UNIVERSITY^{OF} BIRMINGHAM University of Birmingham Research at Birmingham

Unwrapping the mechanisms of ceramide and fatty acid-initiated signals leading to immuneinflammatory responses in obesity

Kucuk, Salih; Niven, Jennifer; Caamano, Jorge; Jones, Simon; Camacho-muñoz, Dolores; Nicolaou, Anna; Mauro, Claudio

DOI: 10.1016/j.biocel.2021.105972

License: Creative Commons: Attribution-NonCommercial-NoDerivs (CC BY-NC-ND)

Document Version Peer reviewed version

Citation for published version (Harvard):

Kucuk, S, Niven, J, Caamano, J, Jones, S, Camacho-muñoz, D, Nicolaou, A & Mauro, C 2021, 'Unwrapping the mechanisms of ceramide and fatty acid-initiated signals leading to immune-inflammatory responses in obesity', *The International Journal of Biochemistry & Cell Biology*, vol. 135, 105972. https://doi.org/10.1016/j.biocel.2021.105972

Link to publication on Research at Birmingham portal

General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

•Users may freely distribute the URL that is used to identify this publication.

•Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.

•User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?) •Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.

- 1 Title: Unwrapping the mechanisms of ceramide and fatty acid-initiated signals leading to immune-inflammatory responses in obesity
- 2 3

4

Authors:

- Salih Kucuk^{a,1}, Jennifer Niven^{a,1}, Jorge Caamano^{b,2}, Simon Jones^{a,2}, Dolores Camacho-Muñoz^{c2}, Anna 5
- 6 Nicolaou^{c,d,2}, Claudio Mauro^{a,2,*}
- 7 ^aInstitute of Inflammation and Ageing, College of Medical and Dental Sciences, University of Birmingham,
- 8 Birmingham, UK.
- 9 ^bInstitute of Immunology and Immunotherapy, College of Medical and Dental Sciences, University of
- 10 Birmingham, Birmingham, UK.
- 11 ^cLaboratory for Lipidomics and Lipid Biology, Division of Pharmacy and Optometry, Faculty of Biology,
- 12 Medicine and Health, School of Health Sciences, The University of Manchester, Manchester Academic Health
- 13 Science Centre, Manchester, UK.
- 14 ^dLydia Becker Institute of Immunology and Inflammation, Faculty of Biology, Medicine and Health, The
- 15 University of Manchester, Manchester Academic Health Science Centre, Manchester, UK.
- 16 ¹ These authors contributed equally to this study – First authors
- 17 ² These authors contributed equally to this study - Senior authors
- 18 *Corresponding author email: c.mauro@bham.ac.uk

20 Abstract

19

- 21 Obesity is considered a global epidemic developed in part as a consequence of the overconsumption of a high
- 22 fat diet. One of the main negative outcomes of obesity is the development of low-grade chronic systemic
- 23 inflammation, induced by dysregulated immune responses, which can lead to multiple obesity-related diseases.
- 24 Ceramides are a group of bioactive lipids known to be elevated in obesity and obesity-associated conditions,
- 25 including cardiovascular disease and type II diabetes. Ceramides may be key players in promoting an obesity-26
- induced inflammatory environment due to their ability to activate key pathways such as TLR4 and Nlrp3, while 27 studies have shown that inhibition of ceramide synthesis gives rise to an anti-inflammatory environment. N-3
- 28 polyunsaturated fatty acids (n-3 PUFA) have been of interest due to their anti-inflammatory actions and shown
- 29 to have beneficial effects in obesity-related diseases. This review will highlight the impact of ceramides in
- 30 promoting an obesity-induced inflammatory microenvironment and discuss how n-3 PUFA could potentially
- 31 counteract these responses and have a regulatory effect promoting immune homeostasis.

33 **Keywords:**

34 Ceramides, obesity, inflammation, fatty acids, N-3 polyunsaturated fatty acids

36 Abbreviations:

- 37 Acid sphingomyelinase (ASM), cardiovascular diseases (CVD), dihydroceramide desaturase (DES),
- 38 dihydroceramide synthase (CerS), docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), fatty acid (FA),
- 39 high fat diet (HFD), obesity-induced inflammatory microenvironment (OIIM), serine palmitoyltransferase
- 40 (SPT), sphingomyelinases (SMase), Type II diabetes (T2D), western diet (WD), World Health organisation
- 41 (WHO), ceramide synthase enzyme (CerS)
- 42 43

32

44 Introduction

- 45 Obesity is one of the major worldwide health concerns of the 21st century. In 2016, 650 million adults were
- 46 classified as obese, 1.9 billion were classified as overweight and the current rising trend estimates that 1 billion
- 47 people would be obese by 2025 (WHO, 2016). Obesity-induced inflammatory microenvironment (OIIM) and
- 48 dysregulated immune responses have been shown to induce, escalate or be associated with most obesity-linked
- 49 diseases including cardiovascular disease (CVD), type II diabetes (T2D), cancer, osteoarthritis, depression and
- 50 autoimmune diseases (Hruby et al., 2016). Obesity can also impact upon many other conditions such as the
- 51 recent severe acute respiratory disease SARS-CoV-2. Meta-analysis of 400,000 SARS-CoV-2 patients has
- 52 shown that obesity increases infection risk by 46%, hospitalisation risk by 113% and death rate by 48%, while
- 53 there are concerns that vaccines might be less effective in obese individuals (Popkin et al., 2020). It is therefore
- 54 crucial to identify the main players driving OIIM and the molecular mechanisms underlying disease initiation.
- 55 The critical link between obesity and diet is well recognised (Bortolin et al., 2018; Almeida-Suhett et al., 2019).
 56 Excessive lipid accumulation because of the overconsumption of high fat (HFD) or western (WD) diets, leads to
- 56 Excessive lipid accumulation because of the overconsumption of high fat (HFD) or western (WD) diets, leads to 57 the expansion of white adipose tissue. Adipose tissue contains many immune cells, including macrophages and
- 57 the expansion of white dupose disde. Adaptive domains many minute cens, meridding inderophages a 58 T cells. Under lean conditions, adipose tissue maintains an anti-inflammatory environment, with M2 like
- 59 macrophage populations and regulatory T cells. However, in obese conditions the adipose tissue environment
- 60 changes, leading to the development of low-grade chronic systemic inflammation, due to shifts in the presence
- 61 immune cell populations (Donohoe et al., 2016).
- 62 Certain bioactive lipids have been shown to be elevated in obesity and modulate multiple critical immune cell
- 63 processes, affecting obesity-induced inflammation (Chaurasia et al., 2016; Hamada et al., 2014 and Chaurasia et
- 64 al., 2019). It is crucial to identify the main bioactive lipid species and explore their involvement into the
- 65 molecular mechanisms leading to OIIM. Sphingolipids, in particular ceramides, have attracted interest due to
- 66 their immunomodulatory effects. Here, we summarise the immunomodulatory effects of ceramides in the
- 67 establishment of OIIM and discuss the therapeutic potential of using n-3 polyunsaturated fatty acids (n-3 PUFA)
- 68 to promote an anti-inflammatory immune response.

69

70 The establishment of obesity-induced inflammatory microenvironment

- 71 The World Health Organisation (WHO) has defined obesity as abnormal or excessive fat accumulation that
- 72 presents a risk to health. Diets rich in high fat, sugar, salt and low fibre content such as HFD and WD have been
- 73 shown to induce obesity (Almeida-Suhett et al., 2019). Overconsumption of these diets with disrupted balance
- in energy homeostasis leads to the accumulation of excessive lipids in adipose and non-adipose tissue of thebody (Donohoe et al., 2016).
- 76 The infiltration of macrophages and lymphocytes into adipose tissues leads to an immune tipping point, with the
- 77 production of inflammatory cytokines and lipid mediators giving rise to a chronic low-grade inflammatory
- 78 environment (Yang et al., 2010 & Christ et al., 2018). Murine models of diet-induced obesity have shown naïve
- 79 adipose-resident T cells decrease and give rise to effector-memory populations, in which the TCR-Vβ repertoire
- 80 were also impacted (Yang et al., 2010). Importantly, these mice had elevated levels of IFN- γ^+ , granzyme and
- 81 other pro-inflammatory mediators which account for the obesity-induced chronic inflammation (Yang et al.,
- 82 2010). WD was shown to be misinterpreted as a threat to the host, in which innate immune system cells became
- 83 hyperactive through inflammasome activation, resulting in the production of pro-inflammatory cytokine
- 84 response via IL-1b forming a hyperactive inflammatory environment (Christ et al., 2018). These studies
- 85 illustrated an extensive pro-inflammatory effect linked to the diet which 'reprogram' or 'prime' both innate and
- 86 adaptive immune cells towards a low-grade chronic inflammatory microenvironment observed in obesity.
- 87 Recent developments in lipidomic techniques have allowed lipid profiling of tissues from overweight and obese
- 88 individuals, identifying ceramides which could potentially act on immune cell processes and promote obesity-
- 89 induced inflammation.
- 90

91 Ceramide Biosynthesis

- 92 Ceramides are sphingolipids, synthesised in the endoplasmic reticulum (ER), that have been considered as key
- 93 players in promoting the OIIM. Ceramides are derivatives of long-chain bases (e.g. sphingosine) and FAs of
- 94 varying lengths (Hannun & Obeid, 2017). Not only are they a universal component of the cellular membrane,

they also play crucial roles in cell proliferation, apoptosis, migration, senescence, autophagy and inflammation
 (Hannun & Obeid, 2017, Chaurasia & Summers, 2020).

97

Dysregulation in sphingolipid metabolism has been associated with various inflammatory diseases including
 cancer, obesity and autoimmune conditions (Chaurasia & Summers, 2020). Elevated ceramide levels within

- 100 specific tissues have been associated with different pathological conditions (Table 1). Lipidomic analysis has
- 101 shown ceramides to accumulate in multiple tissues including liver, skeletal muscle, heart and adipose tissue in
- 102 obesity (Kien et al., 2013, Chaurasia & Summers, 2020).
- 103

Ceramide biosynthesis is regulated by three pathways; *de novo* synthesis, sphingomyelin hydrolysis and the salvage pathway. The *de novo* pathway is the main biosynthetic route, starting with the condensation of palmitate and serine, although other saturated fatty acids and amino acids can be involved (Hannun & Obeid, 2017). The main enzymes of the *de novo* pathway are serine palmitoyltransferase (SPT), that is also the rate limiting step, 3- ketodihydrosphingosine reductase, dihydroceramide synthase (CerS) and dihydroceramide desaturase (DES) (Figure 1). The other two pathways involved in ceramide biosynthesis mainly function as recycling pathways which break down surplus complex sphingolipids via catabolic enzymes including various

sphingomyelinases (SMase). Acid sphingomyelinase (ASM) is one member of the hydrolase enzymes that

112 catalyses the breakdown of sphingomyelin to create ceramide (Xiong et al., 2016). Targeting the ceramide

- biosynthetic pathways can be used to assess the impact of these bioactive lipids on specific immune cell
- 114 populations (Table 2).
- 115

116 Overconsumption of obesity promoting diets containing the key substrates, palmitoyl-CoA and serine, for 117 ceramide synthesis is likely to promote this elevation. In addition, inflammatory modulators, which are elevated 118 in the OIIM, can regulate cellular ceramide metabolism elevating cellular ceramide levels. Inflammatory 119 cytokines IL-1 β and TNF α have been shown to promote the accumulation of ceramides (Gill & Sattar, 2009, 120 Haus et al., 2009, Holland et al., 2011). TNF α has been shown to specifically induce genes involved in the 121 ceramide synthesis pathway, such as serine palmitoyltransferase (SPT) in murine fibroblast (L929) (Meyer& de 122 Groot, 2003). Sphingomyelinase (SMase), a key enzyme in the sphingomyelin hydrolysis pathway for ceramide 123 production has been shown to not only promote ceramide accumulation in bovine cerebral endothelial cells (Xu 124 et al., 1998) but recently, inhibition of neural sphingomyelinase-2 resulted in down regulation of the TNF α -125 mediated expression of CD11c and secretion of inflammatory mediators IL1 β and MCP-1 in 126 monocytes/macrophages via the phosphorylation of JNK, p38 and NF-κB/NF-kB/AP-1 pathways (Al-Rashed et 127 al., 2020). These in vitro observations are also supported by in vivo research where intratracheal administration 128 of TNFa boosted ceramide levels via inducing neutral sphingomyelinase in murine models (Mallampalli et al., 129 1999). Additionally, it is known that TNF α can inhibit insulin signalling via stimulation of p55 TNF receptor 130 and sphingomyelinase activity, which plays a critical role in obesity-induced insulin resistance (Peraldi et al., 131 1996). In addition, it is well documented that the accumulation of adipose tissue during obesity leads to decrease 132 in adiponectin, an important adipokine and their receptors (Nigro et al., 2014). Adiponectin has been described 133 to regulate ceramide metabolism via the adiponectin receptor, expressed endogenously by various cells 134 including T cells (Holland et al., 2011). Previous reports have shown that activation of the adiponectin receptor 135 leads to ceramidase activity and consequently to a decrease of ceramide levels, acting as a negative regulator in 136 cardiomyocyte and pancreatic β cell. As adiponectin levels are reduced in obesity, this may lead to increased 137 ceramide accumulation (Holland et al., 2017). 138 139 TLR-4-stimulated macrophages activate the transcription factor NF- κ B via I κ B kinase (IKK β). A knockout of 140 IKKβ which inhibited the pro-inflammatory signalling mediated via TLR-4, led to a significant reduction in

141 ceramide synthesis (Holland et al., 2011). To further confirm the role of TLR-4-mediated ceramide synthesis,

142 macrophages were supplemented with palmitate but even the palmitate supplementation was not able to restore

- 143 ceramide levels. Signalling pathways modulated by ceramides to induce a pro-inflammatory response can also
- 144 enhance ceramide biosynthesis creating an inflammatory feed forward loop, which could be one of the critical
- 145 mechanisms maintaining a low-grade chronic inflammation.
- 146

147 Immunomodulatory mechanisms of ceramide action

148 Ceramides have been shown to modulate the function of immune system cells. Initially, studies have focused on

149 cells of the innate immune system while recent studies have suggested a link between ceramides and the

adaptive immune cell response (Chaurasia et al., 2016, Hamada et al., 2014, Turpin et al., 2014). Although the

- 151 precise mechanisms are still not fully understood, below we will discuss the immunomodulatory roles ceramides
- play in principal immune cells and how their modulation impacts their function and fate contributing to OIIM(Figure 2).
- 154

155 *Ceramide modulation on macrophages*

156 In obesity, macrophages are pivotal players in maintaining the tissue homeostatic microenvironment, but can

also promote the OIIM via polarising to a pro-inflammatory fate, producing inflammatory cytokines such as IL-

158 1β and TNFα (Chaurasia et al., 2016 & Haus et al., 2009). A clear association between these pro-inflammatory

159 cytokines and increased ceramides levels in obesity, particularly in adipose tissue and circulating serum has
 160 been described (Haus et al., 2009). In peripheral adipose tissue, both genetic and pharmacological inhibition (via

161 myriocin) of serine palmitoyltransferase (Sptlc) enzyme and ceramide synthesis pathway altered macrophage

162 populations, with a reduction of pro-inflammatory cytokines, including IL-6 and TNF α and an increase in the

163 M2 anti-inflammatory macrophage markers, such as IL-10 (Chaurasia et al., 2016).

164

165 Inhibition of ceramide biosynthesis may elicit, including improved glucose tolerance, increased insulin

166 sensitivity, amelioration of hepatic steatosis and reduction in adipocyte size (Chaurasia et al., 2019). Alterations

167 of the M1/M2 macrophage ratio with associated altered gene and cytokine expression, can push OIIM towards

168 an anti-inflammatory homeostatic environment (Chaurasia et al., 2016). Injection of nanoliposome-loaded C6-

169 ceremide to mice models was able to enhance the M1 cytokine production (including IL12, IFN- γ and TNF- α)

170 and inhibit M2 cytokine production in bone marrow-derived macrophages. This observation is important to

171 conceive the flexibility of the immune response in relation to ceramides, which can be easily driven to pro/anti-

172 inflammatory states (Li et al., 2018). Although ceramide synthase enzyme (CerS)-6 deficient mice present

173 reduced macrophage infiltration within gonadal white adipose tissue and improved insulin homeostasis, 174 macrophage-specific CerS6 deletion did not specifically reduce the body weight/adiposity nor improve insulin

174 macrophage-specific CerS6 deletion did not specifically reduce the body weight/adiposity nor improve insulin 175 sensitivity (Turpin et al., 2014). These observations highlight the crucial pathophysiological role of ceramides,

176 demonstrate immunomodulatory effects on OIIM and illustrate the complexity underlying these processes that

177 involve multiple tissues and immune cell types. In addition, reduced macrophage infiltration into white adipose

178 tissue of CerS5 knockout mice fed HFD was observed, as well as a reduction in pro-inflammatory gene

179 expression such as Caspase1, TNF α and IL-1 β (Gosejacob et al., 2016).

180

181 The Nod Like Receptor 3 (Nlrp3) has been shown to be a critical link between ceramide accumulation and

macrophage activation leading to OIIM. Nlrp3 can sense bioactive lipids including ceramides, which trigger the

183 macrophages activation and infiltration to the peripheral OIIMs and ablation of Nlrp3 prevented the obesity–

184 induced inflammasome activation in fat depots and liver together with enhanced insulin–signalling.

Additionally, when Nlrp3 ceramide sensing is interrupted, the levels of pro-inflammatory cytokines (IL-18,

186 IFNγ) are reduced alongside with the number of effector adipose tissue T cells. Importantly, the number of naïve

187 T lymphocytes (CD4⁺CD62L⁺CD44⁻, CD8⁺CD62L⁺CD44⁻) are increased suggesting an ameliorative anti-

188 inflammatory effect on OIIM and obesity-associated pathologies, also supporting the role of ceramides in both

- 189 innate and adaptive immune systems (Vandanmagsar et al. 2011).
- 190

191 *Ceramide modulation on Neutrophils*

192 In addition to macrophage infiltration into adipose tissue, other cells of the innate immune system have been

- 193 shown to infiltrate, promoting the OIIM. Neutrophils have been shown to be elevated in obese individuals (Xu
- 194 et al., 2015) and infiltrate into adipose tissue at the early stages of HFD consumption (Elgazar-Carmon et al.,
- 195 2008 & Hadad et al., 2013). Levels of basal superoxide production and also formyl-methionyl-leucyl-
- 196 phenylalanine (fMLP)-stimulated superoxides were found to be elevated in neutrophils isolated from obese
- 197 indivuals (Brotfain et al., 2015). Both in stimulated and unstimulated conditions, neutrophils from obese

198 microenvironment were primed to increase superoxide production and chemotactic activity that can drive OIIM 199 (Brotfain et al., 2015). Early studies have shown in vitro priming of neutrophils with C2 ceramide lead to 200 enhanced superoxide levels followed by fMLP treatment (Richard et al., 1996). However, this response may be 201 concentration dependent, as in an additional study, in vitro activated neutrophils showed C2 ceramide to 202 increase (< 1 microM) and inhibit (> 1 microM) superoxide generation (Wong K., Li X. B., & Hunchuk N., 1995). Neutrophil extracellular traps (NETs), DNAs components that are known to activate immune responses, 203 204 have been shown at elevated levels in obese individuals (D'Abbondanza et al., 2019). One study has shown how 205 the enhancement of NET production can occur via a ceramide/PKCζ-mediated pathway, and treatment with 206 synthetic ceramide is sufficient to promote NET formation (Corriden et al., 2015). Furthermore, intracellular 207 C16 and C24 levels shown to contribute to spontaneous neutrophil apoptosis via caspase activation, *in vitro*. 208 Pharmacological inhibition of de novo pathway, reducing ceramide accumulation, created an anti-apoptotic 209 effect on neutrophils (Seumois et al., 2007). 210 211 212 Ceramide modulation on T cells

- T cells have also shown to play a role in obesity and promotion of the OIIM. In HFD murine models, elevated
- 214 CD8⁺ effector T cells were found to infiltrate obese epididymal adipose tissue even before macrophage
- accumulation, with reduced numbers of $CD4^+$ helper and regulatory T cells (Nishimura et al., 2009). Genetic
- 216 depletion of CD8⁺ T cells inhibits OIIM cascade with reduced macrophage infiltration and improved systemic
- 217 insulin resistance. It is now clear that CD8⁺T cells play crucial roles in macrophage differentiation, activation
- and infiltration which contribute to the initiation and maintenance of OIIM (Nishimura et al., 2009).
- 219 Ceramide biosynthesis has been shown to impact effective TCR signalling, activation and effector responses. In
- 220 graft versus host murine models, acid sphingomyelinase (ASMase) deletion resulted in reduced CD8⁺ activation
- 221 and effector responses (Rotolo et al., 2009). Sofi et al. showed that CerS6 was as required for optimal T cell
- response, with reduced allogeneic responses observed due to impaired C16 ceramide production (Sofi et al.,
- 223 2017). The ceramide biosynthesis pathways have also been shown to impact CD8 granule-mediated
- 224 cytotoxicity, in which sphingomyelinase (ASMase)-deficient mice are defective in exocytosis of cytolytic
- 225 effector molecules (Herz et al., 2009).
- 226 Other studies have suggested that ceramides can promote T cell differentiation towards a specific pro- or anti-
- inflammatory fate, in a concentration and tissue-specific manner (Martín-Leal et al., 2020). Palmitate, a 16-
- carbon saturated FA, is a main product of de novo fatty acid biosynthesis and common dietary component of
 HFD. In both humans and rodents (including obese and insulin resistance murine models) elevated systemic FA
- availability via acute infusion, can increase plasma ceramide concentrations (Watt et al., 2012 and Tran et al.,
- 231 2016). Palmitate has been shown to promote obesity related low-grade chronic inflammation and can directly
- prime CD4⁺ T cell differentiation into a CD44^{hi}-CCR7^{lo}-CD62L^{lo}-CXCR3⁺-LFA1⁺ effector memory-like
- phenotype (Mauro et al., 2017). This bias in CD4⁺ T cell differentiation leads to a preferential trafficking to non-
- 234 lymphoid periphery sites with an increased effector Th1/Th17 pro-inflammatory function, contributing to the
- creation of OIIM. Palmitate was shown to modulate CD4⁺T cells via enhanced activation of PI3K p110d-Akt-
- dependent pathway (Mauro et al., 2017). Furthermore, ceramides can push homeostasis towards a pro-
- inflammatory environment via reducing the Foxp3⁺ Treg population (Zhou et al., 2016 and Hollmann et al.,
- 238 2016). As mentioned, ASM is a major enzyme in the synthesis of ceramides from sphingomyelin. In ASM
- specific knock out mice, *in vitro* and *in vivo* assays illustrated that ASM ablation leads to a higher number of
- Tregs (Zhou et al., 2016). In addition to elevated Treg frequency, the suppressive activity of Tregs was also
- enhanced both in the ASM knock out mice and in the presence of amitriptyline, a ASM pharmacologicalinhibitor (Hollmann et al., 2016).

243 Ceramide modulation on B cells

Although changes in macrophages and T cells have been the main focus on the development on the OIIM, B

- 245 cells also contribute to the inflammatory environment. In diet-induced obese mice, Winer *et al* showed how B
- 246 cells accumulate in visceral adipose tissue. Depletion of B cells resulted in the suppression of M1 macrophage
- 247 activation, polarisation and CD8⁺ T cell activation with significant reduction in TNF- α and IFN- γ pro-
- 248 inflammatory cytokine levels in adipose tissue stromal vascular cells (Winer et al., 2011). Importantly, B cells

- 249 were shown to promote systemic as well as local (visceral adipose tissue) inflammation in obesity-associated
- 250 diseases such as insulin resistance and glucose intolerance. B^{null} mice showed improved glucose/insulin
- 251 sensitivity, adipose tissue inflammation, reduced adipose tissue hypertrophy and no impact on circulating
- adiponectin levels (DeFuria et al., 2013). Consistent with the reduction observed in inflammatory B- and T-cell
- cytokine levels, reduced inflammation in obese/insulin resistant B cell-null mice associated with an increased
- percentage of anti-inflammatory Tregs (DeFuria et al., 2013). Additionally, ceramides were linked to B cell associated apoptotic program known as activation induced cell death triggered by B cell receptor. Specifically,
- 256 long chain ceramides C16, C18 and C24 synthesised via de novo pathway were shown to cross-link with B cell
- 257 receptor. This cross-linking triggers a specific sequence of biochemical evets leading to caspase-dependent
- apoptosis which potentially involves a mitochondrial damage and loss of function (Kroesen et al., 2001 &
- 259 Kroesen et al., 2003). Furthermore, ceramides (C2) are known to inactivate the anti-apoptotic protein B cell
- 260 lymphoma 2 (Bcl2) via dephosphorylating and reducing the mRNA levels of Bcl2 (Ruvolo et al., 1999 & Chen
- 261 et al., 1995).

262 Therapeutic potential of n-3 PUFA on immune function and OIIM

263 N-3 PUFA could be used to counteract the inflammatory response resulting from the over consumption of a

HFD. Although the extent of their effects is still debated, n-3 PUFA have been shown to have overall anti-

265 inflammatory effects, for example, n-3 PUFA supplementation has been shown to lower blood pressure and

- reduce the levels of triglycerides, leading to a reduced risk of CVD (Hu et al., 2019). In inflammatory diseases,
- such as type II diabetes, n-3 PUFA improved insulin sensitivity reduced the body fat ratio (Martins et al., 2018
- and Gutiérrez et al., 2019).
- 269 The molecular mechanism of the anti-inflammatory action of n-3 PUFA remains of interest and is subject of on-
- 270 going investigations. Increasingly, evidence suggests that n-3 PUFA act in three major ways to help the
- 271 restoration of homeostasis in OIIM. Firstly, n-3 PUFA are recognised by distinct receptors such as TLR4, Nlrp3
- and G protein-coupled receptors, mainly GRP120 expressed by immune cells (such as macrophages) leading to
- reduced inflammatory cytokine and chemokine responses (Oh et al., 2010 and Mildenberger et al., 2017).
- 274 Secondly, n-3 PUFA can modulate the differentiation and motility of T lymphocytes (Cucchi et al., 2019).
- Thirdly, n-3PUFA dilute the availability of pro-inflammatory n-6PUFA substrates (Nicolaou et al., 2014 and
- 276 Kendall et al., 2019)
- 277 As discussed, the saturated FA palmitate and ceramides can directly recognise and activate macrophages via
- TLR4 receptor (Liu et al., 2013 and Eraky et al., 2018). This is a crucial point in which n-3 PUFA could act to
- rebalance the immune response and promote an anti-inflammatory environment and potentially ameliorate
- 280 obesity-associated diseases such as T2D (Eraky et al., 2018). In a human clinical trial, overweight/obese
- pregnant women were daily supplemented with n-3 PUFA which led to down-regulation of TLR4 expression
 and reduction of IL-6, IL-8, and TNFα production in both adipose and placental tissues (Haghiac et al., 2015).
- Further, *in vitro* assays on cells isolated from maternal subcutaneous adipose tissue and placenta illustrated that
- palmitate was responsible for a 10-30-fold increase in the expression of TLR4, IL-6 and IL-8. Whereas n-3
- 285 PUFA supplementation in an ex-vivo cell culture, significantly reduced the transcription of TLR4, IL-6, IL-8
- induced by palmitate, reducing its inflammatory effect by 70% (Haghiac et al., 2015). N-3 PUFA recognition by
- 287 GPR120 significantly reduced the production of pro-inflammatory cytokines such as IL-6, IL-8 and TNF- α
- which is mediated by TLR4 (Haghiac et al., 2015 and Liu et al., 2013). Additionally, activation of GPR120 by
- 289 n3- PUFA reduced the levels of pro-inflammatory cytokines IL-1b, TNFα, MCP-1 and also lead to IL-10-
- 290 mediated promotion of M2 anti-inflammatory genes in adipose tissue (Yan et al., 2013 and Lee et al., 2019).
- 291 Consistently, GPR120 knockout in both murine model and *in vitro* monocytes and macrophages abrogated the
- anti-inflammatory effects of n3- PUFA. After activation by n3-PUFA, the GPR120 coupled to β -arrestin2
- leading to the inhibition of TAB1-mediated activation of TAK1, which may account for the inhibition of both
- the TLR and TNFα pro-inflammatory signalling pathways (Oh et al., 2010).
 Nu 2 in the inflammatory signalling pathways (in the second sec
- Nlrp3 is another important innate immune cell receptor which can drive the anti-inflammatory effect of n-3
- PUFA on OIIM, via inhibition of inflammasome activation (Kumar et al., 2016) and subsequent expression of
- 297 pro-inflammatory cytokines such as IL-1b, TNFα, MCP-1 (Yan et al. 2013). Importantly, n-3 PUFA-dependent

- 298 inhibition of Nlrp3 activation has been shown to exert beneficial effects on HFD-induced T2D murine models
- via reconstitution of a homeostatic cellular microenvironment (Kumar et al., 2016 & Yan et al., 2013) and in
- 300 clinical trials via downregulation of the expression of various pro-inflammatory cytokines such as IL-18 and IL-
- 1 β in adipocyte and macrophages (Lee et al., 2019).
- 302 Furthermore, n-3 PUFA limit ceramide synthesis via diverting their major substrate palmitate (Tachtsis et al.,
- 303 2020) but also via directly inhibiting the ceramide biosynthetic enzyme genes serine palmitoyl transferase long
- 304 chain base (Sptlc) and degenerative spermatocyte homolog 2 (Des2), as shown in the liver of a murine model of
- 305 hepatic steatosis (Dong et al., 2017).

307 Final remarks

- 308 A growing body of evidence implicates various immune cells in sensing exogenous and endogenous metabolic
- 309 signals raised by metabolic dysregulation and obesogenic diets. Ceramides, potent signalling lipids involved in
- 310 energy homeostasis, are found to mediate a number of the cellular and pathophysiological process linked with 311 OIIM and other obesity-associated diseases. Increasing evidence suggests that n-3 PUFA can partly create an
- 311 OIIM and other obesity-associated diseases. Increasing evidence suggests that n-3 PUFA can partly create an 312 anti-inflammatory environment acting via inflammatory receptors, such as TLR4 and Nlrp3 expressed on
- macrophages. As these pathways are activated by ceramides, n-3PUFA could be a tool to counteract their pro-
- inflammatory activities (Figure 3). The extensive modulatory effects ceramides have on various cells and
- 315 tissues, suggests the presence of many more mechanistic interactions still to be unrecovered.

317 Acknowledgements

- 318 This work is supported by funds of the Versus Arthritis- and Medical Research-funded Centre for
- 319 Musculoskeletal Ageing Research at the University of Birmingham to SK, funds of the British Heart Foundation

- Accelerator Award at the University of Birmingham to JN and a University of Birmingham ProfessorialResearch Fellowship to CM.



Figure 1. Ceramide *de novo* biosynthesis pathway. Biosynthesis of ceramides begins in the endoplasmic reticulum with condensation of palmitate and serine by serine palmitoyltransferase (SPT), followed by a series of reactions catalysed by 3-dehydrosphinganine reductase, dihydroceramide synthase (CerS) and dihydoriceramide desaturase (Des). Highlighting Myriocin and Fumonisin b₁, commonly used inhibitors of SPT and CerS respectively.



Figure 2. Schematic representation of interactions between ceramide biosynthesis and intracellular signalling in key immune cells. Ceramide overload triggered by key substrates such as sFFA, palmitate and LPS via TLR4, TNF and CD36 receptors leads to excessive ceramide biosynthesis. Ceramide elicits deleterious effects on insulin signalling and glucose metabolism via inhibiting Akt with PKCζ. Ceramide also induces mitochondrial stress via blocking lipid oxidation and creating ROS that induce cellular apoptosis. Activation of Nlrp3 via ceramides also triggers apoptosis via caspase 1 in addition to its role in obesity-induced inflammatory action. (Akt, Protein Kinase B; CD-36, cluster of differentiation 36; AdipoR, Adiponection receptor; IKK, Ikappa kinase; IL, interleukin; IR, Insulin receptor; LPS, lipopolysaccharide, NF-kβ, Nuclear factor kappa-light-chain-enhancer of activated B cells; Net, Neutrophil extracellular traps; Nlrp3, NLR family, pyrin domain containing 3; PKC, protein kinase C; ROS, reactive oxygen species; sFFA, Saturated fatty acids; TLR4, Toll like receptor-4; TNF-α, Tumour necrosis factor alpha; Tumour necrosis factor alpha receptor).



Figure 3. Schematic illustration highlighting the potential converge of ceramides and n-3 PUFA. Ceramides, when elevated within an obese environment, promote inflammation through the activation of TLR4 and GPR120 receptors on immune cells. N-3 PUFA can regulate this environment, inducing and anti-inflammatory response via potentially blocking the corresponding receptors of ceramides and restore immune homeostasis.

TABLE 1: Recent publications highlighting the association between obesity- induced ceramide accumulation and various pathological conditions.

| Tissue | Experimental model (in vivo/ in vitro) | Ceramide species | Ceramide concentration | Change in OIIM | Change in pathology | Ref. |
|--------------------------|---|--|---------------------------|---|--|--------------------------------------|
| Liver Cardiovascular | In vitro-Human HepG2 liver cells, 0.5 mmol/l palmitate for 4-8 hrs. | Total intracellular and exracellular ceramide | Increased | NA | Obesity and diet-induced diabetes | Watt et al. 2012 |
| | C57BL/6J mice on 60% HFD for 18 weeks | C16:0 | Increased | NA | Glucose intolerance and obesity induced type II diabetes | Raichuretal. 2019 |
| | C57BL/6J mice on 15% ethanol diet for 4 weeks and human VL17A cells Clinical trial- 980 free living human on mediterranian diet followed for 7.4 years | C14, C20, C20:1, C22, C24, and C26:1 | Increased | NA | Promotes alcoholic steatosis | Williamset al. 2018 |
| | | C16:0, C22:0, C24:0 and C24:1 in Plasma | Increased | NA | Higher risk for CVD | Wangetal. 2017 |
| | Clinical trial-462 individuals with familial coronary artery disease | Total serum ceramides (30 out of 32 tested), C16:0, C18:1 and C24:1 most strongly associated | Increased | NA | Coronary artery disease risk | Poss et al. 2020 |
| | In vitro Human AC16 cardiomyocytes, 0.5 mM palmitate and In vivo C57BL/6 mice on 60% for 8 weeks | C16:0, C24:0, C24:1 | Increased | NA | Heart failure-Mitochondrial dysfunction, oxidative stress and cell death in cardiomyocytes. | Law et al. 2018 |
| Adipose tissue Plasma | Clinical Trial-Obese individuals (n=439)- white adipose tissue | C16:0, C18:0 | Increased | NA | Obesity, insulin resistance, diet-induced diabetes | Turpin et al. 2014 |
| | In Vitro-Human 3T3-L1 adipocytes and RAW264.7 macrophages | Total ceramide and C2:0 | Increased | Upregulation of IL6, IFNγ, TNFα and MCP1 | Creation of OIIM | Hamadaet al. 2014 |
| | Wistar rats on 60% HFD for 8 weeks | C18:0, C20:0, and C24:0 | Increased | Upregulation of TNF α | Insulin resistance and obesity-induced type II diabetes | Blachnio- Zabielskaet al. 2018 |
| | Clinical trial- Obese women (n=28) visceral adipose tissue | Total ceramide | Increased | Upregulation of TNF α | Metabolic syndrome | Choromańsk a et al. 2019 |
| | Rhesus monkeys on high-fat and high-fructose (HFFD) 'western' diet for 8 months to 5 years | C14:0, C16:0, C22:0, C24:0 | Increased | NA | Associated with obesity-induced type II diabetes | Brozinick et al. 2013 |
| | C57BL/6J mice on 60% HFD for 18 weeks | C16:0 | Increased | NA | Glucose intolerance and obesity induced type II diabetes | Raichuretal. 2019 |
| | Obese children (n=80, aged 7–17 years) | Total ceramide including C14:0, C16:0, C16:1, C18:0, C18:1, C22:0, C24:0 | Increased | NA | Nonal coholic fatty liver disease | Wasilewska et al. 2018 |
| Skeletal muscle | C57BL/6 mice on 55.2% HFD for 17 weeks | C14:0 and C18:0 | Increased | NA | Contributes to obesity- associated insulin resistance | Turpin et al. 2019 |
| | C57BL/6 mice on 60% HFD for 12 weeks | Total ceramide | Increased | NA | Associated with obesity-induced type II diabetes | Bandeteta. 2018 |
| | Clinical trial-106 individuals with obesity and T2D In Vitro- Human C2C12 myotubes palmitate treatment (0.75 mmol/L) for 16 h | C18:1, C:20, C:22, C:24 and C:24:1 | Increased (by two-fold) | NA | Associated with obesity-induced type II diabetes | al. 2018 |
| | | Total ceramide | Increased (60%) | NA Increased the | Associated with obesity-induced type II diabetes | 2018 |
| | In Vitro- Rat L6 myotubes and mouse C2C12 muscle cells, 0.5 mM | T -1-1 | Increased | expression of pro- inflammatory, Il6 and | | Pillonet al. 2018 |
| | paimitate | Total ceramide | | Cc12 | Associated with obesity-induced type II diabetes | Blachnio- |
| | Wistar rats on 60% HFD for 8 weeks | Total ceramide including C14:0, C18:0, C18:1, C24:1 and C24:0 | Increased | NA | Associated with skeletal muscle insulin sensitivity | Zabielskaa et al. 2016 |

| | Target enzyme | Experimental model (in vivo/ in vitro) | Ceramide species & Tissues | Intervention | Ceramide concentration | Change in OIIM | Change in pathology | Ref. |
|------------------------|-------------------------------------|---|--|--|------------------------|---|---|----------------------------|
| SPT (serine | | C57BL/6J mice on HFD 12-20 weeks | Total ceramide including C24:0,C24:1, C22:0 in Adipose tissue, liver, muscle and serum | Pharmacological inhibition via Myriocin and Sptic knockout model | Reduced | Reduced pro-inflammatory cytokines IL-6, MCP-1, and TNF with increased anti-inflammatory cytokines (IL-10) | Improvemened insulin sensitivity and glucose homeostasis | Chaurasia et al. 2016 |
| | SPT | | | | | More anti-inflammatory M2 macrophages | | |
| | (serine palmitoyitransferase) | | | | | Less macrophage infiltration to periphery | | |
| | | In Vitro- Human 3T3-L1 adipocytes and RAW264.7 macrophages | Total ceramide and C2:0 | Genetic Knockdown of SPT | Reduced | Reduced pro-inflammatory cytokines; IL-6, IFN- $\gamma, TNF\alpha$ and MCP-1 | Restoring the homeostasis of the OIIM | Hamada et al. 2014 |
| | | | | | | | | |
| Ceramide (CerS 1-6) | | C57BL/6 mice on 55.2% HFD for 17 weeks | C16:0, C18:0- adipose tissue, liver | Cerső genetic knockout | Reduced | No macrophage inflatration and reduced pro-inflammatory cytokines IL-6 and IL-1b | Protected from diet-induced obesity, glucose intolerance and type II diabetes | Turpin et al. 2014 |
| | | CS78L/6J mice on 60% HFD for 18 weeks | C16:0- liver and plasma | Cers6 knockdown via antisense oligonucleotides | Reduced (50%) | NA | Improved insulin sensititivity, glucose tolerance with reduced body fat content | Raichur et al. 2019 |
| | Ceramide synthases (CerS 1-6) | In Vitro- Human 3T3-L1 adipocytes and RAW264.7 macrophages | Total ceramide and C2:0 | Pharmacological inhibitior of CERs via Fumonisin B1 | Reduced | Reduced pro-inflammatory cytokines; IL-6, IFN- $\gamma, TNF\alpha$ and MCP-1 | Restoring the homeostasis of the OIIM | Hamada et al. 2014 |
| | | C57BL/6JCrl Cers5 KO mice on 60% HFD for 24 | C16:0 and C18:0- lung, spleen, muscle, liver and white adipose tissue | CerS5 genetic knockout | Reduced | Reduction in pro-inflammatory gene expression such as NF-kB, TNF and IL-1 β | Reduced weight gain, ameliorate OIIM | Gosejacob et al. (2016) |
| | | *Global knockdown of Cers1 in C578L/6 mice on 55.2% HFD for 17 weeks | C18:0 | Cers1 genetic knockout | Reduced (95%) | NA | Decreased body weight and fat content with improved insulin sensitivity and glucose tolerance | Turpin et al. 2019 |
| | Dihydroceramide Desaturase (DES) | C578L/6 mice on 60% HFD for 12 weeks | Total ceramide- Serum, liver, white adipose tissue | Genetic Degsi knockout | Reduced | Reduced pro-inflammatory cytokines; IL-1 and $\mbox{TNF}\alpha$ | Reduced fat density and improved insulin sensitivity | Chaurasia et al. 2019 |

TABLE 2: Intervention methods to target the *de novo* ceramide biosynthesis pathway

Reference

Al-Rashed, F., Ahmad, Z., Thomas, R., Melhem, M., Snider, A. J., Obeid, L. M., . . . Ahmad, R. (2020). Neutral sphingomyelinase 2 regulates inflammatory responses in monocytes/macrophages induced by TNF-α. *Scientific Reports, 10*(1), 16802. doi:10.1038/s41598-020-73912-5

Almeida-Suhett, C. P., Scott, J. M., Graham, A., Chen, Y., & Deuster, P. A. (2019). Control diet in a high-fat diet study in mice: Regular chow and purified low-fat diet have similar effects on phenotypic, metabolic, and behavioural outcomes. *Nutritional Neuroscience*, 22(1), 19-28.doi:10.1080/1028415X.2017.1349359

Bandet, C. L., Mahfouz, R., Véret, J., Sotiropoulos, A., Poirier, M., Giussani, P., ... Hajduch, E. (2018). Ceramide transporter CERT is involved in muscle insulin signaling defects under lipotoxic conditions. *Diabetes (New York, N.Y.), 67*(7), 1258-1271. doi:10.2337/db17-0901

Blachnio-Zabielska, A. U., Hady, H. R., Markowski, A. R., Kurianiuk, A., Karwowska, A., Górski, J., & Zabielski, P. (2018). Inhibition of ceramide de novo synthesis affects adipocytokine secretion and improves systemic and adipose tissue insulin sensitivity. *International Journal of Molecular Sciences*, *19*(12), 3995. doi:10.3390/ijms19123995

Bortolin, R. C., Vargas, A. R., Gasparotto, J., Chaves, P. R., Schnorr, C. E., Martinello, K. B., Moreira, J. C. F. (2018). A new animal diet based on human western diet is a robust dietinduced obesity model: Comparison to high-fat and cafeteria diets in term of metabolic and gut microbiota disruption. *International Journal of Obesity*, 42(3), 525-534. doi:10.1038/ijo.2017.225

Broskey, N. T., Obanda, D. N., Burton, J. H., Cefalu, W. T., & Ravussin, E. (2018). Skeletal muscle ceramides and daily fat oxidation in obesity and diabetes. *Metabolism, Clinical and Experimental, 82*, 118-123. doi:10.1016/j.metabol.2017.12.012

Brotfain, E., Hadad, N., Shapira, Y., Avinoah, E., Zlotnik, A., Raichel, L., & Levy, R. (2015). Neutrophil functions in morbidly obese subjects. *Clinical and Experimental Immunology*, 181(1), 156-163. doi:10.1111/cei.12631

Brozinick, J. T., Hawkins, E., Hoang Bui, H., Kuo, M., Tan, B., Kievit, P., & Grove, K. (2013). Plasma sphingolipids are biomarkers of metabolic syndrome in non-human primates maintained on a western-style diet. *International Journal of Obesity (2005), 37*(8), 1064-1070. doi:10.1038/ijo.2012.191

Chaurasia, B., & Summers, S. A. (2020). Ceramides in metabolism: Key lipotoxic players. *Annual Review of Physiology*, doi:10.1146/annurev-physiol-031620-093815

Chaurasia, B., Kaddai, V. A., Lancaster, G. I., Henstridge, D. C., Sriram, S., Galam, D. L. A., . . . Summers, S. A. (2016). Adipocyte ceramides regulate subcutaneous adipose browning, inflammation, and metabolism. *Cell Metabolism*, 24(6), 820-834. doi:10.1016/j.cmet.2016.10.002

Chaurasia, B., Tippetts, T. S., Mayoral Monibas, R., Liu, J., Li, Y., Wang, L., . . . Summers, S. A. (2019). Targeting a ceramide double bond improves insulin resistance and hepatic steatosis. *Science (American Association for the Advancement of Science)*, *365*(6451), 386-392. doi:10.1126/science.aav3722

Chen, M., Quintans, J., Fuks, Z., Thompson, C., Kufe, D. W., & Weichselbaum, R. R. (1995). Suppression of bcl-2 messenger RNA production may mediate apoptosis after ionizing radiation, tumor necrosis factor {alpha}, and ceramide. *Cancer Research*, 55(5), 991.

Choromańska, B., Myśliwiec, P., Razak Hady, H., Dadan, J., Myśliwiec, H., Chabowski, A., & Mikłosz, A. (2019). Metabolic syndrome is associated with ceramide accumulation in visceral adipose tissue of women with morbid obesity. *Obesity (Silver Spring, Md.), 27*(3), 444-453. doi:10.1002/oby.22405

Christ, A. et al. (2018) Western Diet Triggers NLRP3-Dependent Innate Immune Reprogramming. *Cell*. 172(1-2), 162–175.

Corriden, R., Hollands, A., Olson, J., Derieux, J., Lopez, J., Chang, J. T., ... Nizet, V. (2015). Tamoxifen augments the innate immune function of neutrophils through modulation of intracellular ceramide. *Nature Communications, 6*(1), 8369. doi:10.1038/ncomms9369

Cucchi, D., Camacho-Muñoz, D., Certo, M., Niven, J., Smith, J., Nicolaou, A., & Mauro, C. (2019). Omega-3 polyunsaturated fatty acids impinge on CD4+ T cell motility and adipose tissue distribution via direct and lipid mediator-dependent effects. *Cardiovascular Research*, 116(5), 1006-1020. doi:10.1093/cvr/cvz208

D'Abbondanza, M., Martorelli, E. E., Ricci, M. A., De Vuono, S., Migliola, E. N., Godino, C., . . . Lupattelli, G. (2019). Increased plasmatic NETs by-products in patients in severe obesity. *Scientific Reports*, 9(1), 14678-10. doi:10.1038/s41598-019-51220-x

DeFuria J., Belkina A. C., Jagannathan-Bogdan M., Snyder-Cappione J., Carr J. D., Nersesova Y. R., . . . Nikolajczyk B. S. (2013). B cells promote inflammation in obesity and type 2 diabetes through regulation of T-cell function and an inflammatory cytokine profile. *Proceedings of the National Academy of Sciences - PNAS*, *110*(13), 5133-5138. doi:10.1073/pnas.1215840110

Dong, Y., Zhang, X., Sun, L., Zhang, S., Liu, B., Liu, H., . . . Jiang, C. (2017). Omega-3 PUFA ameliorates hyperhomocysteinemia-induced hepatic steatosis in mice by inhibiting hepatic ceramide synthesis. *Acta Pharmacologica Sinica*, (38(12), 1601-1610) doi:10.1038/aps.2017.127

Donohoe, C. L., Lysaght, J., O'Sullivan, J., & Reynolds, J. V. (2016). Emerging concepts linking obesity with the hallmarks of cancer. *Trends in Endocrinology & Metabolism, 28*(1), 46-62. doi:10.1016/j.tem.2016.08.004

Elgazar-Carmon, V., Rudich, A., Hadad, N., & Levy, R. (2008). Neutrophils transiently infiltrate intra-abdominal fat early in the course of high-fat feeding. *Journal of Lipid Research, 49*(9), 1894-1903. doi:10.1194/jlr.M800132-JLR200

Eraky, S. M., Abdel-Rahman, N., & Eissa, L. A. (2018). Modulating effects of omega-3 fatty acids and pioglitazone combination on insulin resistance through toll-like receptor 4 in type 2 diabetes mellitus. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 136, 123-129. doi:10.1016/j.plefa.2017.06.009

Gill, J., & Sattar, N. (2009). Ceramides: A new player in the inflammation–insulin resistance paradigm? *Diabetologia, 52*(12), 2475-2477. doi:10.1007/s00125-009-1546-x

Gosejacob, D., Jäger, P. S., Vom Dorp, K., Frejno, M., Carstensen, A. C., Köhnke, M., . . . Hoch, M. (2016). Ceramide synthase 5 is essential to maintain C16:0-ceramide pools and contributes to the development of diet-induced obesity. *The Journal of Biological Chemistry*, 291(13), 6989-7003. doi:10.1074/jbc.M115.691212

Gutiérrez, S., Svahn, S. L., & Johansson, M. E. (2019). Effects of omega-3 fatty acids on immune cells. *International Journal of Molecular Sciences*, 20(20), 5028. doi:10.3390/ijms20205028

Haghiac, M., Yang, X., Presley, L., Smith, S., Dettelback, S., Minium, J., . . . Hauguel-de Mouzon, S. (2015). Dietary omega-3 fatty acid supplementation reduces inflammation in obese pregnant women: A randomized double-blind controlled clinical trial. *PloS One*, 10(9), e0137309. doi: 10.1371/journal.pone.0137309

Hamada, Y., Nagasaki, H., Fujiya, A., Seino, Y., Shang, Q., Suzuki, T., . . . Oiso, Y. (2014). Involvement of de novo ceramide synthesis in pro-inflammatory adipokine secretion and adipocyte–macrophage interaction. *The Journal of Nutritional Biochemistry*, 25(12), 1309-1316. doi: 10.1016/j.jnutbio.2014.07.008

Hannun, Y. A., & Obeid, L. M. (2017). Sphingolipids and their metabolism in physiology and disease. *Nature Reviews. Molecular Cell Biology*, *19*(3), 175-191. doi:10.1038/nrm.2017.107

Herz, J., Pardo, J., Kashkar, H., Schramm, M., Kuzmenkina, E., Bos, E., ... Utermohlen, O. (2009). Acid sphingomyelinase is a key regulator of cytotoxic granule secretion by primary T lymphocytes. *Nature Immunology, 10*(7), 761-768. doi:10.1038/ni.1757

Holland, W. L., Bikman, B. T., Wang, L., Yuguang, G., Sargent, K. M., Bulchand, S., . . . Summers, S. A. (2011). Lipid-induced insulin resistance mediated by the proinflammatory receptor TLR4 requires saturated fatty acid–induced ceramide biosynthesis in mice. *The Journal of Clinical Investigation*, 121(5), 1858-1870. doi:10.1172/jci43378

Holland, W. L., Wade, M. R., Scherer, P. E., Bikman, B. T., Brozinick, J. T., Tenorio, V. M., . . . Zhang, B. B. (2011). Receptor-mediated activation of ceramidase activity initiates the pleiotropic actions of adiponectin. *Nature Medicine*, *17*(1), 55-63. doi:10.1038/nm.2277

Holland, W. L., Xia, J. Y., Johnson, J. A., Sun, K., Pearson, M. J., Sharma, A. X., . . . Scherer, P. E. (2017). Inducible overexpression of adiponectin receptors highlight the roles of adiponectin-induced ceramidase signaling in lipid and glucose homeostasis. *Molecular Metabolism, 6*(3), 267-275. doi:10.1016/j.molmet.2017.01.002 Hollmann, C., Werner, S., Avota, E., Reuter, D., Japtok, L., Kleuser, B., . . . Beyersdorf, N. (2016). Inhibition of acid sphingomyelinase allows for selective targeting of CD4 + conventional versus Foxp3 + regulatory T cells. *The Journal of Immunology* (1950), 197(8), 3130-3141. doi:10.4049/jimmunol.1600691

Hruby, A., & Hu, F. (2016). The epidemiology of obesity: A big picture. *PharmacoEconomics*, 33(7), 673-689. doi:10.1007/s40273-014-0243-x

Hu, Y., Hu, F. B., & Manson, J. E. (2019). Marine Omega-3 supplementation and cardiovascular disease: An updated Meta-Analysis of 13 randomized controlled trials involving 127 477 participants. *Journal of the American Heart Association*, 8(19), e013543. doi:10.1161/jaha.119.013543

Haus J. M., Sangeeta R. Kashyap, Takhar Kasumov, Renliang Zhang, Karen R. Kelly, Ralph A. DeFronzo, & John P. Kirwan. (2009). Plasma ceramides are elevated in obese subjects with type 2 diabetes and correlate with the severity of insulin resistance. *Diabetes (New York, N.Y.), 58*(2), 337-343. doi:10.2337/db08-1228

Kendall, A. C., Pilkington, S. M., Murphy, S. A., Carratore, F. D., Sunarwidhi, A. L., Kiezel-Tsugunova, M., . . . Nicolaou, A. (2019). Dynamics of the human skin mediator lipidome in response to dietary ω -3 fatty acid supplementation. *The FASEB Journal*, 33(11), 13014-13027. doi:10.1096/fj.201901501R

Kien C.L., Bunn J.Y., Poynter M.E., Stevens R., Bain J., ... (2013) A lipidomics analysis of the relationship between dietary fatty acid composition and insulin sensitivity in young adults. *Diabetes*. 62(4), 1054–63. doi: 10.2337/db12-0363

Kroesen, B., Pettus, B., Luberto, C., Busman, M., Sietsma, H., Leij, L., & Hannun, Y. (2001). Induction of apoptosis through B-cell receptor cross-linking occurs via de novo generated C16-ceramide and involves mitochondria. *The Journal of Biological Chemistry, 276*(17), 13606-13614. doi:10.1074/jbc.M009517200

Kroesen, B., Jacobs, S., Pettus, B. J., Sietsma, H., Kok, J. W., Hannun, Y. A., & de Leij, Lou F. M. H. (2003). BcR-induced apoptosis involves differential regulation of C16and C24ceramide formation and sphingolipid-dependent activation of the proteasome. *The Journal of Biological Chemistry*, *278*(17), 14723-14731. doi:10.1074/jbc.M210756200

Kumar, N., Gupta, G., Anilkumar, K., Fatima, N., Karnati, R., Reddy, G. V., . . . Reddanna, P. (2016). 15-lipoxygenase metabolites of α -linolenic acid, [13-(S)-HPOTrE and 13-(S)-HOTrE], mediate anti-inflammatory effects by inactivating NLRP3 inflammasome. *Scientific Reports*, 6(1), 31649. doi:10.1038/srep31649

Law, B. A., Liao, X., Moore, K. S., Southard, A., Roddy, P., Ji, R., ... Cowart, L. A. (2018). Lipotoxic very-long-chain ceramides cause mitochondrial dysfunction, oxidative stress, and cell death in cardiomyocytes. *The FASEB Journal*, *32*(3), 1403-1416. doi:10.1096/fj.201700300R

Lee, K. R., Midgette, Y., & Shah, R. (2019). Fish oil derived omega 3 fatty acids suppress adipose NLRP3 inflammasome signaling in human obesity. *Journal of the Endocrine Society*, 3(3), 504-515. doi:10.1210/js.2018-00220

Li, G., Liu, D., Kimchi, E. T., Kaifi, J. T., Qi, X., Manjunath, Y., . . . Staveley-O'Carroll, K. F. (2018). Nanoliposome C6-ceramide increases the anti-tumor immune response and slows growth of liver tumors in mice. *Gastroenterology (New York, N.Y. 1943), 154*(4), 1024-1036.e9. doi:10.1053/j.gastro.2017.10.050

Liu, H., Qiu, Y., Mu, Y., Zhang, X., Liu, L., Hou, X., . . . Wang, F. (2013). A high ratio of dietary n-3/n-6 polyunsaturated fatty acids improves obesity-linked inflammation and insulin resistance through suppressing activation of TLR4 in SD rats. *Nutrition Research* (New York, N.Y.), 33(10), 849-858. doi:10.1016/j.nutres.2013.07.004

Mallampalli, R. K., Peterson, E. J., Carter, A. B., Salome, R. G., Mathur, S. N., & Koretzky, G. A. (1999). TNF-alpha increases ceramide without inducing apoptosis in alveolar type II epithelial cells. *American Journal of Physiology. Lung Cellular and Molecular Physiology*, 276(3), 481-L490. doi:10.1152/ajplung.1999.276.3.L481

Martín-Leal, A., Blanco, R., Casas, J., Sáez, M. E., Rodríguez-Bovolenta, E., Rojas, I., . . . Mañes, S. (2020). CCR5 deficiency impairs CD4+ t-cell memory responses and antigenic sensitivity through increased ceramide synthesis. *The EMBO Journal*, 39(15), e104749-n/a. doi:10.15252/embj.2020104749

Martins, A. R., Crisma, A. R., Masi, L. N., Amaral, C. L., Marzuca-Nassr, G. N., Bomfim, L. H. M., Hirabara, S. M. (2018). Attenuation of obesity and insulin resistance by fish oil supplementation is associated with improved skeletal muscle mitochondrial function in mice fed a high-fat diet. The *Journal of Nutritional Biochemistry*, 55, 76-88. doi:10.1016/j.jnutbio.2017.11.012

Mauro, C., Smith, J., Cucchi, D., Coe, D., Fu, H., Bonacina, F., . . . Marelli-Berg, F. M. (2017). Obesity-induced metabolic stress leads to biased effector memory CD4+ T cell differentiation via PI3K p1108-akt-mediated signals. *Cell Metabolism*, 25(3), 593-609. doi:10.1016/j.cmet.2017.01.008

Meyer, S. G. E., & de Groot, H. (2003). Cycloserine and threo-dihydrosphingosine inhibit TNF- α -induced cytotoxicity: Evidence for the importance of de novo ceramide synthesis in TNF- α signaling. *Biochimica Et Biophysica Acta. Molecular Cell Research, 1643*(1), 1-4. doi:10.1016/j.bbamcr.2003.10.002

Mildenberger, J., Johansson, I., Sergin, I., Kjøbli, E., Damås, J. K., Razani, B., . . . Bjørkøy, G. (2017). N-3 PUFAs induce inflammatory tolerance by formation of KEAP1-containing SQSTM1/p62-bodies and activation of NFE2L2. *Autophagy*, 13(10), 1664-1678. doi:10.1080/15548627.2017.1345411

Nicolaou, A., Mauro, C., Urquhart, P., & Marelli-Berg, F. (2014). Polyunsaturated fatty acidderived lipid mediators and T cell function. *Frontiers in Immunology*, *5*, 75. doi:10.3389/fimmu.2014.00075 Nigro, E., Scudiero, O., Monaco, M. L., Palmieri, A., Mazzarella, G., Costagliola, C., . . . Daniele, A. (2014). New insight into adiponectin role in obesity and obesity-related diseases. *BioMed Research International, 2014*, 658913-14. doi:10.1155/2014/658913

Nishimura, S., Manabe, I., Nagasaki, M., Eto, K., Yamashita, H., Ohsugi, M., . . . Nagai, R. (2009). [CD8.sup.+] effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. *Nature Medicine, 15*(8), 914.

Oh, D. Y., Talukdar, S., Bae, E. J., Imamura, T., Morinaga, H., Fan, W., ... Olefsky, J. M. (2010). GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. *Cell*, 142(5), 687-698. doi:10.1016/j.cell.2010.07.041

Peraldi P., Hotamisligil G. S., Buurman W. A., White M. F., & Spiegelman B. M. (1996). Tumor necrosis factor (TNF)- inhibits insulin signaling through stimulation of the p55 TNF receptor and activation of sphingomyelinase. *The Journal of Biological Chemistry, 271*(22), 13018. doi:10.1074/jbc.271.22.13018

Pillon, N. J., Frendo-Cumbo, S., Jacobson, M. R., Liu, Z., Milligan, P. L., Hoang Bui, H., . . . Klip, A. (2018). Sphingolipid changes do not underlie fatty acid-evoked GLUT4 insulin resistance nor inflammation signals in muscle cells. *Journal of Lipid Research*, *59*(7), 1148-1163. doi:10.1194/jlr.m080788

Popkin, B. M., Du, S., Green, W. D., Beck, M. A., Algaith, T., Herbst, C. H., . . . Shekar, M. (2020). Individuals with obesity and COVID-19: A global perspective on the epidemiology and biological relationships. *Obesity Reviews*, 21(11), e13128-n/a. doi:10.1111/obr.13128

Poss, A. M., Maschek, J. A., Cox, J. E., Hauner, B. J., Hopkins, P. N., Hunt, S. C., ... Playdon, M. C. (2020). Machine learning reveals serum sphingolipids as cholesterolindependent biomarkers of coronary artery disease. *The Journal of Clinical Investigation*, 130(3), 1363-1376. doi:10.1172/jci131838

Raichur, S., Brunner, B., Bielohuby, M., Hansen, G., Pfenninger, A., Wang, B., . . . Tennagels, N. (2019). The role of C16:0 ceramide in the development of obesity and type 2 diabetes: CerS6 inhibition as a novel therapeutic approach. *Molecular Metabolism* (Germany), 21, 36-50. doi:10.1016/j.molmet.2018.12.008

Richard, A., Bourgoin, S., Naccache, P. H., L'Heureux, G. P., Krump, E., McColl, S. R., & Pelletier, G. (1996). C2-ceramide primes specifically for the superoxide anion production induced by N-formylmethionylleucyl phenylalanine (fMLP) in human neutrophils. *Biochimica Et Biophysica Acta, 1299*(2), 259-266.

Rotolo, J. A., Stancevic, B., Van Den Brink, Marcel R, Kolesnick, R., Lu, S. X., Jianjun, Zhang, ... Fuks, Z. (2009). Cytolytic T cells induce ceramide-rich platforms in target cell membranes to initiate graft-versus-host disease. *Blood, 114*(17), 3693-3706. doi:10.1182/blood-2008-11-191148

Ruvolo, P. P., Deng, X., Ito, T., Carr, B. K. & May, W. S. (1999). Ceramide induces Bcl2 dephosphorylation via a mechanism involving mitochondrial PP2A. *The Journal of Biological Chemistry*, *274*(29), 20296-20300. doi:10.1074/jbc.274.29.20296

Seumois, G., Fillet, M., Gillet, L., Faccinetto, C., Desmet, C., Francois, C., . . . Bureau, F. (2007). De novo C sub(16)- and C sub(24)-ceramide generation contributes to spontaneous neutrophil apoptosis. *Journal of Leukocyte Biology*, *81*(6), 1477-1486. doi:10.1189/jlb.0806529

Sofi, M. H., Heinrichs, J., Dany, M., Nguyen, H., Dai, M., Bastian, D., . . . Yu, X. (2017). Ceramide synthesis regulates T cell activity and GVHD development. *JCI Insight, 2*(10) doi:10.1172/jci.insight.91701

Tachtsis, B., Whitfield, J., Hawley, J. A., & Hoffman, N. J. (2020). Omega-3 polyunsaturated fatty acids mitigate palmitate-induced impairments in skeletal muscle cell viability and differentiation. *Frontiers in Physiology*, 11, 563. doi:10.3389/fphys.2020.00563

Tran, T. T., Postal, B. G., Demignot, S., Ribeiro, A., Osinski, C., Pais de Barros, J., . . . Carrière, V. (2016). Short term palmitate supply impairs intestinal insulin signaling via ceramide production. The *Journal of Biological Chemistry*, 291(31), 16328-16338. doi:10.1074/jbc.m115.709626

Turpin-Nolan, S. M., Hammerschmidt, P., Chen, W., Jais, A., Timper, K., Awazawa, M., . . . Brüning, J. C. (2019). CerS1-derived C18:0 ceramide in skeletal muscle promotes obesity-induced insulin resistance. *Cell Reports* (Cambridge), 26(1), 1-10.e7. doi:10.1016/j.celrep.2018.12.031

Turpin, S., Nicholls, H., Willmes, D., Mourier, A., Brodesser, S., Wunderlich, C., . . . Brüning, J. (2014). Obesity-induced CerS6-dependent C16:0 ceramide production promotes weight gain and glucose intolerance. *Cell Metabolism, 20*(4), 678-686. doi:10.1016/j.cmet.2014.08.002

Vandanmagsar, B., Youm, Y., Ravussin, A., Galgani, J. E., Stadler, K., Mynatt, R. L., . . . Dixit, V. D. (2011). The NALP3/NLRP3 inflammasome instigates obesity-induced autoinflammation and insulin resistance. *Nature Medicine*, 17(2), 179-188. doi:10.1038/nm.2279

Wang, D. D., Toledo, E., Hruby, A., Rosner, B. A., Willett, W. C., Sun, Q., ... Hu, F. B. (2017). Plasma ceramides, mediterranean diet, and incident cardiovascular disease in the PREDIMED trial (prevencion con dieta mediterranea). *Circulation (New York, N.Y.), 135*(21), 2028-2040. doi:10.1161/CIRCULATIONAHA.116.024261

Wasilewska, N., Bobrus-Chociej, A., Harasim-Symbor, E., Tarasów, E., Wojtkowska, M., Chabowski, A., & Lebensztejn, D. M. (2018). Increased serum concentration of ceramides in obese children with nonalcoholic fatty liver disease. *Lipids in Health and Disease, 17*(1), 216. doi:10.1186/s12944-018-0855-9

Watt, M., Barnett, A., Bruce, C., Schenk, S., Horowitz, J., & Hoy, A. (2012). Regulation of plasma ceramide levels with fatty acid oversupply: Evidence that the liver detects and secretes de novo synthesised ceramide. *Diabetologia*, 55(10), 2741-2746. doi:10.1007/s00125-012-2649-3

Williams, B., Correnti, J., Oranu, A., Lin, A., Scott, V., Annoh, M., . . . Carr, R. M. (2018). A novel role for ceramide synthase 6 in mouse and human alcoholic steatosis. *The FASEB Journal*, *32*(1), 130-142. doi:10.1096/fj.201601142R

Winer, D. A., Winer, S., Shen, L., Wadia, P. P., Yantha, J., Paltser, G., . . . Engleman, E. G. (2011). B cells promote insulin resistance through modulation of T cells and production of pathogenic IgG antibodies. *Nature Medicine*, *17*(5), 610-617. doi:10.1038/nm.2353

Wong K., Li B. X., & Hunchuk N. (1995). N-acetylsphingosine (C-ceramide) inhibited neutrophil superoxide formation and calcium influx. *The Journal of Biological Chemistry*, 270(7), 3056-3062. doi:10.1074/jbc.270.7.3056

World Health Organization. Global Health Observatory (GHO) data. World Health Statistics 2016. Geneva: WHO; 2016

Xiong, Z., Huang, J., Poda, G., Pomès, R., & Privé, G. G. (2016). Structure of human acid sphingomyelinase reveals the role of the saposin domain in activating substrate hydrolysis. *Journal of Molecular Biology*, *428*(15), 3026-3042. doi:10.1016/j.jmb.2016.06.012

Xu, J., Yeh, C. H., Chen, S., He, L., Sensi, S. L., Canzoniero, L. M., ... Hsu, C. Y. (1998). Involvement of de novo ceramide biosynthesis in tumor necrosis factor-alpha/cycloheximideinduced cerebral endothelial cell death. *The Journal of Biological Chemistry*, 273(26), 16521-16526. Retrieved from <u>https://www.ncbi.nlm.nih.gov/pubmed/9632721</u>

Xu, X., Su, S., Wang, X., Barnes, V., De Miguel, C., Ownby, D., . . . Chen, W. (2015). Obesity is associated with more activated neutrophils in african american male youth. *International Journal of Obesity*, *39*(1), 26-32. doi:10.1038/ijo.2014.194

Yan, Y., Jiang, W., Spinetti, T., Tardivel, A., Castillo, R., Bourquin, C., . . . Zhou, R. (2013). Omega-3 fatty acids prevent inflammation and metabolic disorder through inhibition of NLRP3 inflammasome activation. *Immunity* (Cambridge, Mass.), 38(6), 1154-1163. doi:10.1016/j.immuni.2013.05.015

Yang, H. et al. (2010) Obesity increases the production of proinflammatory mediators from adipose tissue T cells and compromises TCR repertoire diversity: Implications for systemic inflammation and insulin resistance. *J Immunol.* 185(3), 1836-1845.

Zhou, Y., Salker, M. S., Walker, B., Münzer, P., Borst, O., Gawaz, M., . . . Lang, F. (2016). Acid sphingomyelinase (ASM) is a negative regulator of regulatory T cell (treg) development. *Cellular Physiology and Biochemistry*, 39(3), 985-995. doi:10.1159/000447806