Review



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*Review*

# Lipocalin 2 in Obesity and Diabetes: Insights into Its Role in Energy Metabolism

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**Abstract:** Lipocalin 2 (LCN2), also known as neutrophil gelatinase-associated lipocalin, is a 25 kDa protein involved in immune defense, inflammation, and metabolism. LCN2 is widely expressed across various tissues, including immune cells, bone, adipose tissue, liver, kidneys, lung, spleen, and epithelial cells, and exhibits sex- and fat depot-specific expression patterns. Structurally, LCN2 contains a hydrophobic lipid-binding pocket and glycosylation sites, enabling it to interact with diverse ligands and form dimers. In innate immunity, LCN2 plays a critical role by sequestering iron-laden siderophores, thereby restricting bacterial growth. Beyond its role in infection control, LCN2 is implicated in metabolic inflammation and diseases such as obesity and diabetes. Recent research has highlighted a pivotal role for LCN2 in mitochondrial phospholipid metabolism and mitochondrial function. In metabolic diseases and mitochondrial metabolism, LCN2 appears to display paradoxical effects. While some studies link it to improved insulin sensitivity, glucose regulation, and mitochondrial function, others associate it with insulin resistance, obesity, and mitochondrial dysfunction. These inconsistencies may arise from differences in experimental conditions and study populations. This review provides an up-to-date summary of LCN2's multifaceted roles in obesity, diabetes, energy balance, and mitochondrial function, emphasizing its context-dependent effects. LCN2 appears to have dual roles, exerting both protective and detrimental outcomes depending on the physiological or pathological context, sex, cell types, and experimental conditions. Further research is necessary to unravel its complex functions and resolve conflicting findings, particularly in metabolic disorders.

**Keywords:** lipocalin 2; obesity; diabetes; mitochondrial metabolism; metabolic disease

## 1. Introduction

Lipocalin 2 (LCN2), also known as neutrophil gelatinase-associated lipocalin, is a 25 kD secreted protein first characterized in neutrophils [1,2]. This 178-amino acid protein has several notable structural features, including a hydrophobic binding pocket for lipid interactions, glycosylation at asparagine residue 65, and an unpaired cysteine at residue 87, which facilitates dimerization with other proteins [2,3]. Lcn2 is widely expressed in various tissues, including immune cells, adipose tissue, liver, kidneys, bone marrow, prostate, and epithelial tissues exposed to microorganisms, such as the trachea, lungs, stomach, salivary glands, colon, and uterus [4–11]. This broad tissue distribution underscores its roles in immune defense, inflammation, and metabolic regulation.

LCN2 was initially identified for its ability to bind and stabilize matrix-metalloproteinase-9 (MMP-9), an enzyme critical for extracellular matrix remodeling [2,12]. Its expression is induced by toll-like receptor (TLR) activation via Lipopolysaccharide (LPS) [13,14], linking it to the innate immune response. One of its well-established functions is to inhibit bacterial growth by sequestering iron-laden siderophores, thereby limiting bacterial assess to iron [14,15]. Consistent with this, *Lcn2* deficient mice are more susceptible to bacterial infections [14,16].

Beyond infection control, LCN2 has been implicated in metabolic inflammation in diseases such as lung inflammation [17,18], atherosclerosis [19,20], obesity-related adipose tissue inflammation [21,22], intestinal inflammation [21,23–25], fatty liver diseases [26–30], neuroinflmmation [31,32], and cancers [33]. Mechanistically, LCN2 exerts its effects through both ligand binding and receptor-mediated pathways [34–39]. Megalin/gp330, also known as low density lipoprotein-related protein 2

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(LRP2), and SLC22A17, also referred to as the LCN2 receptor or LCN2-R, are two key receptors that mediate LCN2 cellular uptake and regulate iron metabolism [34,35,40]. However, the role of LCN2 SLC22A17 in this process remains controversial [36,37]. LCN2 also interacts with phosphatidylcholine in lipid rafts of the plasma membrane, suggesting a potential role in lipid raft recognition and cellular signaling regulation [38]. These diverse interactions and mechanisms highlight the multifaceted role of LCN2 in regulating cellular processes and maintaining immune and metabolic homeostasis [39].

LCN2 has also been implicated in metabolic processes, particularly obesity and diabetes, where its levels are often upregulated. However, its effects on metabolism appear paradoxical. While some studies indicate LCN2 enhances insulin sensitivity and benefits glucose regulation, others associate it with glucose intolerance, insulin resistance, and obesity [41]. These discrepancies may stem from variations in experimental conditions or differences in population characteristics across human studies. Overall, LCN2 plays diverse roles in inflammation, iron metabolism, and metabolic regulation, exerting both protective and detrimental effects depending on the context. Further research is essential to unravel its complex functions in metabolic disorders. This review offers an up-to-date overview of LCN2's multifaceted role in obesity, diabetes, energy balance, and mitochondrial metabolism. Additionally, it aims to provide a clearer interpretation and understanding of LCN2's paradoxical effects, as characterized by various research groups under differing conditions.

## 2. Lipocalins: The Multigene Family of Up and Down β-Barrel Proteins

The tertiary structural motifs in proteins can be thought of as assemblies of secondary structural elements including α-helices, β-strands, random coils, and assorted turns. One of the most common motifs found in proteins consists of a series of β-strands arranged in anti-parallel manner with adjacent strands hydrogen bonded to each other. This alignment of anti-parallel β-strands is called the up-and-down β-sheet motif because an extended polypeptide chain or β-strand is found adjacent to another segment of extended polypeptide chain running in the opposite direction and as close as possible to the preceding strand. When several of these anti-parallel strands are found together, the main chain atoms appear as a continuous configuration or sheet. When a collection of β-strands are arranged such that the first and last strand are hydrogen-bonded, then a continuous and cylindrical β-structure is formed with an enclosed interior. This structural design is designated the up-and-down β-barrel [42]. This motif is used for a variety of lipid carrier proteins including the extracellular lipocalins and intracellular FABPs. Both the FABPs and the lipocalins have a clearly defined β-barrel motif that forms either an interior cavity (FABP) or a deep pit that forms the lipid binding domain (lipocalins).

The extracellular lipocalins such as LCN2, Retinol Binding Protein (RBP) 4 and $\alpha_2$-microglobulin use a series of β-strands to form a globular protein with a deep depression resembling the calyx of a flower. The word lipocalin in fact means "lipid cup or chalice" in Greek. It is within this depression that hydrophobic ligands are bound. The lipocalins are secreted proteins with varying from 18 to 21 kDa and transport a number of different hydrophobic ligands in extracellular milieus ranging from serum to milk. Included in the compounds that bind to members of the lipocalin family are a variety of retinoids, biliverdin, pheromones, porphyrins, and odorants.

The lipocalins have a conformation that closely resembles the motif used by intracellular FABPs. The structure for LCN2 is shown in Figure 1. The up-and-down β-strand motif is used in a manner that leads to a continuous, nearly cylindrical barrel. However, a total of 8 β-strands are used in the lipocalin motif instead of 10 as in the FABPs. The structure also shows that a single segment of α-helix is found near the COOH terminal of the protein. The eight β-strands in the lipocalin motif are also hydrogen-bonded in a consecutive manner following the $n$, $n$ +1 topology. The overall shape of the lipocalins is slightly more spherical than the somewhat flattened form of the FABPs.

Outside the main up-and-down β-barrel fold of the lipocalins, several secondary structural elements occur. A large loop connects the first two β-strands in the lipocalins. This loop acts like a lid that covers one end of the up-and-down barrel in much the same way the two α-helices do in the FABPs. A second difference in topology occurs at the carboxyl terminus of the lipocalins. Here, there is an α-helix approximately 16 residues in length. This α-helix packs against the barrel parallel next to the last two β-strands. After this α-helix is a terminal β-strand that is not part of the up-and-down barrel.

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The conformations of both the lipocalins and FABPs have an up-and-down barrel core. Perhaps most importantly is that these motifs lead to cavities or pits capable of binding hydrophobic ligands. The nature of these differences is as follows: the lipocalins have a pit-like binding site; the FABPs have a cavity. Unlike the binding site in the FABPs, the residues that line the pit of the lipocalins are predominately hydrophobic. The binding specificity the lipocalins have for their ligands is not mainly the result of polar interactions, as is the case with the FABPs, but hydrophobic, van der Waal (London), interactions that line the pit wall. Of considerable interest is Trp79 that lies in close proximity to the bound ligand. Indeed, a simple assay system based on increases in intrinsic tryptophan fluorescence that occurs upon ligand binding has been used to assess stoichiometry and affinity [43]. Furthermore, the orientation of the ligand is opposite to that observed in the FABPs. In the lipocalin motif, the ligand is bound to a deep pit in the side chain extended towards the surface. In the case of LCN2, the carboxylate of retinoic acid or fatty acid is hydrogen bonded to a pair of tyrosine residues, Tyr52 and Tyr138 (Figure 1). These two tyrosines coordinate the carboxylate group analogously to the Tyr 128 coordinating the carboxylate of the FABPs [44]. A second difference is found in the binding properties of the lipocalins compared to those of the FABPs. In the former there appears to be greater complementarity; the shape of the pit seems to be closely matched to the shape of the ligand. In the FABPs the cavity is larger than it need be for most ligands. The close complementarity between ligand and binding site in the lipocalins is expressed as the degree of available solvent-accessible surface area for the bound ligand. For retinol bound to RBP (a lipocalin), the accessible surface area is approximately 1 $A^2$. In contrast, retinol bound in the cavity of CRBP (an FABP) has an accessible surface area of 16 $A^2$, and oleic acid bound to myelin P2 (an FABP) has an accessible surface area of 33 $A^2$ [45].

Lipocalins are a diverse family of proteins that participate in various biological processes. RBP4, one of the lipocalin subfamily members, has been identified as an adipokine that affect glucose metabolism and insulin sensitivity [46]. RBP4 expression in adipose tissue as well as circulating RBP4 were increased in several models of rodent obesity [46]. Overexpression or loss of function analysis in mice suggests that RBP4 functions as an antagonist of insulin action [46]. The circulating levels of RBP4 were also increased in obese humans, and the levels were correlated negatively with insulin sensitivity [47] and positively with percent abdominal fat [48]. Similarly, as discussed in this review, LCN2 plays a critical role in glucose and lipid metabolism, as well as metabolic diseases.

## 3. Regulation of Lipocalin 2 Expression in Adipose Tissue

The promoter region of the *Lcn2* gene contains binding motifs for nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) and CCAAT enhancer binding protein (C/EBP), as well as several nuclear receptor response elements, including glucocorticoid response element, estrogen response element, and retinoic acid receptor response element [49–51]. These regulatory elements suggest a diverse range of expression controls and biological functions within cells. This section highlights *Lcn2* expression and its regulation in adipose tissue.

### *3.1. Sex- and Depot-Differences in Lipocalin 2 Expression and Regulation by Metabolic Stress*

LCN2 exhibits sex- and depot-specific expression in mouse adipose tissue under steady state conditions [52]. Generally, female mice express significantly higher levels of LCN2 protein in inguinal white adipose tissue (Ing-WAT) than male mice but lower levels in perigonadal white adipose tissue (Gon-WAT) [52]. In female mice, LCN2 expression in Ing-WAT is markedly higher than in the other two depots: Gon-WAT and brown adipose tissue (BAT), a pattern is less pronounced in male mice [52]. Additionally, age seems to regulate LCN2 expression in adipose depots differently based on sex and depot type. Compared to young mice (2-3 months old), middle-aged mice (8-9 months) showed a significant increase in LCN2 protein in Gon-WAT in both sexes, whereas LCN2 protein in Ing-WAT decreases with age in male mice but increases in female mice [52]. A previous study demonstrated that estrogen receptor signaling activation suppressed *Lcn2* expression in gonadal adipose tissue, suggesting that sex hormones may contribute to sex-different regulation of *Lcn2* expression in adipose tissue [53].

*Lcn2* expression in adipose tissue is also highly responsive to metabolic stress and inflammation. Studies by multiple groups, including ours, have consistently demonstrated that *Lcn2* expression is upregulated in the adipose tissue of male mice under various obesity and metabolic stress conditions.

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Specifically, *Lcn2* expression was elevated in genetically obese (*ob*/*ob*) and diabetic (*db*/*db*) mice, as well as in mice with high-fat diet-induced obesity [5,6,54]. While data on *Lcn2* regulation in adipose tissue in obese female mice are limited, human studies have shown a significant upregulation of *Lcn2* expression in the adipose tissue of women with severe obesity [55]. In a separate study, we reported that fasting male mice for 48 hours increased *Lcn2* mRNA expression in the liver, Ing-WAT, and Gon-WAT. Similarly, after 24 hours of fasting, LCN2 protein levels were elevated in WAT, especially in Ing-WAT and BAT [56]. In vitro, treating 3T3-L1 adipocytes with the β-adrenergic agonist norepinephrine (NE) for 24 hours increased intracellular LCN2 protein levels, although LCN2 secretion showed only a modest increase [56]. Furthermore, we found that cold stress (exposure to 4°C for four hours) significantly upregulated *Lcn2* gene expression in BAT, Ing-WAT, Gon-WAT, and the liver of 12-week-old male mice [56]. Collectively, these findings indicate that *Lcn2* expression is regulated by cold stress, high fat diet (HFD)-induced metabolic stress, and aging. These regulatory patterns exhibit notable differences based on sex and adipose tissue depot.

### *3.2. Regulation of Lipocalin 2 Expression by Insulin*

Tan et al. conducted *ex vivo* and *in vivo* studies to investigate the regulation of circulating LCN2 levels and LCN2 expression by insulin in adipose tissue in humans [57]. Six healthy young individuals (mean age 26.5±8 years, gender not specified) participated in a prolonged insulin-glucose infusion study for 26 hours. Results showed an acute rise in serum LCN2 beginning at 4 hours, which then plateaued and was sustained for the entire 26-hour infusion period [57]. Additionally, omental adipose tissue explants from surgical patients (mean age 27.5±7 years, BMI 23.8±2.8 kg/m$^2$) treated with insulin in the presence or absence of phosphoinositide 3-kinase (PI3K) or mitogen-activated protein kinase (MEK) inhibitors. Insulin treatment significantly increased LCN2 protein expression and secretion, while both PI3K and MEK inhibitors blocked this insulin-stimulated LCN2 increase, suggesting that insulin promotes LCN2 expression and secretion via PI3K and MEK signaling pathways [57].

In a separate study using 3T3-L1 adipocytes, we demonstrated that insulin-stimulated LCN2 expression and secretion requires glucose. Using 3-O-methyl-d-glucose, a non-metabolizable glucose analog, we showed that glucose metabolism is essential for insulin to induce LCN2 expression and secretion [56]. Further, blocking the inflammatory pathway with nuclear factor-kappa B (NF-κB) inhibitors (Aspirin and BAY 11-7082) reduced insulin and glucose-stimulated LCN2 expression and secretion [56]. LPS, known to activate NF-κB, was an even more potent stimulator of LCN2 than insulin. Notably, LPS combined with insulin had an additive effect on LCN2 expression and secretion, and BAY 11-7082 could only partially block LCN2 expression and secretion induced by the combined insulin and LPS treatment (Y. Zhang, Foncea, et al. 2014). These findings suggest that LCN2 expression is regulated independently by insulin signaling and inflammatory pathways.

### *3.3. Regulation of Lipocalin 2 Expression by Inflammation*

Zhao et al. investigated the regulation of *Lcn2* expression in adipocytes, both in vitro and in vivo, focusing on the roles of STAT1, NF-κB, and ERK (extracellular signal-regulated kinase) pathways [58]. Using 3T3-L1 adipocytes treated with pro-inflammatory cytokines interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α), they observed that LCN2 secretion was significantly stimulated within the first 24 hours, with TNF-α producing a more robust and sustained response over time. When both cytokines were combined, the increase in LCN2 expression and secretion was greater than the sum of their individual effects, suggesting that each cytokine utilizes distinct pathways, leading to additive effects.

To validate these findings in vivo, Zhao et al. injected male mice with either IFN-γ or TNF-α and sacrificed them after 14 hours [58]. Both cytokines elevated *Lcn2* mRNA levels in Gon-WAT and increased circulating LCN2 levels. Analysis of other insulin-sensitive tissues showed that IFN-γ, TNF-α, or both increased *Lcn2* expression significantly in Gon-WAT, liver, and heart. The combination of cytokines produced a particularly robust response, confirming the additive effect observed in vitro. Notably, either cytokine alone did not significantly impact *Lcn2* expression in Ing-WAT. The most substantial increase in *Lcn2* expression occurred in Gon-WAT following IFN-γ treatment (a 15-fold increase) and in the liver following TNF-α treatment (a 200-fold increase). To

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rule out injection site effects, adipocytes from Gon-WAT and Ing-WAT were isolated for ex vivo analysis, revealing *Lcn2* expression increases of eight-fold and three-fold, respectively [58].

To explore the signaling mechanisms, Zhao et al. identified DNA-binding sites for STAT1 and NF-κB on the *Lcn2* promoter through sequence analysis, EMSA, and CHIP assays [59]. Knockdown of NF-κB p65 and STAT1 via small interfering RNA (siRNA) in adipocytes reduced their respective mRNA levels by 60-70% and 60%, respectively. Adipocytes with knockdowns were serum-starved overnight and then treated with TNF-α or IFN-γ. Both NF-κB and STAT1 knockdowns significantly lowered *Lcn2* mRNA and protein levels compared to controls, suggesting these transcription factors mediate TNF-α-induced *Lcn2* expression. Further, treatment with an ERK-specific inhibitor reduced *Lcn2* expression, indicating that ERK signaling is also involved in the regulation of *Lcn2* expression [59].

To clarify the role of ERK in this process, Zhao et al. applied acute treatments of IFN-γ, TNF-α, or both to differentiated adipocytes. TNF-α treatment led to phosphorylation of p65 at Ser536 and STAT1 at Ser727, independent of IFN-γ. The study ruled out phosphorylation of p65 at Ser276, which was confirmed using a PKA agonist. An ERK inhibitor blocked TNF-α-induced phosphorylation of p65 at Ser536, supporting the role of ERK in directly mediating this phosphorylation [59]. These results indicate that pro-inflammatory cytokines regulate *Lcn2* expression through multiple signaling pathways in a tissue-specific manner, with the potential for additive effects.

## 4. Lipocalin 2 in Metabolic Diseases – Obesity and Diabetes

The role of LCN2 as an adipokine in metabolic regulation has gained significant attention in recent years. Numerous studies have explored its involvement in adipose tissue remodeling and metabolic regulation, particularly in the context of obesity and diabetes.

### *4.1. Circulating Lipocalin 2 Levels Correlate with Obesity and Diabetes*

As discussed earlier, adipose *Lcn2* expression is upregulated in both genetically modified and diet-induced obese mouse models. Studies in mice have demonstrated increased circulating LCN2 levels in obesity. For instance, in HFD-induced obesity, serum LCN2 levels increased by 25% after 16 weeks of HFD feeding in normal mice. Furthermore, approximately twofold increase in serum LCN2 levels was seen in obese insulin-resistant female leptin-deficient ($Lep^{ob/ob}$) mice and male leptin receptor-deficient ($Lepr^{db/db}$) mice compared to wild-type (WT) mice. Similarly, in a human study, women with severe obesity showed significantly elevated circulating LCN2 levels compared to individuals with normal weight [55].

Mosialou et al. explored the systemic role of LCN2 as a secreted protein in glucose metabolism in the context of prediabetes and type 2 diabetes mellitus (T2DM) [60]. Their study involved two cohorts of post-menopausal women (n=88 and n=54) and one male cohort (n=24). Over five years, they found no correlation between fasting plasma glucose and serum LCN2 levels in post-menopausal women. However, serum LCN2 levels showed a strong positive correlation with body mass index (BMI), waist circumference, serum insulin levels, homeostatic model assessment of insulin resistance (HOMA-IR), and β-cell function (using HOMA-β). This correlation intensified with increasing obesity. Serum adiponectin, on the other hand, was inversely correlated with serum LCN2 levels. Logistic regression revealed that increased HOMA-IR was strongly associated with the highest quartile of LCN2 levels. However, baseline LCN2 levels were not predictive of T2DM onset after five years, while HOMA-IR significantly correlated with new T2DM cases [60].

In a separate cohort of 54 postmenopausal women with T2DM, as the disease progressed, the relationship between serum LCN2 levels and insulin as well as hemoglobin A1c (HbA1c) reversed. A negative correlation emerged between LCN2 levels and these factors across different BMI groups [60]. This suggests that while elevated circulating LCN2 indicates metabolic dysregulation during prediabetes, in the context of T2DM, it may reflect better metabolic regulation. The authors proposed that rising LCN2 levels during the onset of insulin resistance (IR) could signify a compensatory mechanism aimed at improving β-cell function and mitigating hyperglycemia.

### *4.2. Lipocalin 2 in Obesity and Insulin Sensitivity*

Several research groups have characterized the role of LCN2 in obesity and insulin resistance using loss- and gain-of-function approaches. The results vary depending on sex, approach of study,

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and experimental design. Most of studies used male mice as an experimental model, while three groups investigated the impact of *Lcn2* deficiency or adipose *Lcn2* overexpression on obesity and insulin resistance in female mice.

4.2.1. Loss of Lcn2 Function in Obesity ad Insulin Sensitivity in Male Mice

Our group reported that male *Lcn2*-/- mice on a HFD exhibited significantly greater body weight gain, increased tissue weights of WAT, BAT, liver, heart, kidneys, and spleen, as well as larger adipocyte size compared to WT mice [61]. In a follow-up study, we demonstrated that *Lcn2* deficiency influenced adipose tissue remodeling induced by HFD. By 15 weeks of age, the adipocyte size distribution in *Lcn2*-/- mice shifted toward larger diameters compared to WT mice on a regular chow diet (RCD), with an even greater increase observed in older mice (30 weeks old). After four weeks on HFD, adipocyte size peaked in both genotypes, with subcutaneous adipose tissue reaching peak size earlier than epididymal WAT [62]. These findings suggest that *Lcn2* deficiency promotes adipocyte hypertrophy, a trend exacerbated with age. Additionally, *Lcn2* deficiency reduced adipogenic gene expression while increasing fibrosis, as indicated by elevated extracellular matrix (ECM) gene expression and collagen deposition, particularly in epididymal WAT of male mice [62].

With regard to glucose homeostasis and insulin sensitivity, our study showed that *Lcn2*-/- male mice on a HFD developed more severe obesity and insulin resistance, characterized by impaired glucose clearance and reduced insulin sensitivity. These effects were evidenced by decreased levels of insulin receptor substrate-1 (IRS1) protein and insulin-stimulated AKT (protein kinase B) phosphorylation in WAT. Furthermore, *Lcn2*-/- male mice exhibited increased hepatic lipid accumulation, elevated triglycerides, and enhanced expression of gluconeogenic and lipogenic genes, indicative of hepatic insulin resistance. However, Law and Jun reported contradictory findings. Law et al observed that *Lcn2*-/- male mice on an 18 week HFD showed increased epididymal fat mass/body weight ratio and larger adipocyte size but displayed improved insulin sensitivity, lower fasting plasma glucose, and reduced weight gain [63]. Meanwhile, Jun et al. suggested that *Lcn2* plays only a minor role in HFD-induced insulin resistance in male mice [64]. These discrepancies may arise from variations in experimental conditions, such as differences in HFD fatty acid composition, housing temperature, and the source of *Lcn2* knockout (KO) mice. For instance, the HFD used by Law at al. and Jun at al. contained 45% fat (soy oil), whereas our studies utilized an HFD with 65% fat (lard).

In an another related study, the Kousteni group silenced *Lcn2* expression systemically in genetically obese *Lepr*$^{db/db}$ mice to assess whether elevated circulating LCN2 levels in obesity are protective against metabolic dysfunction. After 30 days of systemic *Lcn2* siRNA delivery (every two days), *Lcn2* expression was reduced by 30% in bone, 50% in the liver, and 60% in adipose tissue, resulting in 50% reduction in circulating LCN2 levels. This reduction worsened metabolic disturbances in *Lepr*$^{db/db}$ male mice, including increased hyperphagia, gonadal fat accumulation, and body weight gain [60]. Additionally, the Kousteni group identified bone as a significant source of circulating LCN2 in HFD-induced obesity [60]. They demonstrated that *Lcn2* is expressed in bone, with osteoblast-derived LCN2 levels increasing in response to HFD. Using an osteoblast-specific *Lcn2* KO (*Lcn2osb*-/-) mouse model, they showed that WT mice on a HFD had a 30% increase in serum LCN2, while *Lcn2osb*-/- mice exhibited a 64% reduction in circulating LCN2 levels. Consistent with the results obtained from *Lcn2* silencing in *Lepr*$^{db/db}$ male mice, *Lcn2osb*-/- male mice on a HFD developed more severe obesity and insulin resistance, evidenced by increased body weight, fat mass, blood glucose levels, glucose intolerance, and insulin resistance [60]. These findings indicate that *Lcn2* silencing exacerbates metabolic dysregulation in obesity, highlighting its protective role in regulating metabolism and glucose homeostasis.

4.2.2. Gain of Lipocalin 2 Function in Obesity and Insulin Sensitivity in Male Mice

Three research groups, including ours, used distinct gain of function approaches to investigate the role of LCN2 in glucose metabolism, obesity, and diabetes in male mice. Findings from all three studies consistently indicate that overexpressing *Lcn2* gene benefits glucose metabolism and thermogenesis in male mice.

In our recent study, we developed a transgenic mouse model with *Lcn2* overexpression specifically in adipose tissue, driven by the aP2 promoter [65]. Interestingly, LCN2 protein expression levels in male aP2-*Lcn2* Tg mice was significantly higher in both BAT and Ing-WAT but not Gon-WAT

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under normal and cold conditions compared to WT male mice. Male aP2-*Lcn2* transgenic (Tg) mice showed significantly higher LCN2 protein levels in adipose tissue than WT controls and maintained elevated body temperatures during cold exposure, accompanied by increased thermogenic markers such as *Ucp1* and *Pgc-1α* in Ing-WAT [65]. Additionally, male aP2-*Lcn2* Tg mice fed a RCD showed lower fat depot weights and enhanced expression of genes involved in fatty acid oxidation and thermogenesis. Histological analysis revealed smaller adipocytes and an increased number of UCP1-positive cells in Ing-WAT, indicating improved thermogenesis and beiging of WAT, even without cold exposure. In aging studies, male aP2-*Lcn2* Tg mice maintained elevated expression of thermogenic and mitochondrial genes and exhibited increased AMPK activation and GLUT4 expression in adipose tissues by 18 months of age [65]. These changes contribute to improved glucose tolerance, lower fasting blood glucose, reduced blood triglyceride levels, and decreased liver lipid accumulation, although insulin sensitivity remained unchanged. Overall, these findings suggest that *Lcn2* overexpression in thermogenic adipose tissue (BAT and Ing-WAT) enhances mitochondrial metabolism and thermogenesis, promoting sustained metabolic health during aging.

Similarly, Krishnan et al reported that adipose-specific *Lcn2* overexpression in an *Lcn2*-null background, achieved through adeno-associated virus (AAV) delivery using an adiponectin promoter, improved glucose metabolism and insulin sensitivity in young male mice at 8 weeks of age [66], although body weight and fat mass remained unchanged. However, adipose *Lcn2* overexpression in females produced opposite effects, which will be discussed in the following section.

The Kousteni group developed a transgenic mouse model overexpressing *Lcn2* in osteoblasts under the control of the Col1a1 promotor. In this model, Col1a1-*Lcn2*$^{Tg}$ male mice exhibited a 50% increase in circulating LCN2 levels compared to WT controls. Studies on two cohorts of male mice, at 3 and 6 months of age, revealed significant metabolic benefits in Col1a1-*Lcn2*$^{Tg}$ mice, including a 12.5% reduction in food intake, lower body weight and fat mass, decreased blood glucose levels, increased energy expenditure, and enhanced insulin sensitivity compared to control mice [60). Furthermore, these mice displayed elevated expression of thermogenic markers *Ucp1* and *Pgc1a* in BAT, suggesting that osteoblast-derived LCN2 may exert its metabolic effects by regulating adipose tissue thermogenesis. Collectively, these findings suggest that sustained increases in circulating LCN2 levels, in the absence of metabolic stressors like overnutrition confer metabolic benefits in male mice. These benefits include protection against metabolic deterioration, improved glucose homeostasis, and enhanced thermogenesis.

#### 4.2.3. Lipocalin2 in Obesity and Insulin Sensitivity in Female Mice

Krishnan et al reported that *Lcn2* is a sexually dimorphic gene in adipose tissue, with significantly lower expression in gonadal white adipose tissue but higher expression in the liver of females compared to males [66]. In their study of hybrid mouse diversity panel (HMDP) populations, *Lcn2* expression in (gonadal) adipose tissue was strongly correlated with metabolic dysregulation in females, including obesity, dyslipidemia, insulin resistance and liver steatosis. In contrast, this association was absent in males [66]. Interestingly, liver *Lcn2* expression showed only weak associations with metabolic traits. These findings combined with earlier observations of sex- and depot-specific difference in *Lcn2* expression, suggest that LCN2 plays distinct roles in glucose metabolism, obesity, and insulin resistance in females.

Studies from independent groups highlight contrasting roles of LCN2 in metabolism and insulin resistance between male and female mice. For example, Ishii et al. found that female *Lcn2*-/- mice fed a HFD exhibited improved glucose metabolism, enhanced BAT activity, upregulation of thermogenic genes, and increased energy expenditure compared to WT female mice [67]. In another study, Krishnsn et al investigated the effect of *Lcn2* overexpression on obesity and metabolic homeostasis using a gain-of-function approach [66]. As previously noted, overexpressing *Lcn2* in adipose tissue via AAV delivery of *Lcn2* driven by adiponectin promotor improved glucose metabolism in male mice. However, in female mice, *Lcn2* overexpression in either adipose tissue or liver (on an *Lcn2* null or WT background) exacerbated obesity, glucose intolerance, and insulin resistance when subjected to a high-fat/high-sucrose (HF/HS) diet [66]. Furthermore, mitochondria isolated from female *Lcn2*-overexpressing mice showed reduced activity of electron transport chain complexes [66], suggesting that *Lcn2* overexpression-induced metabolic dysregulation in female mice may be mediated by mitochondrial dysfunction in adipose tissue.

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The study by Meyers et al. explored the role of *Lcn2* deficiency in age-related adiposity and obesity in mice under normal chow diet. The researchers observed that female *Lcn2*-/- mice gained more weight and exhibited increased visceral fat deposition as they aged, compared to WT female mice. Interestingly, this trend was not observed in male *Lcn2*-/- mice, which showed no significant difference in body weight compared to WT males. When analyzing overweight mice (defined as females over 25 g and males over 35 g), only about 40–45% of one-year-old female *Lcn2*-/- mice were significantly heavier than WT controls. However, overall, female *Lcn2*-/- mice exhibited more weight gain and increased visceral adipose tissue accumulation compared to WT females [68]. These findings suggest a potential sex-specific role of LCN2 in age-related weight regulation. Additionally, the study linked *Lcn2* deficiency to the inability to induce beiging of WAT, which might contribute to spontaneous obesity development. This aligns with *in vitro* studies showing that recombinant LCN2 enhances thermogenesis and mitochondrial activity in adipocytes [68]. Together, they concluded that LCN2 plays a protective role in regulating age-related weight gain and fat distribution, particularly in females.

Kamble et al. reported that the effect of dexamethasone on *Lcn2* expression in adipose tissue is both sex- and age-dependent in humans. In subcutaneous and omental adipose tissue from pre-menopausal females, dexamethasone significantly induced *Lcn2* gene expression in a dose-dependent manner, with a corresponding increase in LCN2 protein levels. Interestingly, this effect was not observed in adipose tissue from post-menopausal females and males [69]. Additionally, the study demonstrated that 24-hour treatment with recombinant human LCN2 at the high physiological concentration inhibited basal and insulin-stimulated glucose uptake by about 30% in human subcutaneous adipose tissue, an effect consistent across sexes and menopausal status [69]. Notably, rhLCN2 treatment did not influence basal lipolysis, isoproterenol-stimulated lipolysis, or the inhibitory effects of insulin on lipolysis in human subcutaneous adipose tissue [69]. However, whether recombinant human LCN2 exerts distinct effects on insulin-mediated glucose and lipid metabolism in omental adipose tissue remained unexplored.

These findings underscore the complex and sex specific role of *Lcn2* in glucose metabolism and insulin sensitivity. While *Lcn2* overexpression appears to have a protective effect in male mice, it exerts detrimental effects in females. Additionally, the influence of *Lcn2* deficiency or overexpression on glucose metabolism, obesity, and diabetes is significantly modulated by age and diet. Further investigations are necessary to elucidate the mechanisms underlying sexually dimorphic effects of LCN2, particularly in regulating glucose and lipid metabolism.

## 5. Lipocalin 2 in Energy Balance – Fook Intake and Energy Expenditure

### *5.1. Circulating Lipocalin 2 Levels Correlates with Appetite Signals*

Energy imbalance between food intake and energy expenditure plays a central role in the development of obesity. Recent studies indicate that postprandial LCN2 influences appetite regulation and metabolism, and this postprandial LCN2 response becomes dysregulated with obesity. In the earlier studies involving two cohorts of lean women, postprandial serum LCN2 increased over time, peaking between 45-60 minutes after a meal, and strongly inversely correlated with hunger scores [70]. However, subjects with overweight or obesity showed no postprandial LCN2 response, as indicated in a separate study by Petropoulou et al [71). A significant decrease in postprandial serum LCN2 levels was observed in a cohort including overweight or obese men and women [71]. Petropoulou et al. further investigated this phenomenon by studying three human cohorts with varying body mass index (BMI) levels after a meal following an overnight fast. Participants were categorized as either LCN2 “responders” (R), who showed a positive postprandial LCN2 response, or “non-responders” (NR), who exhibited a negative response. The R group displayed a 12% increase in postprandial LCN2 levels, while the NR group showed a 19% decrease, with the lowest levels observed at 60 minutes. NR individuals had larger waist circumferences, among females, higher BMI, body fat, serum glucose, and diastolic blood pressure compared to the R group [71]. Additionally, patients who had undergone gastric bypass surgery demonstrated a significant increase in postprandial serum LCN2 levels within 15 minutes after a meal, independent of GLP-1 (Glucagon-like peptide-1) or insulin levels [71]. These results suggest that substantial BMI reduction may restore the postprandial LCN2 response, improving hunger signaling. Collectively, these findings support the role of LCN2 as an anorexigenic signal involved in appetite regulation.

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Animal studies further support the role of LCN2 in appetite regulation. Mosialou et al. demonstrated that refeeding WT mice after an overnight fast resulted in a threefold increase in serum LCN2 levels over 1-3 hours, accompanied by suppressed food intake. Interestingly, bone tissue showed a 1.6-fold increases in *Lcn2* expression under these conditions [72]. Additionally, fasted *Lcn2*-/- mice injected with LCN2 exhibited food intake suppression within an hour, mimicking the response observed in WT mice after refeeding [72]. Our group also reported that mice overexpressing *Lcn2* in adipose tissue exhibited fewer feeding sessions compared to WT mice, although their total food intake and activity levels remained unchanged [65].

In another study, Mosialou et al. found that bone is a significant source of LCN2, producing levels 10 times higher than WAT [11]. To distinguish the effects of bone- versus fat-derived LCN2, they generated mice lacking *Lcn2* specifically in osteoblasts (*Lcn2osb*-/-) or adipocytes (*Lcn2fat*-/-). *Lcn2osb*-/- mice displayed greater food intake compared to WT mice, with a two-fold increase two hours after refeeding. Intraperitoneal LCN2 injection in fasted *Lcn2osb*-/- mice suppressed their food intake to WT levels within an hour. Notably, compared to *Lcn2fat*-/- mice, *Lcn2osb*-/- mice exhibited higher food intake, increased fat mass, and greater body weight [11] (Mosialou et al. 2017), underscoring the critical role of bone-derive LCN2 in appetite regulation and energy balance.

Olson et al. recently investigated the role of LCN2 in mediating appetite suppression during pancreatic cancer cachexia, a condition characterized by severe weight loss and metabolic dysfunction. Their study revealed that serum and tumor levels of LCN2 were significantly elevated in pancreatic cancer patients and mouse models of cancer cachexia. Higher circulating LCN2 levels were strongly correlated with reduced food intake and body weight loss in both humans and mice. Importantly, mice lacking LCN2 were protected against cancer-induced appetite suppression and weight loss. These mice exhibited higher food intake and maintained fat and lean mass compared to WT mice with the same cancer burden [73]. The researchers also demonstrated that LCN2 crosses the blood-brain barrier and act on the hypothalamus to suppress appetite, specifically through its interaction with the melanocortin-4 receptor (MC4R) pathway. In mouse models, LCN2 administration reduced food intake and activated the anorexigenic pathway in the hypothalamus [73]. These findings position LCN2 as a critical mediator of hypothalamic signaling and anorexia, contributing to appetite suppression during pancreatic cancer cachexia.

In summary, serum LCN2 levels are associated with satiety in individuals with normal BMI but become dysfunctional in obesity. Studies in humans and animals consistently suggest that LCN2 plays a key role in appetite regulation, promoting satiety and suppressing food intake. The administration of LCN2 to deficient mice or its overexpression further supports its anorexigenic effects, highlighting its potential as a regulator of energy balance and a therapeutic target in metabolic disorders.

### *5.2. Mechanism for the Role of Lipocalin 2 in Appetite Regulation*

Mosialou et al. investigated how LCN2 signals the brain to suppress appetite in male mice, focusing on the hypothalamus. Following fasting and refeeding, they found hypothalamic LCN2 levels doubled in WT mice. In *Lcn2*-/- mice treated with LCN2, normal serum levels were restored within two hours, with LCN2 predominantly accumulating in the hypothalamus, brainstem, and thalamus [72]. Intracerebroventricular (ICV) infusion of LCN2 in *Lcn*2-/- mice restored hypothalamic LCN2 levels, normalizing appetite and reducing weight gain, suggesting a direct effect of LCN2 in hypothalamus. Remarkably, LCN2's anorexigenic effects were comparable to those of leptin and melanotan II (an α-MSH analog) [72].

To elucidate the direct effect mechanism, Mosialou et al. treated murine hypothalamus cell cultures with LCN2, which activated cAMP signaling similar to α-MSH but without triggering AMPK (AMP-activated protein kinase), ERK1 (Extracellular signal-regulated kinase 1), ERK2, or tyrosine kinase phosphorylation [72]. In a follow-up study, the researchers explored hypothalamic pathways downstream of the MC4R activated by LCN2 treatment. Biotinylated-LCN2 was found to bind to neurons in the paraventricular hypothalamus (PVH), a key region for satiety regulation [74]. However, this binding was absent in *Mc4r*-/- mice or in hypothalamic cells (GT1-7) with silenced *Mc4r* gene. Supporting these findings, LCN2 increased cAMP levels in cells with active MC4R signaling but had no effect in cells lacking the LCN2 receptors megalin or Slc22a17/24p3R. This suggests that LCN2 signals through MC4R-mediated anorexigenic pathways in the hypothalamus. WT and *Mc4r*-

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/- mice treated with LCN2 further confirmed this mechanism: while LCN2 suppressed appetite and weight gain in WT mice, it had no effect in *Mc4r*-/- mice. Supporting human data showed that three out of six individuals with a *Mc4r* mutation showed a two- to four-fold increase in serum LCN2 compared to controls [72], further implicating LCN2-MC4R interaction.

Petropoulou et al. extended this research by assessing whether LCN2 crosses the blood-brain barrier. Using radio-labeled recombinant human LCN2 (rh-LCN2), they tracked brain distribution in vervet monkeys with MRI and PET scans, employing a "chase/block" paradigm with an MC4R ligand, α-MSH. Results indicated that radio-labeled rh-LCN2 crossed the blood-brain barrier and displaced α-MSH specifically in the hypothalamus, with no spillover into the thalamus. Further studies in baboons and rhesus macaques confirmed hypothalamic rh-LCN2 binding and competition with α-MSH, suggesting rh-LCN2 has a higher binding affinity for MC4R than α-MSH in primates. Immunofluorescence analysis in baboon and human brain sections corroborated these findings [71). Collectively, these studies demonstrate that LCN2 binds specifically to conserved hypothalamic targets and signals through MC4R activation, exerting anorexic effects across species.

### *5.3. Postprandial Lipocalin 2 in Energy Expenditure*

Previous studies in humans have demonstrated that postprandial LCN2 levels correlate with total energy expenditure (TEE) in women with normal weight but not in those with obesity [70). In this study, women with normal weight and obesity consumed a HFD enriched with monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), or saturated fatty acids (SFA). In the normal-weight group, serum LCN2 levels rose significantly between 0-30 minutes and then gradually declined over 150 minutes. A second wave of serum LCN2 increase occurred between 150-300 minutes across all three fatty acid types, with a notably stronger response to PUFA and MUFA diets than to SFA [70]. Additional studies linked to energy expenditure. For instance, LCN2 levels in normal-weight women were positively correlated with TEE. In contrast, the obese group lacked this biphasic postprandial LCN2 response, as serum levels declined across all fatty acid types. Moreover, while LCN2 levels remained elevated for over 300 minutes in normal-weight women, they consistently decreased in obese participants [70]. These findings underscores a key distinction: although fasting serum LCN2 levels are elevated in obesity, the postprandial LCN2 response is blunted or absent, indicating a dysregulated role in appetite regulation and energy metabolism.

Animal studies further support LCN2'role in appetite suppression and energy regulation. In a crossover study, non-human primates treated with recombinant human LCN2 (rh-LCN2) for two weeks exhibited a 28% reduction in food intake from baseline and a 21% reduction compared to controls [71]. Similarly, in female mice, LCN2 treatment increased oxygen consumption ($VO_2$), carbon dioxide production ($VCO_2$), and TEE compared to phosphate-buffered saline (PBS)-treated controls, without significant changes in body weight [70]. In our own work, transgenic mice overexpressing *Lcn2* (aP2-*Lcn2* Tg) exhibited a downward trend in the respiratory exchange ratio (RER) and a significant reduction in $VCO_2$ during the dark phase, despite no changes in $VO_2$ [65]. In addition to mitochondrial oxidation, lactate accumulation can significantly contribute to $VCO_2$ production, as lactate is buffered by plasma bicarbonate, resulting in $CO_2$ release. This suggests that aP2-*Lcn2* Tg mice may exhibit increased mitochondrial oxidation of both glucose and lipids, leading to reduced lactate production, consequently, a decrease in $VCO_2$ and RER.

Our studies underscores the critical role of LCN2 in adipose tissue thermogenesis and adaptive responses to cold stress. Mice deficient in *Lcn2* exhibited impaired adaptive thermogenesis and cold sensitivity [61]. This was associated with significantly reduced expression of key thermogenic and mitochondrial genes, including *Pparg*, *Pgc1α*, *Prdm16,* and *Ucp1,* in BAT. *Lcn2*-/- mice also displayed decreased mitochondrial biogenesis and oxidative metabolism, confirming LCN2's role in maintaining thermogenic and mitochondrial function in BAT [61,75]. In Ing-WAT, *Lcn2* deficiency similarly impaired beiging and mitochondrial function. *Lcn2*-/- mice displayed significantly reduced *Ucp1* and *Pgc1α* expression, whereas transgenic mice overexpressing *Lcn2* in adipose tissue (aP2-*Lcn2* Tg) exhibited enhanced thermogenic gene expression and maintained higher body temperatures during cold exposure [65,76]. These findings highlight the role LCN2 plays in promoting the beiging of white adipose tissue and thermogenic adaptation. Interestingly, LCN2 appears to regulate thermogenesis through a non-β-adrenergic mechanism [75]. Cold exposure in *Lcn2*-/- mice resulted in reduced phosphorylation of p38 MAPK, a key signaling molecule in thermogenesis, while

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phosphorylation of hormone-sensitive lipase was unaffected [75]. This suggests that LCN2 regulates thermogenic signaling via distinct, non-adrenergic pathways.

In vitro studies provide additional insights into LCN2's role in adipocyte metabolism and function. Differentiated inguinal adipocytes from *Lcn2*-/- mice displayed significantly lower *Ucp1* and *Pgc1α* expression, a reduced NAD/NADH ratio, and impaired mitochondrial respiration, indicating diminished capacity for beiging and mitochondrial function [76]. LCN2 also modulates thermogenesis in response to retinoic acid (RA). RA treatment in WT inguinal adipocytes upregulated UCP1 protein and thermogenic gene expression; however this response was blunted in *Lcn2*-/- adipocytes. RA-induced activation of p38 MAPK was also absent in *Lcn2*-/- adipocytes [76], suggesting that LCN2 facilitates RA-induced thermogenic gene expression through p38 MAPK pathway. In summary, LCN2 plays a critical role in regulating energy balance, adaptive thermogenesis, and mitochondrial function. Its deficiency impairs thermogenic capacity, beiging of WAT, and fat oxidation, whereas overexpression enhances these processes.

Collectively, these findings suggest that LCN2 plays a role in modulating appetite and energy expenditure, with potential therapeutic benefits for obesity-related metabolic dysfunctions. In obesity, LCN2 is upregulated as a compensatory mechanism against adiposity, inflammation, and insulin resistance (IR); however, LCN2 signaling becomes dysregulated, losing its functionality in obesity. The evidence suggests that while LCN2 may initially combat metabolic dysfunction, its efficacy diminishes as obesity progresses, highlighting a complex interplay between LCN2 levels and metabolic health.

## 6. Lipocalin 2 in Mitochondrial Metabolism

### *6.1. Effect of Lcn2 Deficiency on Mitochondrial Metabolism*

Numerous studies have linked LCN2 to mitochondrial metabolism and function. The Weiskirchen group showed that *Lcn2* deficiency significantly reduces proteins essential for mitochondrial regulation and function, as well as those involved in managing hepatic steatosis [77]. MALDI-TOF-MS analysis of liver lipids in WT and *Lcn2*-/- mice revealed notable differences in lipid profiles, particularly in phospholipids and lipids containing arachidonic acid, a polyunsaturated omega-6 fatty acid. This indicates that *Lcn2* deficiency may disrupt arachidonic acid metabolism, affecting lipid distribution and possibly contributing to metabolic disease. Additionally, *Lcn2*-deficient mice exhibited altered mitochondrial integrity, with reduced mitochondrial respiration, increased oxidative stress, and impaired liver energy metabolism. The deficiency also impacted peroxisomal function, impairing fatty acid oxidation and leading to the accumulation of long-chain fatty acids. Interestingly, overexpression of *Lcn2* in *Lcn2*-deficient hepatocytes, but not in WT cells, increased peroxisome numbers, lipid peroxidation, mitochondrial membrane potential, activity, and intracellular ROS production [77]. Based on these findings, they proposed that LCN2 might act as a critical modulator that coordinates peroxisomal and mitochondrial activity to meet fatty acid metabolism demands.

In our earlier studies, we found that *Lcn2* deficiency reduces mitochondrial biogenesis in BAT during cold exposure. Mitochondria in *Lcn2*-/- brown adipocytes exhibited fewer cristae, larger lipid droplets, and a reduced number of mitochondria per cell area, highlighting altered mitochondrial morphology and impaired oxidative metabolism [75]. Furthermore, *Lcn2*-/- adipocytes had lower expression of mitochondrial oxidation genes, such as *Pgc1α*, *Cidea*, and *Pparα*, and increased anaerobic glycolysis, suggesting a metabolic shift due to *Lcn2* deficiency [75]. In contrast, aP2-*Lcn2* Tg mice displayed enhanced mitochondrial function, with increased expression of genes involved in mitochondrial biogenesis and fatty acid oxidation in adipose tissue [65].

In our recent studies, we identified that LCN2 associates with mitochondria and mitochondria-associated endoplasmic reticulum membranes (MAMs) in adipocytes, and LCN2 specifically binds to phosphatidic acid (PA), demonstrating strong affinity for this lipid [78].

We further showed that *Lcn2* deficiency alters the acyl-chain remodeling of the mitochondrial phospholipidome, disrupting mitochondrial fission-fusion dynamics and impairing mitochondrial function [78]. PA, beyond its role in mitochondrial phospholipid biosynthesis, regulates enzymes in phospholipid metabolism, including diacylglycerol kinase (DGK), phospholipase D (PLD), phospholipase A2 (PLA2), and LIPIN1 [79–81], all of which influence PA levels. Certain PA species, such as 16:0-18:1 and PA 14:0-16:1, have been linked to DGKδ and type 2 diabetes [82,83], while DGKs

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regulate the conversion of DAG (diacylglycerol) to PA, maintaining cellular PA levels. In *Lcn2*-deficient BAT, the sum of six PA species was significantly reduced, particularly PA (16:0-18:2), PA (16:0-18:1), and PA (18:0-20:2), while DAG levels increased.

Correspondingly, PLD expression was lower, LIPIN1 levels were higher, and four out of six DGK isofroms showed altered expression in *Lcn2*-/- BAT. These findings indicate that LCN2 is essential for the recursive regulation of PA production and enzyme activity within the DAG-PA and PLD-PA pathways, ensuring phospholipid homeostasis. Collectively, this study highlights the critical role of LCN2 in mitochondrial lipid remodeling and dynamics, contributing to overall energy metabolism and thermogenesis in BAT. Disruptions in mitochondrial function and phospholipid remodeling in *Lcn2*-deficient mice impaired mitochondrial respiration and ATP production, ultimately diminishing BAT's thermogenic capacity. Thus, LCN2 plays a key role in promoting energy expenditure through mitochondrial activity.

### *6.2. Effect of Lcn2 Overexpression on Mitochondrial Metabolism*

Song et al studied the effects of mouse recombinant LCN2 on mitochondrial metabolism in cardiomyocytes. They observed that exposure to holo-LCN2, a complex of LCN2, siderophore, and iron in a 1:3:1 ratio, led to the elevation of mitochondrial reactive oxygen species (ROS) production in cardiomyocytes isolated from male rats. This ROS increase impaired oxidative phosphorylation by reducing mitochondrial membrane potential and ATP production, ultimately compromising cellular energy metabolism [84]. These findings suggest that holo-LCN2 and its associated siderophores promote mitochondrial ROS accumulation and oxidative phosphorylation disruption, leading to mitochondrial dysfunction and oxidative stress in cardiomyocytes.

Similarly, Krishnan et al (2019) demonstrated that *Lcn2* overexpression driven by adiponectin promotor induces mitochondrial dysfunction in adipocytes in vitro. Their *in vivo* studies further linked adipose *Lcn2* overexpression to mitochondrial dysfunction in female mice, resulting in reduced energy expenditure and insulin resistance [66].

Adding to this body of evidence, Liu et al. (2022) examined the role of LCN2 in mitochondrial dysfunction under septic conditions. They reported that sepsis-induced *Lcn2* overexpression disrupted mitochondrial membrane potential, reduced ATP production, and elevated mitochondrial ROS levels in the heart. This mitochondrial dysfunction aggravated cardiac injury, emphasizing LCN2's role in exacerbating mitochondrial oxidation and ROS production, leading to cellular damage during sepsis [85].

Marques et al. recently investigated the role of LCN2 in mitochondrial function within renal tubular cells [86]. They found that acute kidney injury, such as renal ischemia-reperfusion injury (IRI), strongly induced *Lcn2* expression in the thick ascending limb (TAL) and collecting duct (CD) segments of the nephron. Using mouse inner medullary CD cells (mIMCD-3) with stable knockdown of *Lcn2* via short hairpin RNA (sh-*Lcn2*) or scramble control cells, they explored LCN2's role in mitochondrial function in vitro. Under unstimulated conditions, *Lcn2* knockdown cells exhibited greater mitochondrial mass and function, as well as larger, elongated, and tubular mitochondria compared to scramble control cells [86]. Conversely, overexpression of *Lcn2* in cells that do not typically express it led to reduced mitochondrial length, suggesting increased mitochondrial fragmentation or fission. However, a limitation of this study was that it only assessed the role of LCN2 under baseline conditions and did not investigate the impact of IRI-induced *Lcn2* expression on mitochondrial function. This distinction is critical, as *Lcn2* is a highly inducible gene with low baseline expression and primarily exerts its functions under stress conditions. Additionally, the study showed that intracellular, rather than secreted LCN2 mediated these effects on mitochondrial dynamics, ruling out an autocrine role in renal tubular cells. In contrast, Qiu et al. demonstrated a protective role of secreted LCN2 against IRI in proximal tubular cells [87]. The discrepancy between these studies likely arises from differences in the forms of LCN2 examined: intracellular versus secreted and the types of nephron cells studied.

## 7. Conclusions

LCN2 shows a sex-dimorphic and depot-dependent expression in adipose tissue, adding complexity to its role and highlighting differences between male and females. While *Lcn2* has low baseline expression, its levels markedly increase in response to stress stimuli such as infection, injury,

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inflammation, and metabolic stress, suggesting its primary function is stress-related. LCN2 plays a significant role in energy balance, including food intake and energy metabolism in obesity and diabetes. Its effects on mitochondrial function appears to be double-edged. Under normal conditions, such as after a meal, LCN2 promotes mitochondrial metabolism to maintain energy balance. However, in obesity, this adaptive response is absent, leading to reduced mitochondrial metabolism and energy expenditure. In *Lcn2*-deficient conditions, the inability to upregulate mitochondrial oxidation under metabolic stress further impairs mitochondrial function. Conversely, overexpression of *Lcn2* in experimental models or pathological conditions (e.g. sepsis) can lead to excessive mitochondrial activation, resulting in overproduction of ROS, oxidative stress, and mitochondrial dysfunction. The detrimental effect of *Lcn2* overexpression appears to be cell-type dependent. For example, thermogenic adipocytes (brown and beige adipocytes) mitigate these detrimental effects via their uncoupling system, whereas cells lacking this system, such as cardiomycytes and renal cells, are more susceptible to damage. However, further research is needed to elucidate the complex and context-dependent functions of LCN2 and resolve conflicting findings, particularly in mitochondrial metabolism and metabolic disorders.

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