S A*, 102: 9577-82.

Rogers, J. T., J. D. Randall, C. M. Cahill, P. S. Eder, X. Huang, H. Gunshin, L. Leiter, J. McPhee, S. S. Sarang, T. Utsuki, N. H. Greig, D. K. Lahiri, R. E. Tanzi, A. I. Bush, T. Giordano, and S. R. Gullans. 2002. 'An iron-responsive element type II in the 5'-untranslated region of the Alzheimer's amyloid precursor protein transcript', *J Biol Chem*, 277: 45518-28.

Roth, G. A., S. Nickl, D. Lebherz-Eichinger, E. M. Schmidt, H. J. Ankersmit, P. Faybik, H. Hetz, and C. G. Krenn. 2013. 'Lipocalin-2 serum levels are increased in acute hepatic failure', *Transplant Proc*, 45: 241-4.

Roudkenar, M. H., Y. Kuwahara, T. Baba, A. M. Roushandeh, S. Ebishima, S. Abe, Y. Ohkubo, and M. Fukumoto. 2007. 'Oxidative stress induced lipocalin 2 gene expression: addressing its expression under the harmful conditions', *J Radiat Res*, 48: 39-44.

Sato, M., Y. Mizoro, Y. Atobe, Y. Fujimoto, Y. Yamaguchi, J. M. Fustin, M. Doi, and H. Okamura. 2011. 'Transportin 1 in the mouse brain: appearance in regions of neurogenesis, cerebrospinal fluid production/sensing, and circadian clock', *J Comp Neurol*, 519: 1770-80.

Saucedo, R., A. Zarate, L. Basurto, M. Hernandez, E. Puello, R. Galvan, and S. Campos. 2011. 'Relationship between circulating adipokines and insulin resistance during pregnancy and postpartum in women with gestational diabetes', *Arch Med Res*, 42: 318-23.

Scheer, F. A., J. L. Chan, J. Fargnoli, J. Chamberland, K. Arampatzi, S. A. Shea, G. L. Blackburn, and C. S. Mantzoros. 2010. 'Day/night variations of high-molecular-weight adiponectin and lipocalin-2 in healthy men studied under fed and fasted conditions', *Diabetologia*, 53: 2401-5.

Scheiermann, C., Y. Kunisaki, D. Lucas, A. Chow, J. E. Jang, D. Zhang, D. Hashimoto, M. Merad, and P. S. Frenette. 2012. 'Adrenergic nerves govern circadian leukocyte recruitment to tissues', *Immunity*, 37: 290-301.

Schmidt-Ott, K. M., K. Mori, J. Y. Li, A. Kalandadze, D. J. Cohen, P. Devarajan, and J. Barasch. 2007. 'Dual action of neutrophil gelatinase-associated lipocalin', *J Am Soc Nephrol*, 18: 407-13.

Schubert, M., D. P. Brazil, D. J. Burks, J. A. Kushner, J. Ye, C. L. Flint, J. Farhang-Fallah, P. Dikkes, X. M. Warot, C. Rio, G. Corfas, and M. F. White. 2003. 'Insulin receptor substrate-2 deficiency impairs brain growth and promotes tau phosphorylation', *J Neurosci*, 23: 7084-92.

Schwartz, M. W., S. C. Woods, D. Porte, Jr., R. J. Seeley, and D. G. Baskin. 2000. 'Central nervous system control of food intake', *Nature*, 404: 661-71.

Serot, J. M., M. C. Bene, and G. C. Faure. 2003. 'Choroid plexus, aging of the brain, and Alzheimer's disease', *Front Biosci*, 8: s515-21.

Shen, F., M. J. Ruddy, P. Plamondon, and S. L. Gaffen. 2005. 'Cytokines link osteoblasts and inflammation: microarray analysis of interleukin-17- and TNF-alpha-induced genes in bone cells', *J Leukoc Biol*, 77: 388-99.

Shi, Y., C. H. Liu, A. I. Roberts, J. Das, G. Xu, G. Ren, Y. Zhang, L. Zhang, Z. R. Yuan, H. S. Tan, G. Das, and S. Devadas. 2006. 'Granulocyte-macrophage colony-stimulating factor (GM-CSF) and T-cell responses: what we do and don't know', *Cell Res*, 16: 126-33.

Shum, H. P., N. Y. Leung, L. L. Chang, O. Y. Tam, A. M. Kwan, K. C. Chan, W. W. Yan, and T. M. Chan. 2015. 'Predictive value of plasma neutrophil gelatinase-associated lipocalin for acute kidney injury in intensive care unit patients after major non-cardiac surgery', *Nephrology (Carlton)*, 20: 375-82.

Sierra, A., A. C. Gottfried-Blackmore, B. S. McEwen, and K. Bulloch. 2007. 'Microglia derived from aging mice exhibit an altered inflammatory profile', *Glia*, 55: 412-24.

Singh, K. P., R. Prasad, P. S. Chari, and R. J. Dash. 1998. 'Effect of growth hormone therapy in burn patients on conservative treatment', *Burns*, 24: 733-8.

Skipor, J., and J. C. Thiery. 2008. 'The choroid plexus--cerebrospinal fluid system: undervaluated pathway of neuroendocrine signaling into the brain', *Acta Neurobiol Exp (Wars)*, 68: 414-28.

Small, G. W., V. Kepe, L. M. Ercoli, P. Siddarth, S. Y. Bookheimer, K. J. Miller, H. Lavretsky, A. C. Burggren, G. M. Cole, H. V. Vinters, P. M. Thompson, S. C. Huang, N. Satyamurthy, M. E. Phelps, and J. R. Barrio. 2006. 'PET of brain amyloid and tau in mild cognitive impairment', *N Engl J Med*, 355: 2652-63.

Sorensen, O. E., J. B. Cowland, K. Theilgaard-Monch, L. Liu, T. Ganz, and N. Borregaard. 2003. 'Wound healing and expression of antimicrobial peptides/polypeptides in human keratinocytes, a consequence of common growth factors', *J Immunol*, 170: 5583-9.

Spuch, C., D. Antequera, C. Pascual, S. Abilleira, M. Blanco, M. J. Moreno-Carretero, J. Romero-Lopez, T. Ishida, J. A. Molina, A. Villarejo, F. Bermejo-Pareja, and E. Carro. 2015. 'Soluble Megalin is Reduced in Cerebrospinal Fluid Samples of Alzheimer's Disease Patients', *Front Cell Neurosci*, 9: 134.

Stawarski, M., M. Stefaniuk, and J. Wlodarczyk. 2014. 'Matrix metalloproteinase-9 involvement in the structural plasticity of dendritic spines', *Front Neuroanat*, 8: 68.

Sultan, S., M. Pascucci, S. Ahmad, I. A. Malik, A. Bianchi, P. Ramadori, G. Ahmad, and G. Ramadori. 2012. 'LIPOCALIN-2 is a major acute-phase protein in a rat and mouse model of sterile abscess', *Shock*, 37: 191-6.

Takada, Y., X. Fang, M. S. Jamaluddin, D. D. Boyd, and B. B. Aggarwal. 2004. 'Genetic deletion of glycogen synthase kinase-3beta abrogates activation of IkappaBalpha kinase, JNK, Akt, and p44/p42 MAPK but potentiates apoptosis induced by tumor necrosis factor', *J Biol Chem*, 279: 39541-54.

Takeuchi, O., and S. Akira. 2010. 'Pattern recognition receptors and inflammation', *Cell*, 140: 805-20.

Tong, Z., X. Wu, and J. P. Kehrer. 2003. 'Increased expression of the lipocalin 24p3 as an apoptotic mechanism for MK886', *Biochem J*, 372: 203-10.

Tong, Z., X. Wu, D. Ovcharenko, J. Zhu, C. S. Chen, and J. P. Kehrer. 2005. 'Neutrophil gelatinase-associated lipocalin as a survival factor', *Biochem J*, 391: 441-8.

Trejo, J. L., E. Carro, and I. Torres-Aleman. 2001. 'Circulating insulin-like growth factor I mediates exercise-induced increases in the number of new neurons in the adult hippocampus', *J Neurosci*, 21: 1628-34.

Triebel, S., J. Blaser, H. Reinke, and H. Tschesche. 1992. 'A 25 kDa alpha 2-microglobulin-related protein is a component of the 125 kDa form of human gelatinase', *FEBS Lett*, 314: 386-8.

Van den Steen, P. E., I. Van Aelst, V. Hvidberg, H. Piccard, P. Fiten, C. Jacobsen, S. K. Moestrup, S. Fry, L. Royle, M. R. Wormald, R. Wallis, P. M. Rudd, R. A. Dwek, and G. Opdenakker. 2006. 'The hemopexin and O-glycosylated domains tune gelatinase B/MMP-9 bioavailability via inhibition and binding to cargo receptors', *J Biol Chem*, 281: 18626-37.

van Dijk, G., S. van Heijningen, A. C. Reijne, C. Nyakas, E. A. van der Zee, and U. L. Eisel. 2015. 'Integrative neurobiology of metabolic diseases, neuroinflammation, and neurodegeneration', *Front Neurosci*, 9: 173.

Vargas, T., M. J. Bullido, A. Martinez-Garcia, D. Antequera, J. Clarimon, M. Rosich-Estrago, A. Martin-Requero, I. Mateo, E. Rodriguez-Rodriguez, E. Vilella-Cuadrada, A. Frank, A. Lleo, L. Molina-

Porcel, R. Blesa, O. Combarros, T. Gomez-Isla, F. Bermejo-Pareja, F. Valdivieso, and E. Carro. 2010. 'A megalin polymorphism associated with promoter activity and Alzheimer's disease risk', *Am J Med Genet B Neuropsychiatr Genet*, 153B: 895-902.

Veroni, C., L. Gabriele, I. Canini, L. Castiello, E. Coccia, M. E. Remoli, S. Columba-Cabezas, E. Arico, F. Aloisi, and C. Agresti. 2010. 'Activation of TNF receptor 2 in microglia promotes induction of anti-inflammatory pathways', *Mol Cell Neurosci*, 45: 234-44.

Viel, J. J., D. Q. McManus, S. S. Smith, and G. J. Brewer. 2001. 'Age- and concentration-dependent neuroprotection and toxicity by TNF in cortical neurons from beta-amyloid', *J Neurosci Res*, 64: 454-65.

Wajant, H., K. Pfizenmaier, and P. Scheurich. 2003. 'Tumor necrosis factor signaling', *Cell Death Differ*, 10: 45-65.

Wang, Y., K. S. Lam, E. W. Kraegen, G. Sweeney, J. Zhang, A. W. Tso, W. S. Chow, N. M. Wat, J. Y. Xu, R. L. Hoo, and A. Xu. 2007. 'Lipocalin-2 is an inflammatory marker closely associated with obesity, insulin resistance, and hyperglycemia in humans', *Clin Chem*, 53: 34-41.

Weinberg, E. D. 1984. 'Iron withholding: a defense against infection and neoplasia', *Physiol Rev*, 64: 65-102.

———. 1996. 'Iron withholding: a defense against viral infections', *Biometals*, 9: 393-9.

Xu, S., and P. Venge. 2000. 'Lipocalins as biochemical markers of disease', *Biochim Biophys Acta*, 1482: 298-307.

Yan, L., N. Borregaard, L. Kjeldsen, and M. A. Moses. 2001. 'The high molecular weight urinary matrix metalloproteinase (MMP) activity is a complex of gelatinase B/MMP-9 and neutrophil gelatinase-associated lipocalin (NGAL). Modulation of MMP-9 activity by NGAL', *J Biol Chem*, 276: 37258-65.

Yan, Q. W., Q. Yang, N. Mody, T. E. Graham, C. H. Hsu, Z. Xu, N. E. Houstis, B. B. Kahn, and E. D. Rosen. 2007. 'The adipokine lipocalin 2 is regulated by obesity and promotes insulin resistance', *Diabetes*, 56: 2533-40.

Yang, J., D. Goetz, J. Y. Li, W. Wang, K. Mori, D. Setlik, T. Du, H. Erdjument-Bromage, P. Tempst, R. Strong, and J. Barasch. 2002. 'An iron delivery pathway mediated by a lipocalin', *Mol Cell*, 10: 1045-56.

Yang, J., K. Mori, J. Y. Li, and J. Barasch. 2003. 'Iron, lipocalin, and kidney epithelia', *Am J Physiol Renal Physiol*, 285: F9-18.

Zettlitz, K. A., V. Lorenz, K. Landauer, S. Munkel, A. Herrmann, P. Scheurich, K. Pfizenmaier, and R. Kontermann. 2010. 'ATROSAB, a humanized antagonistic anti-tumor necrosis factor receptor one-specific antibody', *MAbs*, 2: 639-47.

Zhao, M., D. H. Cribbs, A. J. Anderson, B. J. Cummings, J. H. Su, A. J. Wasserman, and C. W. Cotman. 2003. 'The induction of the TNFalpha death domain signaling pathway in Alzheimer's disease brain', *Neurochem Res*, 28: 307-18.

Zlokovic, B. V., C. L. Martel, E. Matsubara, J. G. McComb, G. Zheng, R. T. McCluskey, B. Frangione, and J. Ghiso. 1996. 'Glycoprotein 330/megalin: probable role in receptor-mediated transport of apolipoprotein J alone and in a complex with Alzheimer disease amyloid beta at the blood-brain and blood-cerebrospinal fluid barriers', *Proc Natl Acad Sci U S A*, 93: 4229-34.