

Cholesterol Metabolism: A Review of How Ageing Disrupts the Biological Mechanisms Responsible for its Regulation

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Abstract

Cholesterol plays a vital role in the human body as a precursor of steroid hormones and bile acids, in addition to providing structure to cell membranes. Whole body cholesterol metabolism is maintained by a highly coordinated balancing act between cholesterol ingestion, synthesis, absorption, and excretion. The aim of this review is to discuss how ageing interacts with these processes. Firstly, we will present an overview of cholesterol metabolism. Following this, we discuss how the biological mechanisms which underpin cholesterol metabolism are effected by ageing. Included in this discussion are lipoprotein dynamics, cholesterol absorption/synthesis and the enterohepatic circulation/synthesis of bile acids. Moreover, we discuss the role of oxidative stress in the pathological progression of atherosclerosis and also discuss how cholesterol biosynthesis is effected by both the mammalian target of rapamycin and sirtuin pathways. Next, we examine how diet and alterations to the gut microbiome can be used to mitigate the impact ageing has on cholesterol metabolism. We conclude by discussing how mathematical models of cholesterol metabolism can be used to identify therapeutic interventions.

Keywords

Cholesterol, ageing, longevity, low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), microbiome

36 1.0 Introduction

37 An intriguing feature of ageing, is that it is often accompanied by the dysregulation of whole body
38 cholesterol metabolism (Mc Auley and Mooney, 2014). A clinical manifestation of this process is an
39 age-related rise in the plasma levels of low density lipoprotein cholesterol (LDL-C) (Abbott et al.,
40 1983). This rise in LDL-C has a significant impact on cardiovascular disease (CVD) risk, due to the
41 association elevated plasma LDL-C has with the mechanisms which underpin atherosclerotic plaque
42 formation (Gould et al., 2007). Conversely, prospective studies have shown that high density
43 lipoprotein (HDL) levels diminish with age (Wilson et al., 1994). This is clinically significant, as HDLs
44 are central to reverse cholesterol transport (RCT) (Groen et al., 2004). This process, which results in
45 the trafficking of HDL-C, or the so-called 'good cholesterol' to the liver for subsequent removal via
46 the intestine, represents the only way of eliminating excess cholesterol from peripheral tissue. There
47 is a plethora of epidemiological evidence supporting an inverse relationship between HDL -C levels
48 and CVD risk, and evidence has consistently shown that HDL-C levels are correlated with longevity in
49 several population groups (Ferrara et al., 1997). It is therefore not surprising, that a healthy ageing
50 phenotype has regularly been associated with the fine tuning of cholesterol metabolism, within
51 certain cohorts of individuals who possess particular genetic variants in tandem with exceptional
52 longevity (Milman et al., 2014). For example, a three-fold increase in the prevalence of homozygosity
53 for the favourable I405V polymorphism, a mutation in the cholesteryl ester transfer protein (CETP), a
54 key enzyme involved in RCT has been observed in those exhibiting exceptional longevity (Barzilai et
55 al., 2003). Individuals with the I405V genotype have significantly larger HDL and LDL particle sizes,
56 leading to the suggestion, that the risk of atherosclerosis development is diminished as a result of
57 the diminished ability of the LDL particle to cross the arterial endothelium (Barzilai et al., 2003;
58 Kulanuwat et al., 2015).

59
60 Many key mechanisms involved in cholesterol metabolism are affected by ageing (**Figure 1**). For
61 instance, ageing has been associated with a decline in the hepatic expression of cholesterol 7-alpha-
62 hydroxylase (CYP7A1), a key regulator of bile acid synthesis, thus resulting in a decreased cholesterol
63 demand for conversion to bile acids (Bertolotti et al., 2007). Furthermore, there is a decline in
64 hepatic LDL receptors (LDLr) with age, leading to a reduction in LDL-C clearance (Ericsson et al.,
65 1991; Millar et al., 1995). Within the small intestine, there is an increase in the number of the sterol
66 transporter Niemann-pick C1-like 1 (NPC1L1), a key mediator of cholesterol absorption (Duan et al.,
67 2006). In addition, there is a decline in the predominant bacterial populations that play a role in the
68 enterohepatic circulation of bile acids (Hopkins and Macfarlane, 2002). Moreover, dysregulation of
69 cholesterol biosynthesis is associated with two key intracellular pathways which are thought to
70 underpin intrinsic ageing and health-span. These pathways are defined by the
71 mammalian/mechanistic target of rapamycin (mTOR) and by the NAD⁺-dependent deacetylase silent
72 information regulator proteins (sirtuins). The former of these pathways has been suggested as a
73 central regulator of intracellular cholesterol homeostasis (Wang et al., 2011), while mammalian
74 sirtuin 6 (Sirt6), has been identified as a critical controller of sterol-regulatory element binding
75 protein (SREBP)-2 in rodents (Tao et al., 2013). These recent findings suggest that it is not one
76 mechanism that is the central driver of cholesterol dysregulation with age, but rather a number of
77 mechanisms interacting with one another to disrupt cholesterol metabolism. Therefore, it is
78 important to view cholesterol metabolism and its relationship with ageing in an integrated way. In
79 this review we will 1) discuss in depth how ageing impacts cholesterol metabolism, 2) discuss a

80 number of the genes involved in cholesterol metabolism which have been implicated with longevity,
81 3) discuss the role of oxidative stress in disrupting cholesterol metabolism, 4) describe the role of
82 caloric restriction (CR) in modulating cholesterol metabolism, 5) describe recent evidence that
83 demonstrates the role mTOR and sirtuins play in cholesterol biosynthesis, 6) provide an overview of
84 diet and its impact on cholesterol metabolism, 7) discuss the interactions between cholesterol
85 metabolism and the gut microbiome, 8) propose therapeutic strategies based around the gut
86 microbiome which could help to prevent the dysregulation of cholesterol metabolism with age, and
87 lastly we will provide an overview of mathematical models that have been used to gain an increased
88 insight into the dynamics of cholesterol metabolism.

89

90 **2.0 Overview of Cholesterol Metabolism**

91 Cholesterol plays a vital role in the human body, as it is an essential component of all cell
92 membranes. In addition, it is the precursor of steroid hormones, which control a range of
93 physiological functions. Cholesterol is also the precursor to bile acids, which are necessary for the
94 intestinal absorption of cholesterol, fats and lipophilic vitamins. Cholesterol can be obtained from
95 the diet as well as being endogenously synthesised, the latter being the main source in humans
96 (Gylling, 2004). A subtle balancing act between ingestion, absorption, synthesis and excretion
97 maintains whole body cholesterol metabolism (Figure 1). Briefly, 1) the average daily intake of
98 cholesterol is 304 and 213mg/day, for males and females respectively, living in the UK (Henderson et
99 al., 2003). Of this, 85-90% is free cholesterol while 10-15% is in the esterified form (Iqbal and
100 Hussain, 2009). Ingested cholesterol then enters the small intestine, where absorption occurs
101 (Tanchaorenrat et al., 2014). 2) Cholesterol in the free form is more readily incorporated into a bile
102 acid micelle for absorption. Therefore, cholesterol ester hydrolase (CEH) converts the esterified
103 cholesterol into free cholesterol, which can then be incorporated into a bile acid micelle (Ikeda et al.,
104 2002). This enables NPC1L1 to absorb the cholesterol by clathrin-mediated endocytosis (Betters and
105 Yu, 2010). Upon entry to the enterocyte, acetyl CoA acetyltransferase 2 (ACAT2) converts the
106 cholesterol into the esterified form in order to maintain the concentration gradient (Chang et al.,
107 2009). Microsomal triglyceride transfer protein (MTP) then shuttles the esterified cholesterol with
108 apo B-48, while triacylglycerol and phospholipids are also incorporated to form a chylomicron (Jamil
109 et al., 1995). 3) The chylomicron is then exported to the lymphatic system via exocytosis, and enters
110 the blood stream, where it can deliver fatty acids to the tissues before being removed by hepatic
111 remnant receptors and degraded in the liver (Cooper, 1997). 4) Cholesterol is also synthesised
112 endogenously in all nucleated cells in the body, including the hepatocytes and enterocytes from
113 acetyl CoA (Bloch, 1965). 5) From the hepatic cholesterol pool, very low density lipoprotein
114 cholesterol (VLDL-C) is formed, to enable the transport of endogenously synthesised triacylglycerol
115 to the tissues. Partial hydrolysis of VLDL-C by lipoprotein lipase (LPL) forms the LDL-C precursor,
116 intermediate density lipoprotein cholesterol (IDL-C). IDL-C is further hydrolysed by hepatic lipase to
117 form LDL-C (Havel, 1984). 6) Following this, VLDL-C, IDL-C and LDL-C are removed from the
118 circulation by hepatic LDLr (Veniant et al., 1998). In addition, LDL-C can also be absorbed by receptor
119 independent means (Spady et al., 1985). 7) Accumulation of LDL-C can develop into atherosclerosis
120 the major clinical manifestation of CVD (Baigent et al., 2010). 8) Cholesterol can be removed from
121 the tissues by HDL in RCT, via receptors including ATP-binding cassette subfamily A member 1
122 (ABCA1), and scavenger receptor class B member 1 (SRB1), or independently. CETP then acts to
123 facilitate the 1:1 exchange of cholesterol from HDL-C for triacylglycerol from VLDL-C and LDL-C

124 (Ohashi et al., 2005). 9) Cholesterol can be removed from the body by two mechanisms, as
125 cholesterol can be removed directly via the ATP-binding cassette subfamily G5/G8 (ABCG5/G8)
126 receptor and effluxed to the gall bladder (Repa et al., 2002) or alternatively, cholesterol can be
127 converted to bile acids for faecal excretion. Bile acids are usually conjugated to glycine or taurine
128 (3:1) before being effluxed to the gallbladder by receptors, including bile salt export pumps (BSEP)
129 (Soroka and Boyer, 2014) for release into the small intestine postprandially in response to
130 cholecystokinin (CKK) (Marciani et al., 2013). 10) On average, 500mg/day of both cholesterol and
131 bile acids are excreted (Lu et al., 2010). Of the 5% of circulating bile acids that are excreted daily,
132 98% are in the unconjugated form due to a lower reabsorption efficiency in the ileum (Batta et al.,
133 1999; Gérard, 2014). Conjugated bile acids are deconjugated by bacterial modification (Joyce et al.,
134 2014). Bacterial species such as *Lactobacillus* and *Bifidobacterium* produce bile acid hydrolase (BSH)
135 in order to remove the associated amino acid (Oner et al., 2014). There are several survival-
136 promoting motives for bacteria to respond in this way; these include providing a nutrition source
137 and bile acid detoxification (Begley et al., 2006). This modulation of bile acid circulation indicates
138 that the gut microbiome also plays an important role in maintaining cholesterol metabolism.
139 Collectively the mechanisms we have discussed coordinate together to maintain whole body
140 cholesterol balance and age-related changes to such mechanisms have important implications for
141 health.

142

143 **3.0 Impact of Ageing on Cholesterol Metabolism**

144 **3.1 Lipoprotein Dynamics and Ageing**

145 It is well established that LDL-C levels rise with age (Abbott et al., 1983). Evidence from the
146 Framingham Study demonstrates LDL-C steadily rises from 97.08 and 100.44mg/dL in 15-19 year
147 olds, to 132.25 and 156.91mg/dL in 75-79 year olds in males and females, respectively (Abbott et al.,
148 1983). An increase in LDL-C is correlated with an increased risk of CVD; every 1mmol/L of LDL-C is
149 associated with a 28% increased risk of coronary heart disease (CHD)-mortality (Gould et al., 2007).
150 Paradoxically, this is not always the case, as higher levels of LDL-C was associated with a lower risk of
151 all causes of mortality in a Chinese cohort of 935 ≥ 80 year old males and females. In this cohort each
152 1mmol/L increase in LDL-C reflected a 19% decrease in mortality (Lv et al., 2015). Furthermore,
153 abnormally high LDL-C (≥ 3.37 mmol) resulted in a 40% reduction in mortality risk. Participants that
154 survived the three year survey-based study were also found to have a higher prevalence (39.0% vs.
155 27.7%) of central obesity (Lv et al., 2015). This phenomenon in the oldest old could be explained by
156 several factors. Firstly, it is possible that individuals susceptible to the effects of increased LDL-C
157 levels had already died before the age of 80 years, and are consequently not included in studies of
158 the oldest old. It has also been suggested increased LDL-C enhances the immune response to
159 pathogens (Biswas et al., 2015; Netea et al., 1996).

160 A mechanistic explanation for the correlation between advancing age and increased LDL-C is that
161 over time there is a reduction in its rate of clearance from the circulation. Under normal
162 circumstances, apo B-100 containing lipoproteins, LDL-C and VLDL-C, are removed from the
163 circulation by hepatic LDLr (Veniant et al., 1998). From the hepatic pool, cholesterol can be directly
164 effluxed to the small intestine for excretion, or first be converted to bile acids. This process occurs in
165 order to maintain the levels of circulating cholesterol, by counteracting the synthesis and ingestion

166 of cholesterol. Deficiency in LDLr results in severe hypercholesterolaemia (type II), as cholesterol
167 cannot be removed from the plasma and into the liver for excretion (Hasan et al., 2014; Kowala et
168 al., 2000). Murine models have shown LDLr deficiency increases the residence time of LDL-C and
169 VLDL-C by decreasing the clearance rate (Ishibashi et al., 1993). For example, Ishibashi et al. (1993)
170 demonstrated LDLr deficiency increased the half-life of ¹²⁵I-LDL and ¹²⁵I-VLDL by 2.5- and 30-fold
171 respectively, while the half-life of ¹²⁵I-HDL was unaffected. Furthermore, LDLr deficiency induced a 2-
172 fold increase in total cholesterol, a 7- and 9-fold increase in IDL-C, and LDL-C respectively, in addition
173 to a modest 1.3-fold rise in HDL-C (Ishibashi et al., 1993). In humans the number of hepatic LDLr
174 decrease with age, thus reducing the rate of LDL-C clearance, and augmenting LDL-C residence time
175 (Millar et al., 1995). Furthermore, the rate of VLDL apo B-100 synthesis increases (Millar et al., 1995).
176 This age-related decline in LDLr is possibly a contributing factor to LDL-C accumulation. It is likely
177 there are several factors influencing the decline in LDLr with age, the primary factor being the
178 decline in the rate of bile acid synthesis, as discussed in section 3.2. Briefly, a decline in bile acid
179 synthesis, results in a decline in cholesterol utilisation from the hepatic pool. Thus, less cholesterol is
180 required to maintain the hepatic pool, resulting in down regulation of LDLr and plasma cholesterol
181 accumulation. More recently, proprotein convertase subtilisin kexin-9 (PCSK9) has also been
182 associated with LDLr degradation. PCSK9, regulated by SREBP-2, acts by binding to the epidermal
183 growth factor like repeat A domain of LDLr leading to receptor degradation. Levels of PCSK9 have
184 been shown to rise with age, and may account for the age-related reduction in LDLr and LDL-C
185 clearance (Cui et al., 2010; Dubuc et al., 2010).

186 HDL-C levels are also affected by the ageing process (Wilson et al., 1994). Typically, HDL-C is
187 observed to decrease by 1% per year (Ferrara et al., 1997). The age-related decline of the
188 atheroprotective HDL-C is linked with the pathogenesis of CVD (Cooney et al., 2009). For instance, a
189 favourable HDL-C profile is often observed in the offspring of centenarians (Barzilai et al., 2001). Due
190 to the lack of controls, to compare the lipoprotein protein of long lived individuals with age-matched
191 controls, offspring studies are utilised. By using this approach, inherited elevated HDL-C levels can be
192 observed (Barzilai et al., 2001). Therefore, increased levels of HDL-C have been highlighted as a
193 potential mechanism conferring exceptional longevity. This is substantiated by evidence detailing
194 individuals with familial hyperalphalipoproteinaemia, whereby the production rate of apo A-I is
195 markedly increased. These individuals display increased HDL-C levels, and exhibit reduced rates of
196 CHD, which may play a role in promoting exceptional longevity (Rader et al., 1993).

197

198 **3.2 Cholesterol Absorption and the Synthesis and Enterohepatic Circulation of Bile Acids**

199 Cholesterol from both the diet and bile is absorbed in the small intestine (Repa et al., 2002;
200 Tanchaorenrat et al., 2014). Cholesterol absorption is regulated by two receptors on the apical
201 membrane, NPC1L1 and ABCG5/G8. NPC1L1 is predominantly located in the jejunum, although this
202 is found the length of the small intestine, and is responsible for the absorption of sterols from the
203 intestinal lumen into the enterocytes (Masson et al., 2010; Sane et al., 2006). ABCG5/G8 is located
204 primarily in the jejunum and ileum and to a lesser extent, the duodenum, and is responsible for the
205 efflux of non-cholesterol sterols from the enterocyte into the intestinal lumen (Masson et al., 2010;
206 Wang et al., 2007). Murine models have demonstrated that NPC1L1 expression significantly
207 increases in the duodenum and jejunum with age, while ABCG5/G8 expression is suppressed. These
208 age-related changes to receptor expression represented a 19-40% increase in cholesterol absorption

209 between young adult and aged adult mice. This effect was amplified in response to high levels of
210 oestrogen (Duan et al., 2006). These findings are intriguing, as it has long been suggested that an
211 increase in cholesterol absorption is an important factor in the rise in LDL-C which accompanies
212 ageing (Hollander and Morgan, 1979).

213 Bile acid synthesis declines with age in humans (Bertolotti et al., 2007; Einarsson et al., 1985). This is
214 due to a reduction in the hepatic expression of the rate limiting enzyme for bile acid synthesis,
215 CYP7A1 (Bertolotti et al., 2007). This in turn reduces cholesterol utilisation, which is accompanied by
216 a rise in plasma cholesterol (Uchida et al., 1996). Significantly, it has been estimated that with every
217 10 years, there is a decrease of 60mg/day in cholesterol converted to bile acids (Bertolotti et al.,
218 1993). Thus, a decline in bile acid synthesis is another factor which could contribute to the
219 dysregulation of whole body cholesterol metabolism with age.

220 In rodents a mechanistic explanation for the decline in CYP7A1 activity has been postulated. It is
221 suggested the reduction in its activity is in part, due to neuroendocrine dysfunction which causes an
222 age dependent decrease in growth hormone, which is known to act pleiotropically on lipoprotein
223 metabolism (Parini et al., 1999). Synthesised bile acids are effluxed from the liver primarily by BSEP,
224 and stored in the gall bladder, with BSEP expression remaining fairly consistent with age in mice (Fu
225 et al., 2012). Following release into the small intestine postprandially, bile acids aid in the absorption
226 of dietary lipids, and undergo bacterial modification before being reabsorbed or excreted. Therefore,
227 any age related alterations to these processes will have consequences for whole body cholesterol
228 metabolism.

229 Digestive microflora play a vital role in the enterohepatic circulation of bile acids, by modifying bile
230 acids and influencing feedback mechanisms. For example, conventionally grown mice have a 71%
231 reduction in the size of their bile acid pool compared to germ free mice. Furthermore, these
232 conventionally grown mice excrete over 4 times the amount of bile acids (Sayin et al., 2013). This
233 emphasises the comprehensive role of the gut microbiota in regulating enterohepatic circulation. It
234 is therefore logical changes to the gut microbiota with age will have an impact on overall cholesterol
235 metabolism. Within the digestive tract, bile acids are metabolised by the digestive microbiota and
236 converted to secondary bile acids. Deconjugation of primary bile acids by bacterial BSH is essential
237 for this conversion to secondary bile acids. Deconjugated bile acids are more readily excreted than
238 conjugated bile acids, as they are less readily reabsorbed by the apical sodium dependent bile acid
239 transporter (ASBT) (Dawson, 2011). The excreted bile acids need to be replenished from the
240 conversion of cholesterol (Joyce et al., 2014). With age, the rise in LDL-C can in part be explained by
241 the decline in BSH⁺ species, such as *Lactobacillus* and *Bifidobacterium* species (Hopkins and
242 Macfarlane, 2002). A decline in BSH results in fewer bile acids being deconjugated, and thus more
243 are reabsorbed, and fewer are excreted. This results in a decline in the need for bile acid synthesis,
244 and thus cholesterol utilisation is reduced (Joyce et al., 2014). One way to combat this decline in BSH
245 is via the administration of probiotic strains (Al-Sheraji et al., 2012). However, caution is needed
246 when suggesting this strategy as a therapeutic intervention for the treatment of
247 hypercholesterolaemia, as increased concentrations of secondary bile acids can increase
248 inflammation and cancer risk in the colon (Salemans et al., 1993). This is emphasized in older
249 individuals, where intestinal transit time is elevated, and reabsorption of conjugated bile acids is
250 decreased, thus increasing the exposure of the intestinal mucosa to bile acids (Salemans et al.,

251 1993). This elevated exposure time results in the promotion of colorectal cancer in the elderly (Ajouz
252 et al., 2014).

253

254

255 **4.0 Impact of Genetic Variation on Cholesterol Metabolism and Healthy Ageing**

256 There are several key genes involved in cholesterol metabolism; mutations to these genes can
257 impact on plasma cholesterol levels; the response to pharmaceutical intervention; and the
258 pathogenesis of age-related disease. In this section we will discuss several of the key genetic
259 polymorphisms responsible for the dysfunction of cholesterol metabolism, as well as those
260 promoting exceptional longevity. Asselbergs et al. (2012) describe 122 single nucleotide
261 polymorphisms (SNPs) which could account for ~9.9% of the variance in HDL-C levels. Furthermore,
262 104 SNPs could explain ~9.5% of the variance in LDL-C, 142 SNPs could explain 10.3% of variance in
263 total cholesterol, while 110 SNPs could explain 8.0% of the variance associated with triglyceride
264 levels (Asselbergs et al., 2012). In addition, genetic factors can also influence the lipoprotein
265 response to extrinsic factors, such as pharmaceutical intervention or diet. For example, in response
266 to increases in dietary cholesterol, individuals can be categorised as either a hypo-responder, where
267 plasma total cholesterol increases <0.05mmol/L, or as hyper-responders, where there is an increase
268 of ≥0.06mmol/L per each additional 100mg dietary cholesterol, respectively (Herron et al., 2003).
269 Likewise, Herron et al. (2003) demonstrated ingestion of ~640mg/day resulted in a 30% increase in
270 LDL-C and an 8% increase in HDL-C in individuals classified as hyper-responders, whereas LDL-C and
271 HDL-C were unaffected in individuals classed as hypo-responders. Thus, it is not surprising that
272 previously Bosner et al. (1999) demonstrated cholesterol absorption varies from 29.0 to 80.1% in
273 healthy subjects aged between 17 and 80 years of age. Ethnicity also plays a role in this variation,
274 with African-Americans on average absorbing larger amounts of cholesterol than Caucasians or
275 those from Asian descent (63.4% vs. 56.2%). Although, dietary intake, rather than absorption
276 efficiency, appeared to be the dominant factor in cholesterol absorption (Bosner et al., 1999). In
277 addition, the response to pharmaceutical intervention, such as the administration of cholesterol
278 biosynthesis inhibitors or cholesterol absorption inhibitors is highly variable (Barber et al., 2010;
279 Simon et al., 2005). For example, the presence of at least 1 minor allele at g.-18C resulted in a 15%
280 improved reduction in LDL-C in response to ezetimibe + statin therapy (Simon et al., 2005).

281 **4.1 Cholesteryl Ester Transfer Protein**

282 Mutations to the gene encoding for the CETP enzyme can influence CETP activity and size (Cefalu et
283 al., 2009). This affects both the amount of esterified cholesterol transported from HDL to LDL and
284 VLDL, as well as lipoprotein size and number (Wang et al., 2002). There are several mutations within
285 the CETP gene that have been discovered. Of these polymorphisms, several have been associated
286 with lower CETP levels, reduced risk of CVD, and increased longevity. Murine models transfected
287 with CETP undergo extensive lipid profile remodelling resulting in an increased risk for CVD
288 (Westerterp et al., 2006). Therefore, any mutation resulting in decreased CETP, is thought to reduce
289 CVD risk and increase life-span. For example, homozygosity for the common I405V polymorphism is
290 associated exceptional longevity (Barzilai et al., 2003). In one case, a three-fold increase in
291 homozygosity for the I405V genotype was observed in long lived individuals (24.8% vs. 8.6%). This
292 homozygous amino acid substitution of 405 isoleucine for valine reflected a 17% reduction in CETP

293 levels, elevated HDL concentrations by 3.63%, and decreased LDL levels by 7.31%, in comparison to
294 individuals homozygous for the isoleucine codon. Furthermore, LDL and HDL particles were
295 significantly larger (Barzilai et al., 2003). These larger lipoproteins have been associated with a
296 decreased incidence of CVD, hypertension, metabolic syndrome and neurodegeneration (Barzilai et
297 al., 2006; Barzilai et al., 2003). It is likely that larger LDL molecules are less readily able to penetrate
298 the arterial tissue, and therefore result in a decreased risk for atherosclerosis pathogenesis (Barzilai
299 et al., 2003). Homozygosity for the I405V polymorphism is therefore regarded as a protective
300 phenotype for healthy ageing (Atzmon et al., 2005; Barzilai et al., 2006).

301

302

303 The D442G mutation has also been described as an atheroprotective genotype, as the D442G
304 mutation has been shown to increase LDL-C particle size, and HDL-C levels (Wang et al., 2002), in
305 addition to decreasing the risk for CVD mortality (Koropatnick et al., 2008). However, Zhong et al.
306 (1996) demonstrated an increase in HDL-C associated with this genotype, was correlated with an
307 increase in CHD risk (Zhong et al., 1996). Alternatively, Hirano et al. (1997) demonstrated that a G to
308 A mutation in intron 14, which induced a rise in HDL-C exhibited a U-shaped curve of the incidence
309 risk of ischemic change (Hirano et al., 1997). Moreover, Agerholm-Larsen et al. (2000) demonstrated
310 the A373P/R451Q genotype resulted in a decrease in HDL-C in both males and females from the
311 Danish general population. Homozygosity for the mutation resulted in the effect being more
312 pronounced than in heterozygotes, with HDL-C levels of 1.19 and 1.38mmol/L in males and females
313 respectively compared to 1.26 and 1.62mmol/L. Non-carrier males and females had HDL levels of 1.4
314 and 1.74mmol/L, respectively. Although this CETP genotype induced reduced HDL-C levels, they
315 were not associated with ischemic heart disease (IHD). Furthermore, when the authors adjusted for
316 a group of risk factors in addition to HDL-C, the mutation resulted in a 36% reduction in risk of IHD
317 (Agerholm-Larsen et al., 2000).

318

319 **4.2 Niemann-Pick C1-Like 1**

320 Intestinal absorption of cholesterol varies significantly from person to person. In healthy individuals,
321 cholesterol absorption can range from 29.0-80.1% (Bosner et al., 1999). This is due, in part to the
322 genetic variation in the genes encoding for the NPC1L1 receptor, which is responsible for the
323 clathrin-mediated endocytosis of cholesterol from the digestive tract. Cohen et al. (2006) discovered
324 20 polymorphisms within individuals classified as hypo-absorbers, compared to only 5 for the hyper-
325 absorber category. Of the 20 mutations conferring a low cholesterol absorption efficiency, 18 were
326 observed in African-Americans. This reflected the findings that these hypo-absorber phenotypes
327 were more prevalent in African Americans (6.2%) than white (1.8%) or Hispanic (1.7%) populations.
328 These hypo-absorber phenotypes conferred an average 8.6% reduction in LDL-C (Cohen et al., 2006).

329 In individuals with autosomal dominant hypercholesterolaemia, lacking LDLr or apo B mutations,
330 NPC1L1 mutations may play a role in the hypercholesterolaemic phenotype displayed. For example,
331 it has been shown that the -133A>G polymorphism, significantly increases NPC1L1 promoter activity
332 (Martín et al., 2010). More recently, NPC1L1 SNPs have been linked with CVD. For instance, Polisecki
333 et al. (2010) demonstrated that homozygous carriers for the minor alleles at -18A>C, L272L, V1296V

334 or U3_28650A>G exhibited a 2-8% increase in LDL-C, while the risk of developing a fatal or nonfatal
335 CHD event escalated by 50-67% (Polisecki et al., 2010). Muendlein et al. (2015) determined that 24
336 variants, particularly rs55837134 were associated with future cardiovascular events. Homozygosity
337 for the rare rs55837134 variant was associated with a 3-fold increase in cardiovascular event
338 incidence, compared with carriers homozygous for the common allele (Muendlein et al., 2015). In
339 contrast, Stitzel et al. (2014) demonstrated that the presence of 1 of 15 NPC1L1 inactivating
340 mutations, as observed in 1/650 individuals, corresponded to a 12mg/dL decline in LDL-C, and a 53%
341 reduction in cardiovascular event risk (Stitzel et al., 2014). In addition to affecting baseline
342 lipoprotein characteristics, mutations to the NPC1L1 gene also influence the lipoprotein profile
343 response to therapeutic intervention. For example, Simon et al. (2005) demonstrated that
344 individuals homozygous for the common allele g.-18C>A exhibited a 24.2% decline in LDL-C from
345 baseline levels with ezetimibe treatment, compared with 27.8% for individuals heterozygous for the
346 minor allele. Thus, heterozygosity for the minor allele represented a 15% increased response to
347 ezetimibe treatment (Simon et al., 2005). In addition to NPC1L1 mutations leading to an altered
348 response to the NPC1L1 inhibitor ezetimibe, statin treatment efficiency is also affected. Polisecki et
349 al. (2010) demonstrated the -133A>G SNP influenced the LDL-C response to Pravastatin treatment.
350 Males homozygous for the minor -133A>G allele had the greatest decline in LDL-C with pravastatin
351 treatment, while females with the major -133A>G allele exhibited the greatest response to
352 treatment (Polisecki et al., 2010).

353

354 **4.3 Apolipoprotein E**

355 Apo E is present on chylomicrons, VLDL, IDL, and HDL and acts as a ligand for hepatic LDLr and LRP to
356 enable lipoprotein uptake. There are three major alleles associated with the *APOE* gene. These are,
357 $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$, which have a population frequency of 6.9, 76.2 and 16.9%, respectively in a Belgian
358 cohort (Engelborghs et al., 2003). The $\epsilon 3$ allele is most commonly observed, and is considered as the
359 'neutral' apo E genotype. Along with $\epsilon 2$, $\epsilon 3$ preferentially binds to HDL-C, while the $\epsilon 4$ allele has a
360 preference for VLDL-C (Dong and Weisgraber, 1996). The presence of the $\epsilon 4$ allele confers a 15 and
361 25% decline in plasma apo E in males and females, respectively, compared to those with the $\epsilon 3$
362 allele. This decline in apo E is associated with a 2 and 5% increase in LDL-C in males and females,
363 respectively. In comparison, those with the $\epsilon 2$ allele exhibit a 27 and 32% increase in apo E, which is
364 associated with a 10% decrease in LDL-C levels (Larson et al., 2000). The presence of an $\epsilon 4$ allele is
365 considered a risk factor for the development of many conditions including atherosclerosis (Zende et
366 al., 2013), Alzheimer's Disease (Rhinn et al., 2013), and multiple sclerosis (Horakova et al., 2010), in
367 addition to accelerating telomere shortening (Wikgren et al., 2012). On the other hand, this allele
368 has been associated with a higher vitamin D status (Huebbe et al., 2011), and has been identified as
369 a possible protective genotype against macular degeneration (Kovacs et al., 2007). The $\epsilon 2$ allele in
370 contrast has been associated with an increased risk for the disease, or for its earlier onset (Tikellis et
371 al., 2007). Furthermore, homozygosity for the $\epsilon 2$ allele is found in 90% of individuals with
372 hyperlipoproteinaemia type III (Mahley and Rall, 2000). The $\epsilon 2$ isotope results in defective
373 lipoprotein binding to LDLr, which in turn leads to incomplete catabolism of chylomicrons and VLDL-
374 C, resulting in an accumulation of cholesterol rich lipoprotein remnants (Phillips, 2014). However,
375 only 5% of $\epsilon 2$ homozygotes have this disease, and therefore there are other factors involved in the
376 development of the disease (de Beer et al., 2002). With the exception of hyperlipoproteinaemia type

377 III, this $\epsilon 2$ allele has been associated with a protective phenotype against CHD (Bennet et al., 2007).
378 Furthermore, the $\epsilon 2$ allele is positively associated with exceptional longevity in Italian, Danish, US,
379 and Japanese cohorts. In contrast, the presence of the $\epsilon 4$ allele reduced the chance of reaching
380 exceptional longevity in Spanish, Italian, Danish, US and Japanese cohorts (Garatachea et al., 2014;
381 Schupf et al., 2013).

382

383 **4.4 Lipoprotein and Hepatic Lipase**

384 Another enzyme that is effected by genetic mutation is LPL. LPL is primarily found on the endothelial
385 wall of capillaries and is responsible for the hydrolysis of triacylglycerol in chylomicrons and VLDL
386 into FFA and MAG (Goldberg et al., 2009). A common polymorphism in the LPL gene is S447X. In a
387 cohort of middle-aged and elderly American subjects, 44.0 and 50.6% of males and females,
388 respectively exhibited homozygosity for the common allele, while only 12.6 and 7.6% were
389 homozygous for the rare allele (Larson et al., 1999). Heterozygosity was displayed in 43.4 and 41.8%
390 of males and females respectively. Females, but not males, exhibiting homozygosity for the rare
391 allele had lower total cholesterol and LDL-C levels, when compared to heterozygotes and
392 homozygotes for the common allele (Larson et al., 1999). This alteration to cholesterol metabolism
393 could play a role in the association of this genotype with age-related conditions such as
394 hypertension, type 2 diabetes mellitus and coronary artery disease (Daoud et al., 2013; Muñoz-
395 Barrios et al., 2012). Hepatic lipase is responsible for the conversion of IDL to LDL, and can also be
396 effected by genetic mutation. In contrast, the -C480T polymorphism in the hepatic lipase gene have
397 been shown to elevate HDL-C levels. Homozygosity for the common allele was observed in 53.2% of
398 control individuals, while 40.3% of these individuals were observed to be heterozygous.
399 Homozygosity for the -C480T polymorphism was observed in 6.5% of healthy individuals, whereas,
400 this was reduced to 4.7% for individuals with a paternal history of myocardial infarction before the
401 age of 55 years, although this was not statistically significant (Murtomäki et al., 1997). Furthermore,
402 McCaskie et al. (2006) found that although HDL-C levels were raised in an Australian population with
403 this polymorphism, it was not associated with a decrease in CHD risk (McCaskie et al., 2006). In
404 contrast, Fan et al. (2006) found that this polymorphism was associated with a lower coronary flow
405 reserve, which is an early indicator of atherosclerosis (Fan et al., 2006).

406

407 **4.5 HMG CoA Reductase**

408 HMG CoA reductase is the enzyme responsible for the rate limiting step in cholesterol biosynthesis,
409 and is therefore the main target for pharmaceutical intervention by statins (Istvan and Deisenhofer,
410 2001). Chasman et al. (2004) demonstrated that two genetic polymorphisms were not only able to
411 influence the baseline characteristics of the lipoprotein profile, but also influence the efficacy of
412 statin treatment. The presence of one copy of SNP 12 (rs17244841) induced an 18.9% reduction in
413 LDL-C and 4.6% increase in HDL-C, compared with individuals homozygous for the major allele.
414 Whereas, heterozygotes for SNP 29 (rs17238540), exhibited 18.9 and 2.4% reduction in LDL-C and
415 HDL-C, respectively. The presence of one of the SNPs also resulted in the diminished efficacy for
416 cholesterol lowering treatment by pravastatin. For individuals with either SNP, the total cholesterol
417 and LDL-C lowering efficacy was reduced 22 and 19% respectively (Chasman et al., 2004). Thus,
418 genetic polymorphisms in certain enzymes and receptor genes associated with cholesterol

419 biosynthesis can provoke the dysregulation of cholesterol metabolism, lipoprotein profile, alter CVD
420 risk, and the response of cholesterol metabolism to pharmaceutical intervention.

421

422 **5.0 Oxidative Stress and Cholesterol Metabolism**

423 The free radical theory of ageing is underpinned by the belief, that the gradual accumulation of
424 oxidative stress with time is responsible for the ageing process (Harman, 1956, 2009). Reactive
425 oxygen species (ROS) play a key role in the development of oxidative stress (Kandola et al., 2015).
426 ROS are produced during mitochondrial oxidative phosphorylation, and by cells exposed to
427 xenobiotics (Berthiaume and Wallace, 2007), pathogen associated patterns (PAMPs) (Tassi et al.,
428 2009) or pro-inflammatory cytokines (Yang et al., 2007). Despite the processed role ROS may play in
429 the ageing process, ROS also have useful roles in processes such as phagocyte derived bactericidal
430 and tumouricidal activity (Li et al., 2013; Vatanserver et al., 2013), nitric oxide (NO) production (Shen
431 et al., 2014), and in insulin signalling (Bashan et al., 2009). Atherosclerosis is suggested to be a
432 condition mediated by ROS, LDL-C and intrinsic ageing (Vogiatzi et al., 2009). Briefly, LDL-C migrate
433 across damaged artery endothelium into the tunica intima, where an accumulation of LDL-C,
434 immune cells, and proliferative smooth muscle cells occlude the artery lumen restricting blood flow
435 (Hansson and Hermansson, 2011). This endothelial damage and dysfunction can be influenced by a
436 variety of factors including smoking (Ambrose and Barua, 2004), hypertension (Li and Chen, 2005),
437 hyperglycaemia (Popov, 2010), hyperlipidaemia (Kerenyi et al., 2006), ageing (Wang and Bennett,
438 2012), infection (Rosenfeld and Campbell, 2011), and hyperhomocysteinaemia (Guthikonda and
439 Haynes, 2006). This damage results in increased ROS production, and a more permeable membrane
440 in which LDL-C and immune cells can more freely migrate. Oxidation of LDL by ROS forms the
441 cytotoxic and immunogenic oxLDL (Mahmoudi et al., 2011). Release of monocyte chemotactic
442 protein-1 (MCP-1) by endothelial smooth muscle cells and macrophage that have already localised in
443 the tunica intima, leads to the migration of monocytes across the endothelium where they
444 differentiate into macrophage (Dewald et al., 2005). These macrophage then engulf oxLDL via
445 scavenger receptors SR-A and CD36, forming lipid-laden foam cells (Korporaal et al., 2007).
446 Meanwhile, T cells, mainly Th1, migrate across the endothelium and release pro-inflammatory
447 cytokines such as IL-2, IL-12 and IFN- γ to intensify the immune response (Baidya and Zeng, 2005).
448 Foam cells, macrophage, and T-cells then combine to form a fatty streak. The macrophage also
449 secrete the pro-inflammatory cytokines TNF α , IL-1 β , IL-6, and IL-12, in addition to the mitogen
450 platelet derived growth factor (PDGF), which induces the proliferation of smooth muscle cells of the
451 tunica media forming a cap for the plaque (Ross et al., 1990). This segregates the plaque from the
452 blood, however the plaque cause the artery to harden and narrow, restricting blood flow.
453 Subsequent instability in the plaque can result in it rupturing; which can block the supply of blood to
454 the heart causing a myocardial infarction, or to the brain, triggering an ischaemic stroke (Bentzon et
455 al., 2014). In addition to the effects of ROS on LDL, it has also been shown to interact with the
456 atheroprotective particle HDL, it has been suggested HDL is oxidised during the pathogenesis of
457 atherosclerosis, causing HDL to lose its protective properties and transform into a proinflammatory
458 and proatherogenic mediator. These oxidised HDL, oxHDL, have been shown to promote smooth
459 muscle cell proliferation and migration in a dose dependent manner, thus aiding in the progression
460 of atherosclerosis pathogenesis (Wang et al., 2014). Further to this, oxHDL, have also been shown to
461 induce ROS production, upregulate the expression of the proinflammatory cytokine TNF- α , and

462 upregulate the expression of prothrombotic cyclooxygenase-2 (COX-2) and plasminogen activator
463 inhibitor-1 (PAI-1) (Callegari et al., 2006; Norata et al., 2004; Soumyarani and Jayakumari, 2012).

464

465 **6.0 Caloric Restriction**

466 CR, a dietary regime defined by a 20-40% reduction of calories, which does not induce malnutrition
467 (Taormina and Mirisola, 2014), has been demonstrated to extend life-span in a diverse range of
468 organisms, however its effect on humans has not be fully established (Barzilai et al., 2012; Guarente,
469 2013). CR has been associated with many metabolic effects linked to ageing and longevity. For
470 example, CR has been associated with a reduction in the release of ROS from complex I of
471 mitochondria within the cardiac tissue of rodents (Gredilla et al., 2001). Therefore, there is a
472 prevailing hypothesis within gerontology, that the positive effects of this dietary regime are
473 mediated through a reduction in ROS. However, it is possible that the beneficial effects of CR on
474 health-span extend beyond this particular aspect of ageing, as evidence suggests, that metabolic
475 rate is unaffected by CR in murine models (Hempenstall et al., 2010). Moreover, it is considered that
476 ageing is associated with the accumulation of ROS and oxidative damage. Conversely, recent
477 evidence has suggested that low grade oxidative damage may be beneficial. As an example, glucose
478 restriction has been associated with an increase in oxidative stress in *Caenorhabditis elegans*, which
479 is thought to increase resistance to further oxidative stress, and thus extend life-span via
480 mitochondrial hormesis (Schulz et al., 2007). Alternatively, murine models have demonstrated that
481 calorie restriction can prevent the age-related decline of heat shock proteins (HSPs), which are
482 induced following exposure to stress to protect cells and organs from the stressor (Colotti et al.,
483 2005). CR has also been shown to have a positive effect on cholesterol metabolism in mammals. For
484 instance, Edwards et al. (1988) investigated the effect of CR on LDL-C over a five year period in
485 Rhesus monkeys and found this regime reduced LDL-C levels when compared to a control group
486 (Edwards et al., 1998). Much more recently, it has also been suggested CR improves metabolic
487 health generally (Ristow and Zarse, 2010). For instance, Colman et al. (2014) demonstrated a 2.9
488 times increased risk for all age-related causes of death, in Rhesus monkeys undertaking a control
489 diet, when compared to those undertaking a 30% CR diet. CR also increased the survival rate of
490 those animals by 3.63 times (Colman et al., 2014). The Comprehensive Assessment of Long-Term
491 Effects of Reducing Calorie Intake (CALERIE) study provides information on the effect of CR in
492 humans. Phase one of this program examined healthy, but overweight individuals (BMI 25-
493 29.9kg/m²) from three centres across America who underwent 20-25% CR. From these studies it was
494 determined two biomarkers of longevity, fasting insulin and body temperature were reduced
495 following 6 months of 25% CR. The authors of this study postulated that CR increases longevity via a
496 reduction in metabolic rate (Heilbronn et al., 2006). In terms of a direct impact on lipid metabolism,
497 CR was shown to decrease weight, fat mass and visceral adipose tissue in participants. These
498 changes were associated with an increase in insulin sensitivity (Larson-Meyer et al., 2006). The
499 project has recently progressed to phase 2 trials, to examine the effects of CR on healthy nonobese
500 (BMI 22-28kg/m²) individuals (Stewart et al., 2013).

501 The effects of CR in humans has also been investigated by Fontana et al. (2004). In this study, the
502 lipoprotein profile and carotid artery intima-media thickness of 18 members of the Caloric
503 Restriction Society, whose members practice long term self-imposed CR (3-15 years), was compared
504 with 18 control individuals. This investigation revealed a number of interesting findings about the

505 interaction of CR with lipid metabolism, including a decline in total cholesterol, LDL-C, and
506 triacylglycerol by 19.1, 29.5 and 63.8%, respectively following CR. HDL-C was also affected by CR,
507 with a 51.2% elevation in levels. This was in addition to a reduction in other risk factors associated
508 with CVD including, blood pressure and the inflammatory marker C-reactive protein (CRP). Together
509 with the carotid intima-media thickness reduction of approximately 40%, CR appears to have an
510 atheroprotective effect (Fontana et al., 2004). We can conclude from these studies, although it is
511 clear that CR increases life-span in many species, the underlying mechanisms are still ambiguous.
512 However, in mammals a favourable lipid profile could be one component of a much broader
513 cardioprotective protective effect brought on by CR which ultimately contributes to life span
514 extension.

515

516 **7.0 Sirtuins, mTOR and Cholesterol Biosynthesis**

517 Mechanistic target of rapamycin (mTOR) is an evolutionarily conserved serine/threonine protein
518 kinase of the phosphatidylinositol-3-OH kinase (PI(3)K)-related family that regulates an array of
519 anabolic and catabolic pathways at the mRNA expression level (Johnson et al., 2013). mTOR acts as a
520 key metabolic sensor in a wide range of biological activities, both at a cellular and organism level.
521 This ability to act as a regulator causes it to respond to a plethora of both intrinsic and extrinsic
522 cellular signals (Mc Auley et al., 2015). These metabolic cues include changes to oxygen, nutrient and
523 hormonal levels. mTOR forms the catalytic subunit of two discrete signalling complexes, known as
524 mTOR complexes 1 and 2 (mTORC1 and mTORC2). The mTOR pathway impacts cell growth and
525 proliferation by provoking anabolic processes, including biosynthesis of proteins, lipids and
526 organelles, and by restricting catabolic processes, such as autophagy. There is a large body of
527 evidence which has been generated from several animal models that link the activities of mTORC1 to
528 the beneficial effects of CR, and thus longevity. Discussing these studies is beyond the scope of this
529 review, rather we will focus on how mTOR impacts cholesterol biosynthesis. Central to the
530 regulation of cholesterol biosynthetic gene expression is the SREBP family of transcription factors
531 (Horton et al., 2002). It has been observed that silencing of SREBP inhibits Akt (Protein kinase B
532 (PKB)) dependent lipogenesis. Akt is an upstream regulator of mTOR, and it has been suggested
533 PI3K/Akt/TOR pathway regulates protein and lipid biosynthesis in an orchestrated manner
534 (Porstmann et al., 2008). More recently, Peterson et al. (2011) demonstrated TORC1 regulates SREBP
535 by controlling the nuclear entry of lipin 1, a phosphatidic acid phosphatase. It was found that
536 inhibition of hepatic mTORC1 impaired SREBP function and resulted in mice becoming tolerant in a
537 lipin 1-dependent fashion, to hepatic steatosis and hypercholesterolemia induced by a high-fat and
538 cholesterol diet (Peterson et al., 2011). Moreover, a recent study that examined non-alcoholic fatty
539 liver disease under conditions of inflammation in apolipoprotein E knockout mice, demonstrated the
540 inhibition of mTORC1 activity blocked the translocation of SCAP/SREBP-2 complex from the
541 endoplasmic reticulum to the Golgi, and decreased the expression of LDLr and SREBP-2. These
542 effects were accompanied by an increase in LDLr degradation (Liu et al., 2015). Thus, this study
543 suggests that there could be an important link between mTOR and LDLr turnover, which has
544 significant implications for whole body cholesterol balance and healthy ageing.

545 Sirtuins have also been shown to impact cholesterol biosynthesis. There are 7 known mammalian
546 sirtuins, that function as NAD⁺-dependent deacetylases, which are involved in a wide range of
547 cellular activities including nutrient sensing and DNA repair (Chang et al., 2009; de Magalhães et al.,
548 2012). The most well studied of the sirtuins, SIRT1, plays a role in various metabolic processes that
549 enable the cell to adapt to changes in nutrient levels. For instance, SIRT1 plays a part in modulating

550 hepatic gluconeogenesis, insulin secretion, fat mobilisation, and stress responses (Satoh et al., 2011;
551 Wei et al., 2011). SIRT1 also deacetylates the nuclear receptor liver X receptor α (LXR α) to induce
552 synthesis of the transporter ABCA1, a mediator of HDL and RCT. SIRT1 KO mice display reduced
553 plasma HDL-C levels in addition to an accumulation of cholesterol in the liver (Li et al., 2007). SIRT1
554 has also been suggested to be cardioprotective. For instance, evidence indicates it has a role in
555 preventing cardiac hypertrophy (Planavila et al., 2011). In contrast, it has been demonstrated that
556 inhibition of SIRT2 can reduce sterol biosynthesis by decreased trafficking of SREBP-2, as a
557 mechanism of neuroprotection in cellular and invertebrate models of Huntingtons Disease (Luthi-
558 Carter et al., 2010). Moreover, Tao et al. (2013) have suggested that Sirt6 is a critical factor for
559 Srebp2 gene regulation. Hepatic deficiency of Sirt6 in mice resulted in elevated serum and hepatic
560 cholesterol levels. Sirt6 is recruited by forkhead box O (FoxO)3 to Srebp2, where Sirt6 deacetylates
561 histone H3 at lysines 9 and 56, thus promoting a repressive chromatin state. It was found that Sirt6
562 or FoxO3 overexpression improved hypercholesterolemia in diet-induced or genetically obese mice
563 (Tao et al., 2013). Therefore, Sirt6 and FoxO3 could have a crucial role to play in the regulation of
564 cholesterol homeostasis

565

566 **8.0 Can Diet Mitigate the Effect Ageing has on Cholesterol Metabolism?**

567 During the 1950s, the Seven Countries Study (SCS) began exploring the role of diet and lifestyle on
568 disease rates in populations from various countries. Amongst the findings reported from these
569 studies were the causal association between, serum cholesterol, blood pressure and smoking and
570 CHD mortality rates (Menotti et al., 1998; Menotti et al., 2004a; Menotti et al., 2004b), whereas,
571 diets high in saturated fat, and trans fats were associated with higher serum cholesterol and thus
572 CHD risk (Kromhout et al., 1995). Conversely, diets high in vegetables, rich in fibre and antioxidants,
573 promoted significant reductions in CHD risk (Buijsse et al., 2008; Streppel et al., 2008). Dietary
574 regime is therefore an important factor that should be analysed and adjusted in order to reduce CHD
575 risk and promote longevity. The important role of dietary and other lifestyle interventions on life-
576 span can be emphasised by analysing the North Karelia Project. Internationally, Finnish males,
577 especially those in the province of North Karelia, had the highest rate of CHD in the late 1960s, as a
578 result of a diet high in salt and saturated fat, and low in vegetables, in addition to high rates of
579 smoking and physical inactivity (Puska, 2008). In order to combat this burden, a low-resource,
580 community-based intervention study titled the North Karelia Project was implemented in 1972
581 (Puska, 1973). The North Karelia Project aimed to reduce CHD morbidity and mortality rates by
582 reducing LDL-C concentrations and blood pressure by improving diet and exercise patterns; and
583 reducing smoking rates. The project resulted in the most rapid decline in CHD mortality in the world.
584 Within 5 years, a 4.1 and 1.2% reduction in serum cholesterol was exhibited in men and women,
585 respectively (Puska et al., 1979). These figures increased further to a 21% and 23% decline in total
586 cholesterol under re-examination in 2007 (Vartiainen et al., 2010). The initial five year study resulted
587 in a 17.4 and 11.5% reduction in CHD risk in males and females, respectively. Following a further 25
588 years of implementation, this decline was amplified to a 60% reduction (Puska et al., 1979;
589 Vartiainen et al., 2010). This 30 year project reflected an 85% decrease in CHD-related mortality
590 (Puska, 2008). The impact of lifestyle on cholesterol metabolism, and consequently CVD risk is
591 therefore significant. The role diet and lifestyle plays in reducing risk of age related diseases and in
592 extending life-span is also apparent in those who consume a Mediterranean diet. This dietary

593 pattern has been studied extensively, particularly, the role it plays in optimising lipoprotein profile
594 and reducing CVD risk

595

596 **8.1 Mediterranean Diet**

597 The Mediterranean diet is characterised by a high intake of vegetables, fruits, legumes, nuts, cereals
598 and olive oil, and a low intake of dairy, and red and processed meats (Trichopoulou and Lagiou,
599 1997). Richard et al. (2012) demonstrated a five week Mediterranean diet decreased LDL-C by 9.9%,
600 even in the absence of weight loss in men with metabolic syndrome. It was suggested this dietary
601 pattern was able to effect LDL-C levels, by increasing LDL-C clearance as well as reducing cholesterol
602 absorption. This was thought to be due to an increase of dietary phytosterols, nutrients,
603 monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), fish oils and fibre (Richard
604 et al., 2012; Woodside et al., 2015). The Mediterranean diet affects cholesterol metabolism as
605 follows. Firstly, it is postulated PUFA increases LDLr expression (Fernandez and West, 2005).
606 Furthermore, studies have indicated plant sterols can reduce cholesterol absorption by 30-50% (Law,
607 2000), although the expression of ABCG5/G8 and NPC1L1 are thought to be unaffected by sterol
608 ingestion (Field et al., 2004). Consumption of a Mediterranean diet has not only been associated
609 with a reduction in the incident rate of the age related diseases, type II diabetes mellitus, CVD, and
610 cancer, by 52, 30, and 12%, respectively (Benetou et al., 2008; Estruch et al., 2013; Salas-Salvadó et
611 al., 2011). Furthermore, individuals, from Spain or Italy for example, born in 2000, are expected to
612 live on average 2 years longer than individuals from the UK or USA. In addition, the healthy life
613 expectancy of these individuals is also 2 years more (WHO, 2015). Thus, the Mediterranean diet is
614 believed to play a role in prolonging both health-span and life-span. The Mediterranean diet has also
615 been utilised as a strategy to treat age-related disease onset. For example, de Lorgeril et al. (1999)
616 reported a 9.11% reduction in the rate of secondary cardiovascular events in patients who adhered
617 to a Mediterranean diet compared to those that followed a standard diet. It was determined that
618 each 1mmol/L increase in total cholesterol resulted in a 20-30% increase in the risk of recurrence (de
619 Lorgeril et al., 1999). Therefore, a Mediterranean diet that results in decreased cholesterol levels is
620 not only protective against primary cardiovascular events but also secondary events. The substantial
621 evidence demonstrating the potential benefit of a Mediterranean diet on prolonging health-span as
622 well as life-span has resulted in large-scale studies, such as the NU-AGE project arising (Santoro et
623 al., 2014). The NU-AGE project aims to utilise the Mediterranean diet as a treatment strategy to slow
624 the rate of inflammaging, in addition to establishing the molecular mechanisms underpinning the
625 anti-inflammaging effect of this dietary approach (Santoro et al., 2014).

626

627 **9.0 The Recent Emergence of the Gut Microbiome**

628 The gut microbiome has a range of metabolic roles which maintain host health, including; facilitating
629 the digestion of starch, fibre, and sugars (Szilagyi et al., 2010); producing short-chain fatty acids (den
630 Besten et al., 2013; Yu et al., 2010); vitamin absorption (Beulens et al., 2013); enhancing host
631 immunity; preventing allergies (Shen and Clemente, 2015) and facilitating enterohepatic circulation
632 of bile acids (section 3.2). Alteration to the microbiome can impact host health and this has
633 increasingly been investigated as a contributor to disease. The close relationship between the
634 microbiome and its human host has resulted in humans being described as metaorganisms (Biagi et

635 al., 2012). The impact of the microbiome on overall health was recently illustrated by a female
636 subject that underwent a faecal transplant from her overweight, but otherwise healthy daughter for
637 the treatment of recurrent *Clostridium difficile* infection. Post-transplant, the recipient experienced
638 substantial weight gain, resulting in a weight gain of 41 pounds and an increase in BMI from 26 to
639 34.5 at 36 months observation (Alang and Kelly, 2015). This suggests 'obesity promoting' microbiota
640 can be transmitted from human to human, as previously observed in rodents (Ridaura et al., 2013).
641 Understanding the role of the microbiome in health is challenging, due to complex bidirectional
642 interactions with many biological systems. For example, it has been implicated in enhancing alveolar
643 macrophage function in lung infections (Schuijt et al., 2015) and is thought to influence brain
644 morphology and function (Fernandez-Real et al., 2015). A decrease in Actinobacteria with age is
645 associated with amygdala disruption and thalamic microstructure, reduced motor speed and
646 attention, in addition to increased intra-abdominal fat (Fernandez-Real et al., 2015). Conversely, in a
647 classic study, Killian et al. (1998) showed mice exposed to stress exhibited altered intestinal function
648 (Kiliaan et al., 1998). Moreover, administration of probiotic strains impact behaviour by improving
649 mood and decreasing anxiety symptoms in both rodent and humans (Messaoudi et al., 2011;
650 Savignac et al., 2015; Steenbergen et al., 2015). Thus, a bidirectional relationship exists between the
651 gut and brain and it is likely that a similar relationship exists for other organ systems.

652 **9.1 The Gut Microbiome and CVD**

653 There is an association between the microbiota and CVD risk. This could be mediated via its effects
654 on bile acid metabolism, or by its contribution to choline diet-induced trimethylamine N-oxide
655 (TMAO) production (Joyce et al., 2014; Koeth et al., 2013). Susceptibility to atherosclerosis has also
656 been demonstrated to be transferable by microbiota transplantation in murine models (Gregory et
657 al., 2015). Moreover, gut microbiota dysbiosis has been associated with increased low-grade
658 inflammation, which is linked with the development of atherosclerosis (Chistiakov et al., 2015). To
659 examine the role of the gut microbiome on CVD risk, Fu et al. (2015) explored the potential
660 relationships between operational taxonomic units (OTUs) with BMI, and blood lipids. High bacterial
661 diversity was associated with a decreased BMI, and triglyceride levels, whilst a positive correlation
662 was observed with HDL-C levels. A total of 66 OTUs were associated with BMI, while 114 were
663 associated with triglycerides, and 34 OTUs with HDL. In particular *Clostridiaceae/Lachnospiraceae*
664 was able to modulate LDL-C levels. Fu et al. (2015) estimated that the gut microbiota is
665 independently responsible for $\leq 6\%$ of blood lipid level variation (Fu et al., 2015).

666

667 **9.2 The Gut Microbiome and Ageing**

668 Due to inter-individual variation, there is conflicting evidence on microbiome changes during ageing.
669 In an elderly Irish cohort (65-96 years), the proportion of Bacteroidetes ranged from 3-92%, while
670 Firmicutes ranged from 7-94% (Claesson et al., 2011). Further differences in the gut microbiome
671 have also been observed in other population groups. For example, *Clostridium* cluster XIVa has been
672 observed to decrease with age in Japanese, Finnish, and Austrian cohorts (Hayashi et al., 2003; Hippe
673 et al., 2011; Makivuokko et al., 2010), whereas an increase has been observed in German and Italian
674 cohorts (Mueller et al., 2006). Biagi et al. (2010) reported higher levels of the *Clostridium* cluster
675 XIVa in elderly Italians (49%), when compared to younger individuals (44%), although the levels did
676 reduce slightly in centenarians (34%) (Biagi et al., 2010). These conflicting results make it difficult to

677 establish an overall picture of how ageing effects the microbiome. However, it is likely that diet,
678 lifestyle, antibiotic usage, and host health status accounts for much of this variation (Candela et al.,
679 2014; Claesson et al., 2012; O'Sullivan et al., 2013). For example, the reduction in species diversity
680 witnessed with age in humans (Biagi et al., 2010), is amplified in those housed in long-term
681 residential care (Claesson et al., 2012). Furthermore, a carnivorous or herbivorous diet can induce
682 changes to the microbiome composition to favour metabolism of protein or carbohydrates (David et
683 al., 2014). Moreover, Evard et al. (2012) demonstrated that a high fat diet decreased the expression
684 of regenerating islet-derived 3 gamma (Reg3g), an antimicrobial lectin with activity against Gram-
685 positive species. This reduction of Reg3g increases colonisation of the intestinal epithelium, causing
686 alterations in the microbiome, including a decrease in the Firmicutes/Bacteroides ratio. However,
687 prebiotic administration is able to counteract this decrease in Reg3g (Everard et al., 2014).

688 Bacteria from the phyla Bacteroidetes and Firmicutes contribute to 95% of faecal microbiota across
689 ages, however a slight decline has been observed in centenarians (93%) (Biagi et al., 2010), while the
690 Firmicutes/Bacteroidetes ratio also lowers with age (Park et al., 2015). In addition, Claesson et al.
691 (2011) demonstrated Firmicutes increased from 40% to 51%, and Bacteroidetes decreased from 57%
692 to 41%, when comparing a young cohort (28-46 years old) to an elderly cohort (≥ 65 years old)
693 (Claesson et al., 2011). In contrast, Biagi et al. (2010) found that the Firmicutes/Bacteroidetes ratio
694 increased from 3.9 in young individuals to 5.1 in elderly individuals, before decreasing to 3.6 in
695 centenarians (Biagi et al., 2010). Furthermore, species diversity and number of *Bifidobacterium* and
696 *Lactobacillus* species commonly declines with age (Hopkins and Macfarlane, 2002; Wang et al.,
697 2015). Hopkins and Macfarlane (2002) found that species diversity of *Bifidobacterium* and
698 *Lactobacillus* decreased by 57.1 and 45.5% respectively between healthy young adults aged 21-34,
699 and healthy elderly individuals, aged 67-73 years old. The number of *Bifidobacterium* and
700 *Lactobacillus* species, measured as \log_{10} CFU/g wet weight of faeces, decreased by 53.2 and 52.2%
701 respectively with age (Hopkins and Macfarlane, 2002). In addition, with age, there is an increase of
702 potentially pathogenic facultative anaerobes. For example, Proteobacteria increased from 1.2% to
703 2.6% in human centenarians, whilst bacilli increased from 5% to 12% (Biagi et al., 2010).

704 Evidence suggests centenarians have further altered gut microbiota than elderly cohorts (Biagi et al.,
705 2010). For example, when comparing the gut microbiota of cohorts exhibiting 'normal life-spans'
706 (urbanised town communities, UTC) with those exhibiting exceptional longevity (longevity village
707 communities, LVC) in South Korea, LVC individuals displayed significantly higher numbers of
708 *Bacteroides*, *Prevotella*, and *Lachnospira*, while levels of *Dialister*, *Subdoligranulum*, *Megamonas*,
709 EF401882_g, and AM275436_g were greater in UTC individuals. The content of pro-inflammatory
710 LPS was also significantly lower in the faecal samples of the LVC cohort. Higher LPS levels were
711 associated with increased meat intake, decreased vegetable intake, and the presence of several
712 bacterial species found only in the UTC cohort (Park et al., 2015). These factors could influence the
713 progression of low-grade inflammation. This view is consolidated as bacteria associated with anti-
714 inflammatory effects were significantly higher in the LVC cohort, making it possible that factors such
715 as diet, influence microbiome composition, and result in a drop in pro-inflammatory LPS and a
716 concomitant reduction in inflammaging. Additionally, Biagi et al. (2010) found that an age-related
717 increase in potentially pathogenic Proteobacteria was correlated with the upregulation of pro-
718 inflammatory IL-6 or IL-8 (Biagi et al., 2010). This further consolidates the belief, that reducing
719 proinflammatory mediators such as LPS/cytokines could reduce inflammaging and promote healthy
720 ageing (Biagi et al., 2010; Park et al., 2015).

721 The microbiome also affects metabolism. By investigating the bacterial genetic material in human
722 faecal samples, Rampelli et al. (2013) revealed an increase in the bacterial genes involved in
723 tryptophan metabolism with age. It is plausible that this age-dependent increase in bacterial
724 tryptophan metabolism, decreases host bioavailability, a phenomenon which is implicated in a
725 variety of inflammatory related conditions (Capuron et al., 2011; Murr et al., 2015). Furthermore,
726 the abundance of genes involved in SCFA production reduced with age. Moreover there was a
727 decrease in bacterial saccharolytic potential, while an increase in proteolytic potential, diverted
728 metabolism towards putrefaction. Furthermore, increasing age corresponded with the enrichment
729 of genes relating to pathobionts such as *Escherichia* (Rampelli et al., 2013). Future investigations will
730 no doubt explore further bidirectional relationships between the regulation of lipid metabolism, the
731 gut microbiome and intrinsic ageing.

732

733 **10.0 Current and Future Therapeutic Strategies**

734 The emerging bi-directional relationship between the gut microbiome and human host promotes
735 this as a potential therapeutic target for the regulation of many host systems. Probiotic
736 administration has been highlighted as an effective immunomodulator, which can have potential
737 benefits on many diseases (Patel et al., 2015). For example, Makino et al. (2010) demonstrated that
738 a daily probiotic intake for 8-12 weeks resulted in a 2.6 times lower risk of becoming infected with
739 the influenza virus in individuals ≥ 40 years old (Makino et al., 2010). Furthermore, it has been
740 demonstrated that administration of probiotics for several weeks prior to a flu vaccination, increases
741 initial antibody titres in addition to maintaining these enhanced levels for increased lengths of time
742 in elderly cohorts (Boge et al., 2009; Nagafuchi et al., 2015). As well as this, probiotics have been
743 found to influence cholesterol metabolism. Al-Sheraji et al. (2012) demonstrated an 8 week probiotic
744 supplementation in an elderly murine model significantly reduced plasma total cholesterol,
745 triglycerides, LDL-C, and VLDL-C, in addition to increasing HDL-C levels. Moreover, probiotic
746 supplementation significantly reduced the atherosclerotic index of these animals (Al-Sheraji et al.,
747 2012). These alterations in plasma cholesterol levels could be due to a number of factors, including,
748 the generation of SCFAs which may reduce the rate of hepatic cholesterol synthesis, the increase in
749 bile acid deconjugation resulting in reduced cholesterol absorption, and the increase in bile acid
750 excretion (Al-Sheraji et al., 2012; Begley et al., 2006; Hara et al., 1999).

751 Furthermore, dietary interventions such as the Dietary Approaches to Stop Hypertension (DASH) and
752 portfolio diets, which target the risk factors for CVD, hypertension and hypercholesterolaemia
753 respectively, can be utilised (Jenkins et al., 2015; Keith et al., 2015; Rifai and Silver, 2015). For
754 example, a recent meta-analysis determined the DASH diet lowered systolic pressure by 6.74mmHg,
755 and diastolic blood pressure by 3.54 mmHg (Saneei et al., 2014). Although the portfolio diet is less
756 successful in lowering blood pressure, it is effective at modifying the lipoprotein profile. Jenkins et al.
757 (2011) observed a 13.1 and 13.8% reduction in LDL-C in individuals undertaking the routine and
758 intensive portfolio diets over a 6 month period. Adherence to the routine or intensive portfolio diet
759 resulted in a respective calculated 10 year CHD risk reduction of 10.8 and 11.3% respectively (Jenkins
760 et al., 2011). As there is a significant risk reduction for CHD, and few adverse reactions associated
761 with these diets, wide-scale utilisation in elderly individuals may play a role in maintaining good
762 health in later years. Further to this, dependence on pharmaceutical intervention may be reduced.
763 Moreover, many of the food items associated with these diets contain phytochemicals that can

764 positively modulate infection and/or inflammaging and its related diseases (London and Beezhold,
765 2015; McCarthy and O'Gara, 2015; Shayganni et al., 2015). Another viable therapeutic avenue could
766 be to inhibit PCSK9. Recently inhibition of this enzyme has proven to be effective at lowering LDL-C
767 in patients with hypercholesterolaemia. By inhibiting PCSK9, the rate of LDLr degradation is reduced,
768 and the rate of LDL-C clearance can be maintained. A systemic review and meta-analysis of phase 2
769 or 3 randomised controlled trials revealed treatment with monoclonal antibodies targeting PCSK9
770 lowered LDL-C levels by 47.49%, and reduced all-cause mortality and myocardial infarction risk,
771 although cardiovascular mortality was unaffected (Navarese et al., 2015).

772

773 **11.0 The role of Mathematical Modelling in Identifying Future Therapeutic Strategies**

774 It is clear from the biological mechanisms and complex interactions outlined in this review that
775 studying their dynamics is challenging. In recent years, research in this area has benefitted from
776 adopting a systems biology paradigm to study the inherent complexities associated with ageing and
777 metabolism (Mc Auley and Mooney, 2015a; Mc Auley et al., 2013; McAuley et al., 2009). The
778 systems biology approach provides a framework for dealing with this intrinsic complexity. Central to
779 this approach is the use of mathematical models, which work in tandem with experimental work by
780 integrating experimental data and enabling dynamic behaviour to be modelled in a holistic manner
781 (Enrique Salcedo-Sora and Mc Auley, 2016; Kilner et al., 2016; Mooney et al., 2016). This contrasts
782 with the often reductionist approach that is commonly used in experimental biology, which
783 generally focuses on a small number of processes operating in isolation. The utility of mathematical
784 modelling lies in its inherent ability to facilitate hypothesis exploration, and to make predictions
785 about the behaviour of the biological systems in question, and can often lead to a deeper
786 understanding of the biology. Recently, there has been three excellent reviews of mathematical
787 models in this area (Mc Auley and Mooney, 2015b; Paalvast et al., 2015; Parton et al., 2015),
788 therefore our aim here is not to review each of these models, but to provide a synopsis of how
789 mathematical models of cholesterol metabolism, and its associated processes can be used to
790 enhance our understanding of how ageing impacts this core biological system. We addressed this
791 problem recently by constructing a whole body mathematical model of cholesterol metabolism and
792 its age associated dysregulation (Mc Auley et al., 2005; Mc Auley et al., 2012). Within this framework
793 we included several key mechanisms, including LDLr turnover, intestinal cholesterol absorption, and
794 endogenous cholesterol synthesis. Using the model, a number of mechanisms were explored. Firstly,
795 using an *in silico* simulation we gradually reduced the efficiency of cholesterol absorption.
796 Interestingly, by increasing cholesterol absorption from 50% to 80% by 65 years, we were able to
797 show that LDL-C increased by 34 mg/dL from its baseline value of 100mg/dL at 20 years of age in a
798 healthy adult male. However, the key finding of the model centred on hepatic LDLr. Using the model
799 we were able to show that by decreasing the activity of the LDLr to 50% by age 65 years, this
800 produced a rise in LDL-C of 116 mg/dL from a base line value of 100mg/dL at age 20 years in a
801 healthy male. Our model is coded in the Systems Biology Markup Language, SBML (Hucka et al.,
802 2003), and is archived in the BioModels database (Le Novere et al., 2006)
803 (<http://www.ebi.ac.uk/biomodels-main/BIOMD0000000434>). This makes the model straightforward
804 to adapt and update.

805 Recently other groups have adapted the model, for example, Mishra et al. (2014) included the
806 variables body weight and physical activity and explored cholesterol absorption in depth (Mishra et

807 al., 2014). Moreover, Paalvast and colleagues used the model to conduct an *in silico* experiment
808 utilizing the statin, simvastatin (Paalvast et al., 2015). To simulate this effect, the authors reduced
809 hepatic cholesterol synthesis by 75%. This resulted in a reduction in LDL-C of 14% and 33% in six
810 weeks and one year respectively. In recent years a number of other models have mathematically
811 represented various aspects of cholesterol metabolism. Briefly, these include models of cholesterol
812 biosynthesis (Bhattacharya et al., 2014; Kervizic and Corcos, 2008; Mazein et al., 2013; Watterson et
813 al., 2013), lipoprotein dynamics (Chapman et al., 2010; Hübner et al., 2008; Shorten and Upreti,
814 2005; Sips et al., 2014), LDLr regulation (Shankaran et al., 2007), hepatic LDL-C endocytosis (Wattis et
815 al., 2008), and RCT (Lu et al., 2014). Most of these models do not focus on the ageing process as
816 such, but it is possible they could be adapted and merged to explore in depth some of the changes
817 that occur within cholesterol metabolism during ageing, discussed in this review, in particular the
818 interaction of the gut microbiome with cholesterol metabolism.

819

820 **12.0 Discussion**

821 Developed populations are ageing, resulting in an increase in the diseases associated with ageing. Of
822 the diseases whose prevalence increases with age, CVD related morbidity is by far the most
823 common. The risk factors for CVD are many, however together with classic factors such as
824 chronological age, smoking, sex, blood pressure and diabetes; lipid biomarkers have become the
825 cornerstone in determining CVD risk. It is generally accepted the relationship between CVD risk and
826 the dysregulation of lipid metabolism is at least in part due to the strong association that exists
827 between elevated total cholesterol/LDL-C and atherosclerotic plaque formation. Conversely, due to
828 its role in RCT, HDL-C is widely regarded as being anti-atherogenic, and evidenced by the inverse
829 correlation between HDL-C levels and CVD. Fundamentally, cholesterol metabolism is maintained by
830 a subtle balancing act between dietary ingestion, intestinal absorption, whole-body synthesis and
831 excretion. These processes work in a coordinated fashion over a diverse range of spatial and
832 temporal scales to help maintain whole body cholesterol balance. Changes to any of these processes
833 can have a direct impact on the levels of LDL-C and HDL-C, thus indirectly influencing CVD risk.
834 Changes to any of these processes can have a direct impact on the levels of LDL-C and HDL-C, thus
835 indirectly influencing CVD risk, a finding of paramount importance, when considering the complex
836 interactions that exist between cholesterol metabolism and the ageing process. This review has
837 highlighted the ageing process does not affect cholesterol metabolism at solely one, or even a
838 number of sites, but rather each regulatory component of cholesterol metabolism is affected by the
839 ageing process. Worryingly, there is a paucity of studies detailing the mechanistic changes that occur
840 during metabolism of this nutrient and ageing, and of those that exist, the majority tend to focus on
841 murine models and were completed several decades ago. Despite this, our review uncovered a
842 number of important findings about how cholesterol metabolism affects ageing. It was revealed that
843 NPC1L1 expression significantly increases in the duodenum and jejunum with age, while ABCG5/G8
844 expression is suppressed. Moreover, in humans it has been found that the rate of bile acid synthesis
845 declines with age and occurs with a concomitant reduction in the hepatic expression of the rate
846 limiting enzyme of bile acid synthesis, CYP7A1. Also, from an intestinal perspective it has been
847 suggested that the rise in LDL-C that accompanies ageing is due to a decline in BSH⁺ species, such as
848 *Lactobacillus* and *Bifidobacterium*. However, when we examined how lipoprotein dynamics change
849 with age, it was suggested that the mechanistic explanation for the rise in LDL-C during ageing is due

850 to a reduction in the clearance rate for LDL-C from the circulation. This assertion is certainly in line
851 with the central finding from our recent mechanistic model of whole body cholesterol metabolism,
852 which revealed that a reduction in the hepatic clearance rate of LDL-C is the central driver in
853 dysregulating cholesterol metabolism. However, for the purposes of abstraction our model did not
854 incorporate many of the mechanisms outlined in this review. Therefore, it is our opinion that the
855 dysregulation of cholesterol metabolism is the cumulative effect of ageing on all the components of
856 cholesterol metabolism and it is naïve to single out any one aspect in particular. This view is
857 supported by additional findings from this review that revealed how other important aspects of
858 cholesterol metabolism are effected by the ageing. For instance, oxidative stress was shown not only
859 to be involved in the progression of atherosclerosis but to also be involved in the oxidation of HDL
860 particles. Moreover, various molecular mechanisms involved intracellular cholesterol homeostasis
861 and biosynthesis have been shown to be effected by the metabolic regulators mTOR and sirtuins.
862 These cellular metabolic hubs are widely regarded as having a key role to play in intrinsic ageing and
863 health-span. For instance, mTORC1 regulates SREBP levels which in turn results in altered LDLr
864 expression. In addition, Sirt6 has been identified as being involved in Srebp2 gene regulation.
865 Collectively these findings emphasize that it not the dysregulation of one or even a few biological
866 mechanisms; rather, age related dyslipidaemia is likely to be the result of a combination of several
867 factors and future therapeutic interventions should be underpinned by this.

868 This review also revealed diet has a key role to play in modulating cholesterol metabolism and could
869 be a key therapeutic avenue to mitigate the effects ageing has on lipid metabolism. The central
870 dietary paradigm of ageing research has been CR. This regime has been shown to have a positive
871 cardioprotective effect in humans, part of which is brought about by an improvement in blood lipid
872 profile in subjects undertaking this diet. More conventional diets also affect cholesterol metabolism.
873 The high levels of dietary phytosterols, MUFA, and PUFA typically found in the Mediterranean diet
874 for instance, have been shown to modulate cholesterol metabolism, by increasing hepatic
875 expression of LDLr, in addition to reducing cholesterol absorption. Thus, experimental evidence
876 suggests employment of healthy diets such as the Mediterranean diet, and supplementation with
877 probiotics for example, could be utilised to slow the rate of LDL-C accumulation, associated with the
878 ageing process.

879 One way in which we could explore the relationship between diet, ageing and cholesterol
880 metabolism further would be to use mechanistic mathematical models. Recently, mathematical
881 models have been used to explore the dynamics of cholesterol metabolism and the effect that both
882 ageing and dietary changes have on it. One area that a mathematical model could be used to explore
883 in greater depth, is the bi-directional relationship between the gut microbiome and cholesterol
884 metabolism. Thus, modelling could help to identify alternative therapeutic targets, which could
885 reduce the dependence on pharmaceutical intervention in older people to improve blood lipid
886 profile.

887

888 **13.0 Conclusion**

889 It is evident, the breakdown of cholesterol metabolism associated with ageing results in increased
890 LDL-C and has important implications for health-span. Dietary intervention offers a potential non-
891 pharmacological avenue that could be invaluable for mitigating the insidious effects ageing has on

892 this system. In recent years, there have been an increase in the use of mechanistic mathematical
 893 models to explore complex systems such as cholesterol metabolism in a more integrated and non-
 894 reductionist fashion. Such models should be increasingly used to determine new targets for
 895 therapeutic intervention.

896

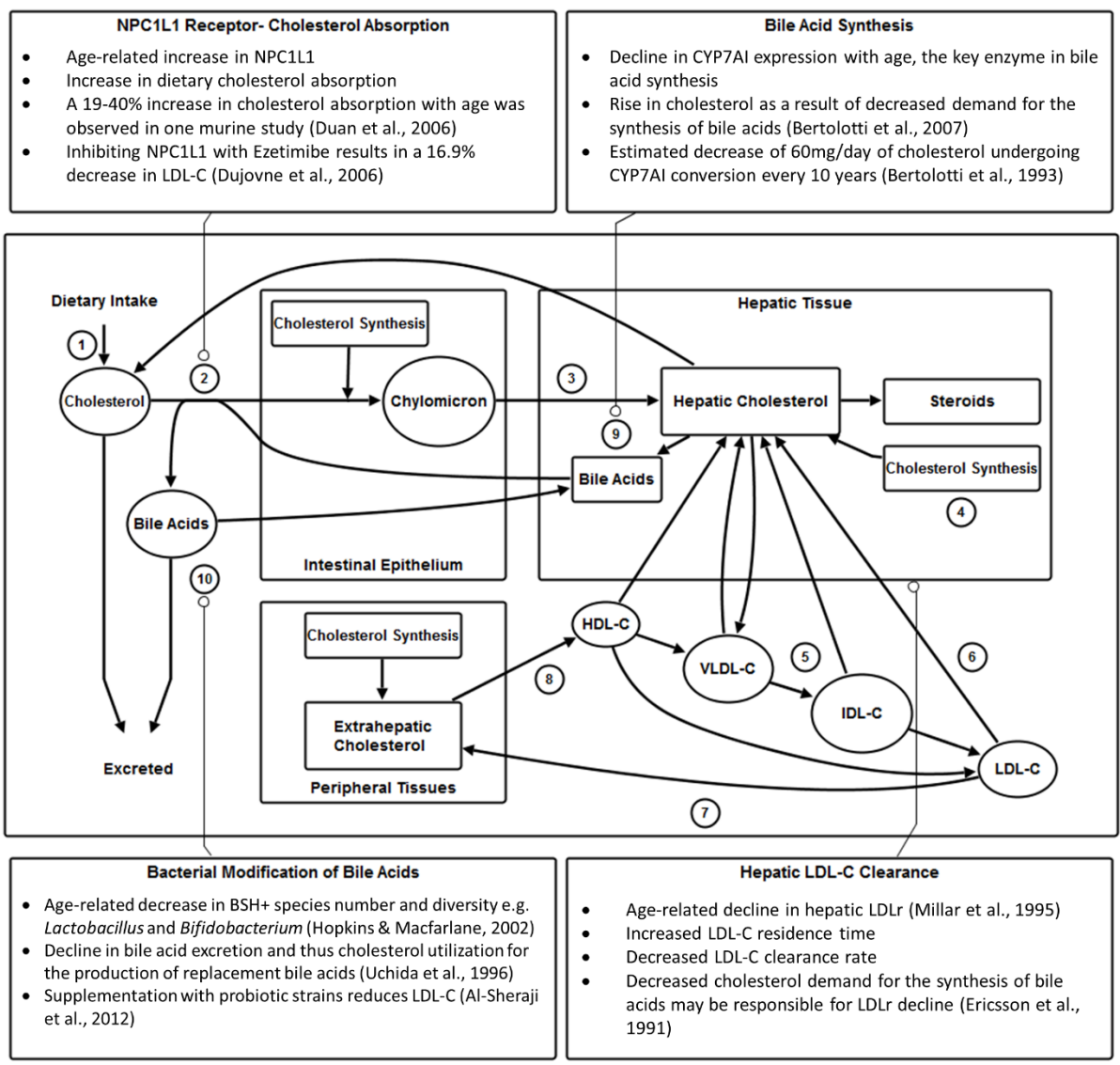
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900

901 **Figures**

902



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904

905 **Figure 1. Overview of cholesterol metabolism and age associated changes to mechanisms.** Briefly
906 outlined is 1) ingestion of dietary cholesterol, 2) intestinal absorption, 3) chylomicron transport, 4)
907 cholesterol biosynthesis, 5) VLDL-C production and hydrolysis to IDL-C and LDL-C, 6) hepatic uptake
908 of LDL-C, 7) peripheral uptake of LDL-C, 8) reverse cholesterol transport, 9) bile acid synthesis, and
909 10) enterohepatic circulation of bile acids and bacterial modification. The age-related changes
910 highlighted centre on some of the mechanisms responsible for the rise in LDL-C with age; the
911 increase in intestinal absorption of cholesterol, the reduction of bile acid synthesis, the decrease in
912 LDL-C clearance, and the decrease in BSH⁺ species in the digestive microbiome.

913

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