Effects of obesity on cholesterol metabolism and its implications for healthy ageing.

Mark Tomás Mc Auley

[1]Faculty of Science and Engineering, University of Chester, Thornton Science Park, Chester, CH2 4NU, UK

Abstract

The last few decades have witnessed a global rise in the number of older people. Despite this demographic shift, morbidity within this population group is high. Many factors influence healthspan; however an obesity pandemic is emerging as a significant determinant of older peoples' health. It is well established obesity adversely effects several metabolic systems. However, due to its close association with overall cardiometabolic health, the impact obesity has on cholesterol metabolism needs to be recognised. The aim of this review is to critically discuss the effects obesity has on cholesterol metabolism and to reveal its significance for healthy ageing.

Keywords: Ageing, older people, cholesterol metabolism, obesity, oldest old

1. Introduction

In 2030 older people (persons aged ≥60 years) are projected to account for almost 20% of the global population[1]. Despite this demographic shift in favour of older people, morbidity among this group is high[2]. Many factors impact older peoples' health; however an obesity pandemic is emerging as a significant global health concern[3; 4; 5; 6; 7]. Microcosmically, the UK illustrates the extent of the global obesity problem. Among females aged 65-74 years in the UK 30% have a body mass index (BMI) ≥30 kg/m^2, and are categorised as obese[8]. The problem is even more pronounced among their male counterparts, as 33% of males are categorised as obese within this age group[8]. The problem extends to those aged ≥75 years, as 28% of females are obese, while 23% of males are obese in this age group. From a public health perspective these figures are alarming because obesity adversely effects several metabolic systems, and is synonymous with many conditions including, cancer, type 2 diabetes mellitus (T2DM), hypertension and dyslipidemia[9; 10; 11]. However, due to its close association with overall cardiometabolic health, the impact obesity has on cholesterol metabolism needs to be recognised[12; 13]. The aim of this review is to critically discuss the effects obesity has on cholesterol metabolism and to reveal its significance for healthy ageing.

2. An overview of cholesterol metabolism

Figure 1 outlines that cholesterol balance is maintained by the body responding to changes in ingestion, absorption, synthesis and excretion[14]. Humans ingest a modest amount of dietary cholesterol (DC), which mixes with intestinal cholesterol[15]. Absorption is controlled by cholesterol ester hydrolase which liberates cholesterol esters (CE), facilitating the inclusion of free cholesterol (FC) into bile acid micelles[16]. The intestinal protein Niemann-Pick C1-Like 1 (NPC1L1) mediates cholesterol absorption into the enterocyte by clathrin-mediated endocytosis[17]. ATP-binding cassette (ABC) transporters G5 and G8 (ABCG5/G8) control the efflux of cholesterol from the enterocytes to the lumen[18]. Within the enterocyte, acetyl CoA acetyltransferase 2 (ACAT2) re-esterifies cholesterol[19], which is combined with apolipoprotein B-48 (apoB-48), triglycerides (TGs) and phospholipids, to generate a chylomicron[20]. Upon entering the bloodstream, chylomicrons are acted on by lipoprotein lipase (LPL), which catalyses their TGs, liberating free fatty acids (FFAs)[21]. Chylomicron remnants are removed from the circulation by hepatic remnant receptors[22].

The liver is the main site of cholesterol synthesis[23], providing cholesterol and TGs for the assembly of very low density lipoproteins (VLDLs)[24]. In the plasma, LPL hydrolyses VLDLs to low density lipoproteins (LDLs), via intermediate density lipoproteins (IDLs)[25]. LDL-cholesterol (LDL-C) is removed by the LDL receptor (LDLr)[26] and LDLr-related protein 1 (LRP1)[27]. This process is governed by intracellular sterol levels[28]. Increasing intracellular cholesterol activates, insulin-induced genes (Insigs) proteins. Insig-1 and Insig-2 bind to sterol regulatory element-binding protein cleavage-

64 activating protein (SCAP) in the endoplasmic reticulum (ER), restricting the migration of the SCAP/

65 sterol regulatory element-binding protein (SREBP) complex to the Golgi[29; 30]. When sterol levels drop,

66 SREBP-2 migrates to the Golgi where it is cleaved by subtilisin kexin isozyme/Site-1 protease (SKI-

67 1/S1P), and the intramembranous metalloprotease Site-2 protease (S2P)[31]. This releases the NH-

68 terminal domain of SREBP-2 from the membrane. Two N-terminal fragments dimerize, then interact

69 with importin-β, before entering the nucleus, to activate SREBP-2-regulated gene promoters[32]. A

70 further regulatory point involves LDLr synthesis. Nuclear SREBP-2 increases the transcription of

71 proprotein convertase subtilisin/kexin type 9 (PCSK9)[33]. PCSK9 reduces the number of LDLrs by

72 increasing their metabolism, and subsequent degradation, restricting LDL uptake[33]. High cellular

73 cholesterol levels suppress SREBP-2 release from the ER, thus PCSK9 transcription is reduced, which

74 subsequently increases LDLRr levels[34]. The synchronised interplay of SREBP-2 induced transcription

75 of both LDLr and PCSK9 regulates circulating LDL-C levels. Additionally, cholesterol entering a

76 hepatic cell as part of LDL triggers ACAT2[26], which catalyses FC to CE[19], and this cholesterol also

77 activates cholesterol 7α - hydroxylase (CYP7A1), the rate-limiting enzyme of bile acid synthesis[35].

78 By disrupting any of the mechanisms I have discussed, obesity has the potential to provoke a rise in

79 plasma LDL-C. Elevated LDL-C levels are inexorably linked to an increased risk of atherosclerotic-

80 cardiovascular disease (CVD)[36; 37; 38]. Moreover, emerging evidence suggests suboptimal LDL-C

81 levels, in tandem with elevated serum uric acid, could present an increased risk of developing

82 hypertension or metabolic syndrome[39; 40].

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85 A further important aspect of cholesterol metabolism is reverse cholesterol transport (RCT). RCT

86 removes excess cholesterol from peripheral tissue[41]. High density lipoproteins (HDLs) are central to

87 this. HDLs "mop up" excess cholesterol, generating HDL-cholesterol (HDL-C)[42]. Central to RCT is

88 the ferrying of FC and phospholipids to lipid-free apo A-I to form nascent pre-β HDL particles, in a

89 process primarily regulated by ABCA1[43; 44]. Nascent HDLs progress to mature HDLs due to the

90 esterification of cholesterol by lecithin-cholesterol acyltransferase (LCAT)[45]. Cholesterol within

91 HDLs can follow one of two routes to the liver. HDLs can go directly to the liver and deposit their

92 cholesterol by interacting with scavenger receptor class B, type 1 (SR-B1) receptors[46]. Secondly,

93 cholesterol can be transferred to the liver via the action of cholesteryl ester transfer protein (CETP),

94 which redistributes cholesterol to LDL and VLDL[47]. Regardless of the route, HDLs transfer cholesterol

95 to the liver, where it can be effluxed directly as cholesterol or converted to bile salts[48]. It can then be

96 excreted during enterohepatic circulation. Consequently, RCT is regarded as antiatherogenic[49];this is

97 underscored by studies which have revealed an inverse relationship between HDL-C levels and the

98 onset of premature CVD[50; 51]. Intriguingly, HDL's antiatherogenic role is thought to be enhanced

99 further by possessing antioxidant properties[52]. As with the mechanisms which regulate LDL-C levels,

100 if obesity interferes with the processes underpinning RCT, this has the potential to modulate an
101 individual's risk of CVD.
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105 **3. Obesity and cholesterol metabolism**
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107 *3.1 Cholesterol absorption*
108 In pioneering work, Miettinen and Kesäniemi (1989) identified a negative correlation between the
109 fractional absorption of DC and obesity in middle aged men; although, a mechanistic explanation for
110 this result was not immediately apparent[53]. In a follow up investigation cholesterol absorption was also
111 found to be inhibited in obese middle aged males (BMI >31 kg/m^2)[54]. On this occasion two
112 explanations were proposed for this finding. It was posited labelled DC could have contributed to sub-
113 normal cholesterol absorption. Secondly, it was suggested that cholesterol absorption was inhibited by
114 expanded biliary secretion. However, the precise reason why obesity inhibited cholesterol absorption
115 remained unclear. More recent studies have added further intrigue to this puzzle. It has been found that
116 treatment of obese hypercholesterolemic subjects with the NPC1L1 inhibitor, Ezetimibe, improved the
117 lipid profile and insulin resistance (IR) in these subjects[55; 56]. This suggests obesity could in fact
118 increase cholesterol absorption in obese subjects rather than inhibiting it, and an arbiter of this change
119 could be NPC1L1. If obesity does increase cholesterol absorption, this effect could also be induced by
120 provoking a rise in circulating bile acids. For example, Vincent et at. (2013) found that the post-prandial
121 bile acid response is increased in obese male and female patients with T2DM compared to age-matched
122 normoglycaemic individuals[57]. Further evidence that obesity influences bile acid metabolism comes
123 from studies of the gut microbiome[58]. For instance, in one study it was found that bile salt hydrolase
124 (BSH) is the arbiter of host-microbiome interactions which modulated weight gain, and lipid
125 metabolism in a murine model[59]. Specifically, the expression of cloned BSH enzymes in the
126 gastrointestinal tract of gnotobiotic or conventionally raised mice significantly modified plasma bile
127 acid signatures and regulated the transcription of important genes involved in cholesterol metabolism
128 (Abcg5/8) both hepatically and intestinally. Moreover, high-level expression of BSH in conventionally
129 raised mice resulted in a significant drop in host weight gain, plasma cholesterol levels, and hepatic
130 TGs. As an adjunct to this finding it has been shown that the farnesoid X receptor (FXR) has a central
131 role to play in modulating host-microbiome dialogue. For instance, mouse models of diet-induced
132 obesity have shown that both the microbiome and FXR signalling are required for weight gain[60; 61].
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3.2 Cholesterol synthesis

Cholesterol synthesis is also affected by obesity; sterol-balance studies have shown increased weight gain results in a higher rate of cholesterol synthesis[62; 63]. It is uncertain how weight gain induces a higher rate of cholesterol synthesis. However, it is important to acknowledge that hepatic HMGCR increases in obese subjects[64]. This would naturally result in an increase in hepatic cholesterol production; something which has been observed in obese subjects[65]. For example, in a study involving 17 morbidly obese middle-aged males, it was found that the activity, and mRNA levels of HMGCR, was higher in the obese subjects, when compared to lean control group[66]. Moreover, the activity and mRNA level of cholesterol 7 alpha-hydroxylase, also increased compared to controls. The activity of ACAT2, and LDLr mRNA levels were elevated in these subjects. Such alterations have the potential to impact the normal functioning of hepatic LDLr. For example, it was also found in this study that the binding of LDL to the LDLr was reduced by fifty percent when compared with controls[66].

3.3 Hepatic free cholesterol/ bile acid accumulation and Non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) encompasses a number of hepatic pathologies, from fatty liver disease to non-alcoholic steatohepatitis (NASH); a condition which can progress to cirrhosis[67; 68; 69; 70; 71]. NAFLD has a higher occurrence in males than females, and its prevalence increases with age[72; 73]. NAFLD is closely associated with IR and hyperinsulinemia and is prevalent in 70-80% of obese individuals[74]. Moreover, it has recently been associated with key parameters of cardiovascular health, including arterial stiffness[75]. Traditionally, NAFLD has been associated with increased hepatic TGs, however in recent years there has been growing evidence linking altered cholesterol metabolism with the aetiology of NAFLD[76; 77; 78]. For instance, hepatic FC accumulates in obese diabetic mice and results in steatohepatitis[79]. Most recently in mice it has been found that found that hepatic cholesterol, but not hepatic TG, increased with age[80]. Focusing on humans the intake of high levels of DC have been associated with NASH[81; 82]. Mechanistically, it would appear cholesterol synthesis is upregulated in NAFLD. This was demonstrated by a study which examined the expression of an array of genes associated with cholesterol metabolism[83]. In the investigation, 20 middle aged subjects with NAFLD (mean BMI 34.1 kg/m^2) and NASH (mean BMI 34.2 kg/m^2) were compared to 20 obese controls (33.2 kg/m^2) and 6 lean normal controls (mean BMI 21.4 kg/m^2). It was found NAFLD was associated with increased SREBP-2 maturation, HMGCR expression and decreased phosphorylation of HMGCR. Additionally, cholesterol ester hydrolase was increased, while ACAT2 remained unchanged. Moreover, LDLr expression decreased significantly. Also, HMGCR expression was correlated with FC, histologic severity of NAFLD and LDL-C levels. Bile acid homeostasis is also affected as a result of NAFLD[84]. Individuals with NASH have elevated levels of bile acids which can accumulate hepatically[85]. Bile acid signalling is regulated by the Farnesoid X receptor (FXR), which contributes to overall cholesterol metabolism [84]. Interestingly, it has been shown in mice that the gut microbiota can modulate obesity via this receptor. The gut microbiota promoted weight gain and hepatic steatosis in an FXR-dependent

174   manner between Fxr-/- and wild-type mice [60]. Moreover, bile acid profiles and the composition of

175   faecal microbiota differed between Fxr-/- and wild-type mice. The finding that the gut microbiota

176   induced liver steatosis in an FXR-dependent manner was suggested to be induced by the increased

177   expression of CD36, Apolipoprotein C2, and VLDL receptor, all of which are involved in lipoprotein

178   uptake. Thus, increased steatosis was thought to be attributed to the augmented expression of lipogenic

179   genes or diminished expression of genes associated with fatty acid oxidation. In terms of promoting

180   obesity mechanistically the authors showed that the gut microbiota of Fxr-deficient mice was defined

181   by a phylum-wide rise in Bacteroidetes and phylum-wide reduction of Firmicutes. Thus, the gut

182   microbiota changes in response to diet and is associated with an obesity phenotype, which is mediated

183   by FXR signalling.

184   *3.4 Lipoprotein dynamics and RCT*

185   Lipoprotein processing is significantly impaired due to obesity. Morbidly obese middle aged patients

186   have been found to have lower expression of LPL and LRP1 in their visceral tissue[86]. LPL expression

187   was also lower in the subcutaneous adipose tissue of these subjects. The decrease in the expression of

188   LRP1 is likely a contributing factor to the increase in plasma LDL-C, which often, but not always,

189   accompanies obesity[87]. More strikingly, obesity has regularly been associated with an increase in

190   atherogenic small dense LDL[88; 89; 90]. Obesity also lowers HDL-C regardless of age, sex or ethnic

191   background, while an inverse association between HDL-C levels and BMI has also been observed[91; 92;

192   93]. Intriguingly, in the Framingham offspring study, the effect of increased BMI on total cholesterol

193   and LDL-C was not as strong as it was for HDL-C[94]. More recently, an inverse association has been

194   found between LDL-C and BMI in morbidly obese subjects[95]. Low levels of the major apolipoprotein

195   component of HDL (Apo A-I) have also been found to be associated with obesity in the Framingham

196   Offspring Study in men and women[96]. Moreover, it has been revealed in a mouse model that increased

197   Apo A-I could have an anti-obesity effect. Increased energy expenditure and up-regulation of

198   uncoupling protein 1 in brown fat were associated with high levels of Apo A-I[97]. In addition, other

199   apolipoproteins have been shown to be influenced by obesity. Elevated levels of fasting and

200   postprandial apo B-48 have been measured in obese human subjects[66]. Obesity could also impact the

201   functional capacity of HDL-C. For example, the antioxidant capacity of HDL appears to be

202   compromised in obese subjects[98]. Obesity interferes with other components of RCT, for example,

203   obesity causes impairment in RCT due to reduced plasma cholesterol uptake and efflux by hepatocytes

204   and adipocytes in *ob/ob* mice[99]. Plasma CETP levels have also been positively correlated with

205   obesity[100]. The likely reason for this finding, is that adipose tissue is a significant source of CETP[101].

206   Intriguingly, LCAT deficiency has been suggested to confer a degree of protection from the

207   development of obesity. Tentative evidence for this finding comes from a study of LCAT null mice

208   which appeared to be protected from diet-induced obesity[102].

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4. Commonality between obesity and ageing

It is possible obesity superimposed on ageing could expedite the age associated dysregulation of cholesterol metabolism[103; 104; 105]. Taking LDL-C as an example, increasing age is associated with a rise in LDL-C in males and females, in both cross-sectional[106; 107] and prospective studies[108; 109]. It is not known how ageing contributes to a rise in LDL-C; however, there are solid reasons to believe a decline in the hepatic clearance rate of LDL-C is a factor[110; 111]. For example, human ageing is associated with a drop in the number of hepatic LDLr[112]. Obesity can also results in a rise in LDL-C[113; 114], although in some observational studies it is only weakly associated[115; 116], or not associated at all[117]. An obesity induced rise in LDL-C could have the same mechanistic underpinning as ageing, because it is also associated with a decline in hepatic LDLr numbers. Also similar to obesity, HDL-C levels decrease with age in humans in certain studies[118]. For instance, HDL-C levels have been observed to decrease with age in both men and women in prospective studies[109; 119]. Although HDL-C levels do not change with age in most cross-sectional studies[120; 121], they have been shown to decrease in others[122]. A decline in HDL-C with age could be due to disrupted CETP activity. If this is the case it would be mechanistically similar to the putative effect obesity has on RCT[100]. Ageing also impacts lipoprotein processing by reducing the activity of plasma LPL by as much as 55-60%[123; 124]. This is similar to the metabolic affect obesity has on LPL[125]. In rodents it has been found that bile acid synthesis diminishes with age[126]. This is also similar to obesity because in men and women a decrease in the conversion of cholesterol to bile acids has been identified in certain studies[127; 128]. However, other studies conflict with this and suggest obesity results in an increase in bile acid synthesis[129].

The landscape of an obese state also resonates with the free radical theory of ageing[130]. For example, obesity is associated with oxidative stress via increased generation of reactive oxygen species (ROS)[131]. As visceral fat stores increase, adipocytes generate increasing levels of ROS [132]. Consequently, oxidative stress results in IR within adipose and peripheral tissue[133]. It has been suggested that high levels of ROS impinge on intracellular cholesterol homeostasis[134]. ROS have been found to upregulate the activity of hepatic HMGCR in rodent hepatic tissue, leading to an increase in cholesterol synthesis[80; 135; 136; 137] ROS are also implicated in the pathogenesis of atherosclerosis, where oxidation of LDL is regarded as a key event in the initial stages of atherosclerosis formation[138]. Despite the many parallels between obesity and ageing a number of difference do exist. In contrast to obesity it has been revealed that cholesterol absorption efficiently increases with age, however there is a paucity of evidence for this in humans and findings are confined to rodent studies[139]. Mechanistically it appears ageing suppresses the expression of *Abcg5* and *Abcg8*, and upregulates the expression of *Npc1l1* in murine models[140]. In rodents it has been found that bile acid synthesis declines with age[126]. This contrasts with obesity. Also, unlike obesity, it has been found that hepatic ACAT2 activity decreases with age in Watanabe heritable hyperlipidemic rabbits[141].

5. Diet: A key modulator of obesity and cholesterol metabolism

An obese state is the result of an imbalance between the amount of calories consumed by an individual and the amount of energy they expend[142]. Moreover, it is generally regarded that excess consumption of dietary fat plays a role in the development of obesity[143]. Taking this a step further, a clear link between diet, cholesterol metabolism and obesity centres on the excessive intake of DC. For instance, DC has been shown to exacerbate hepatic steatosis and inflammation in obese LDLr-deficient mice[144]. Moreover, in this study, the consumption of DC exacerbated hepatic macrophage infiltration, apoptosis, and oxidative stress. Excessive intake of DC has also been shown to result in the accumulation of hepatic cholesterol in obese diabetic mice[79]. The accumulation of cholesterol was attributed to changes in some of the regulator mechanisms discussed previously, including the up-regulation of LDLr, via activation of SREBP-2, a drop in the conversion of cholesterol to bile acids, and suppression of bile acid excretion in bile.

In addition to the intake of fat/DC, high intakes of dietary sugars have been associated with obesity and unfavourable lipid levels in both men and women[127; 128]. In particular dietary fructose has emerged as an important dietary factor which contributes to the hepatic dysregulation of cholesterol metabolism [69; 145]. For instance, high fructose consumption increases serum PCSK9 concentrations and reduces liver LDLr protein levels in hyperlipidemic hamsters[146]. In humans, it has been found that the consumption of fructose and high fructose corn syrup increases LDL-C, and apo-B in both men and women[147]. Moreover, NASH is associated with animal models fed a high fat, high fructose diet[148; 149]. Consumption of excessive dietary fructose has also been associated with cognitive decline in older adults[150]. This is intriguing because obesity can be correlated with poor cognitive performance in older adults[151]. Taking this a step further, it is possible fructose makes a mechanistic contribution to the pathogenesis of Alzheimer's disease by interfering with lipid metabolism. For example, animal models of dementia suggest excessive consumption of fructose induce IR and promote dementia pathogenesis[152; 153; 154]. This is thought to occur as follows, IR is associated with elevated plasma ceramides which interfere with lipoprotein metabolism and amyloidogenic processing resulting in the deposition of β-amyloid peptides, which is a hallmark of Alzheimer's disease[155].

In certain circumstances age could potentially offer a degree of protection against diet-induced obesity. In a recent study which compared the response of young and old mice to a Western diet (WD), it was found that old mice did not show a higher body weight or adipose tissue mass, when compared to their young counterparts[156]. Significantly, and of direct relevance to the underlying hepatic health of older people, it was found that the aged mice did have a build-up of hepatic lipid on the WD. As well as the type and amounts of nutrients consumed, it has been found that meal frequency, timing, and regularity are also associated with obesity[157]. Recently this has been shown to have important implications for cholesterol metabolism. In a cross-sectional study of non-institutionalized and non-pregnant healthy

Taiwanese adults (≥19-years-old) found that higher energy intake at night time is associated with elevated total and LDL-C levels[158]. This was an interesting finding, although a mechanistic explanation for this discovery was not posited. Regardless, the study presents the possibility that meal timing and frequency could impact both obesity and cholesterol metabolism which is an intriguing prospect.

Alcohol consumption also effects cholesterol metabolism. Evidence suggests moderate to low alcohol consumption increases levels of HDL-C and decreases levels of LDL-C [159]. However, it is important to be cautious as alcohol has a limited therapeutic range and only modest drinking appears to be beneficial. This is partly due to the caloric richness of alcohol, as its excessive consumption can result in increased weight gain in certain individuals[160]. Moreover, chronic alcohol intake affects lipid metabolism broadly by provoking the increased synthesis of TGs and subsequent hypertriglyceridemia[161]. In addition, it has been observed that alcohol consumption decreases the activity of LPL, which is also associated with hypertriglyceridemia[162]. From the perspective of HDL metabolism, in individuals with alcohol dependence syndrome no association between plasma-HDL-C and the number of drinks consumed per day has been observed in these individuals, indicating that only low level consumption of alcohol is beneficial[163]. Furthermore, in a longitudinal study of alcohol consumption, the long-term effect of total alcohol consumption on the change in HDL-C was observed to be a nonlinear relationship[164]. The mechanistic explanation for this is thought to centre on the pathophysiological effect of alcohol on the liver which results in a decrease in the hepatic production of HDL in these subjects[165].

Alarmingly, the excessive intake of alcohol has been increasing among older people in certain populations[166]. In a recent study involving a cohort of Australian males (≥ 65 years), which explored the association between alcohol intake and body composition, it was found that participants who consumed ≥5 alcoholic drinks/day had a greater BMI, fat mass index, waist circumference, percent body fat and lower lean mass than non-drinkers[167]. This has metabolic consequences for older people because alcohol consumption augments lipid synthesis via sterol SREBP-1[168]. This is possibly mediated by acetaldehyde, which contributes to an increase in the synthesis of SREBP-1, which in turn augments cholesterol and fat synthesis[168]. Alcohol consumption has also been shown to dysregulate hepatic fatty acid oxidation[169] and decrease the secretion of VLDL[170]. A drop in VLDL secretion could result in a decrease in the conversion of VLDL-C to LDL-C, and be responsible for the decrease in LDL-C associated with low to moderate alcohol intake[171]. Furthermore, this mechanism could explain the decreased risk of alcohol dependence with increased LDL-C levels identified among some of the participants of a recent case control study investigating alcohol consumption and obesity[172].

On the flip side of the coin, emerging research has revealed that certain novel dietary components improve both cholesterol metabolism and have anti-obesity effects. For example, a diet high in fruit and

322 vegetables is associated with a lower risk of obesity/body adiposity[173; 174; 175]. Moreover, certain

323 components of fruits and vegetables have a favourable effect on cholesterol metabolism. For instance,

324 soluble fibre exerts a favourable effect by decreasing LDL-C levels[176]. It has been found that 3g per

325 day of soluble fibre can lower total and LDL-C by ~0.13 mmol/L[177]. Several mechanisms have been

326 suggested to account for this effect, including the inhibition of bile salt intestinal re-absorption, and a

327 diminished glycemic response, which results in a drop in insulin stimulated hepatic cholesterol

328 synthesis[178]. The gut is also thought to be the site of action of plant sterols. Plant sterols are naturally

329 occurring compounds found in fruit and vegetables which are structurally related to cholesterol differing

330 only in the structure of their side chains[179]. Consumption of 1.8-2.0 g/day of plant sterols has been

331 shown to lower both total and LDL-C concentrations by 10%-15% in different population groups[180;

332 181]. Although the precise mechanism by which LDL-C is lowered is uncertain, it is generally regarded

333 that plant sterols inhibit intestinal cholesterol absorption[182]. Not only have plant sterols been associated

334 with decreased LDL-C. More recently, the consumption of high levels of phytosterols, which includes

335 plants sterols, have been associated with decreased rates of obesity. For example, a recent cross-

336 sectional study of Chinese adults (18-60 years) revealed that higher consumption of phytosterols was

337 associated with lower BMI, waist circumference, and a lower prevalence of

338 overweight/obesity/abdominal obesity in this population group[183]. Intriguingly the administration of

339 both phytosterols and red rice were recently studied in mildly hypercholesterolemic subjects[184]. In

340 tandem these two nutraceuticals had a more significant impact on LDL-C levels, when compared to

341 either phytosterols or red rice on their own. Diet also has an important role to play in terms of alleviating

342 the metabolic consequence of obesity. Most recently, it has been shown that medium chain saturated

343 fatty acids (MCSFA) could illicit a degree of protection against obesity-induced comorbidities such as

344 diabetes in obesogenic mice[185]. Moreover, in a rat model it has been found that MCSFA could help

345 prevent NAFLD[186]. Mechanistically, it is thought the beneficial effects of MCSFA consumption could

346 be induced via their preferential β-oxidation over long chain saturated fatty acids[187]. A further way

347 diet has been suggested to provide a means of treating obesity is by modulating the gut microbiome.

348 For instance, in an obese population which adhered to a Mediterranean diet (MD) for a year, it was

349 found that the MD exerted a protective effect against T2DM development by modulating specific

350 changes in the gut microbiota. Specifically, this involved increasing the abundance of *Faecalibacterium*

351 *prausnitzii* and *Roseburia* species.

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354 **6. Cholesterol metabolism and obesity in older people**

355 Certain individuals have a metabolic profile which does not appear to be overtly affected by obesity.

356 Such individuals are known as "metabolically healthy obese" (MHO)[188]. In a study which investigated

357 obesity in the US population, it was found 31.7% of obese adults (~19.5 million), were MHO[189]. The

358 prevalence of metabolically healthy older individuals was 14.3% among those aged 65-79 years, and

359　22.1% among those ≥80 years. Healthy participants were defined by having 0 or 1 cardiometabolic

360　abnormality. Unhealthy individuals were defined as having ≥2 cardiometabolic abnormalities. Among

361　individuals with ≥2 metabolic abnormalities, the two most common cardiometabolic risk factor

362　combinations, were high TG level/low HDL-C level and high blood pressure/high glucose level. In a

363　similar investigation it was found that among participants with ⩽1 metabolic abnormality, obesity was

364　associated with a greater risk of developing multiple metabolic abnormalities[190]. Significantly, lipid

365　metabolism was also key, as TG and HDL-C levels predicted an individual's progression to a

366　metabolically unhealthy obese (MUHO) state. Within obesity research, a further puzzle exists; there are

367　situations where being overweight/mildly obese appears to be beneficial. This is known as the "obesity

368　paradox"[191]. In an ageing context, the obesity paradox appears to confer a survival advantage in older

369　patients (generally those >50 years) who have conditions such as, CVD, arthritis and kidney disease[192].

370　Focusing specifically on cholesterol metabolism, and its intersection with the obesity paradox, normal

371　total serum cholesterol levels have been reported in morbidly obese individuals. In a study of 3,312

372　women (≥ 18 years) it was found the percentage of individuals with normal total serum cholesterol

373　levels (<200 mg/dL) decreased with increasing BMI, from 55% in those with a BMI <20 kg/m^2 to 28%

374　in women with a BMI of 30-35 kg/m^2[193]. Total serum cholesterol >7.75 mmol/l was found in 2% of

375　individuals with a BMI <20 kg/m^2, but in 6% of the group with a BMI between 30 and 35 kg/m^2. Among

376　morbidly obese women (BMI >40 kg/m^2), 39% had total serum cholesterol levels <5 mmol/l. Thus, it

377　would appear in morbidly obese women, there is a significant number of individuals with normal total

378　serum cholesterol levels. Such findings were also identified in a study which examined individuals (20–

379　64 years) with a BMI in the range 34-77 kg/m^2[194]. It was found that mean total cholesterol levels in the

380　obese group fell with increasing BMI. Moreover, LDL-C levels were lower in obese men (3.65 mmol/l

381　versus the control group, 4.17 mmol/l).

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383　The association between obesity and cholesterol metabolism has been studied to a limited extent in the

384　oldest old (individuals ≥80 years). Despite this, the oldest old who are obese have a higher prevalence

385　of morbidity[195]. In a study which examined the oldest old among a group aged 60-85 years, it was

386　reported that obesity was associated with shorter survival plus a higher incidence of coronary heart

387　disease and T2DM[196]. When cholesterol metabolism has been examined in the oldest old some

388　intriguing findings have been revealed. In the Leiden 85-Plus Study it was observed that both high and

389　low levels of LDL-C had a similar impact on mortality risk[197]. Interestingly, this finding occurred

390　despite CVD being the main cause of mortality in these subjects. Similar observations have been

391　identified in several other studies which have examined the lipoprotein profile of the oldest old[198; 199].

392　Interestingly, during a three year follow up study involving the Chinese oldest old it was found that for

393　each 1 mmol/L increase of LDL-C concentration there was a corresponding 19% decrease in 3-year all-

394　cause mortality [200]. These findings are intriguing and require a biological explanation. The oldest old

395　in general are in a state of multi-morbidity[201]. Based on this premise it is logical that low levels of

396 LDL-C could be one particular clinical manifestation of underlying multi-morbidity. Ageing

397 superimposed on cholesterol metabolism in a MUHO individual or a metabolically unhealthy normal

398 weight individual could theoretically contribute to a drop in LDL-C. The conceptual framework

399 outlined in figure 2 suggests an obese state/poor metabolic health combined with an age associated rise

400 in hepatic ROS levels results in a rise in HMGCR activity[202; 203]. In a normolipidemic individual this

401 would result in rise in LDL-C due to the homeostatic down-regulation of LDLr synthesis. However, if

402 there is also an age associated decrease in ACAT2 activity, this reduces the conversion of FC to CE.

403 Consequently, VLDL-C production would drop and there would be a concomitant reduction in LDL-

404 C. As intracellular levels of FC accumulate and oxidative stress progresses, this state could advance to

405 NAFLD[204]. As there is a strong association between NAFLD[205] and CVD[206], this in theory could

406 increase an older persons risk of mortality, and help to account for the association between low levels

407 of LDL-C and increased risk of mortality, which has been observed in certain studies involving the

408 oldest old.

409

410 **7. Conclusions**

411 Obesity among older people has increased significantly. This review has revealed that obesity has a

412 pleiotropic effect on cholesterol metabolism. Obesity affects cholesterol absorption, synthesis,

413 lipoprotein processing, and results in the accumulation of cholesterol hepatically. Many of the changes

414 are similar to how ageing intersects with cholesterol metabolism, and it can be suggested an obese state

415 superimposed on ageing has the potential to exacerbate the dysregulation of cholesterol metabolism,

416 which occurs with advancing age. This review also revealed diet as a key factor which links an obese

417 state to important changes which occur in hepatic cholesterol metabolism. In particular the excessive

418 intake of dietary lipids and fructose were highlighted as key factors which underpin conditions such as

419 NAFLD. Careful attention needs to be placed on this association. This review also highlighted a number

420 of anomalies which exist in this field. Firstly, there are certain individuals who, despite being in an

421 obese state, appear to be normolipidemic, and it is not immediately clear why this is the case. The

422 second anomaly centres on the oldest old. In particular the association between low levels of LDL-C

423 and an increased risk of mortality, which has been observed in a number of studies. A tentative

424 explanation for this association was presented, which centred on obesity/an unhealthy metabolic state

425 and its intersection with ageing as important factors underpinning this anomaly. However, this is only

426 one possible explanation and to fully elucidate this intriguing anomaly, it is necessary for the dynamics

427 of cholesterol metabolism in the oldest old to be investigated to a much greater extent. To date there

428 has been paucity of research in this area. Finally, this review has raised a broader question which relates

429 to the public health challenge surrounding an ageing global population which is becoming increasingly

430 obese.   There is no straightforward solution to this problem. However, one possible strategy could

431 involve adopting public health initiatives which target middle aged individuals and educating them

432 about the deleterious health implications of being overweight/obese. Increased awareness among this

group could lead to better health in later life. To this end it is vital public health interventions are initiated which make both younger people and middle-aged individuals cognisant of appropriate lifestyle choices which optimise their chances of growing old healthily. If this issue is not addressed in coming years, more and more people will reach old age in poor metabolic heath due to being obese.

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913 **List of Figures**

914 **Figure 1:** Overview of whole-body cholesterol metabolism. Cholesterol metabolism is maintained by

915 an array of regulatory processes which control absorption, synthesis, hepatic lipoprotein production,

916 lipoprotein uptake and reverse cholesterol transport. The signs indicate where obesity has been shown

917 to impact cholesterol metabolism. Note: cholesterol absorption has both a positive and negative sign

918 associated with it, to indicate that certain studies have found that obesity decreases cholesterol

919 absorption while other have found the opposite effect. Abbreviations: ABCA1, ATP-binding cassette

920 transporter; Acetyl-CoA, acetyl coenzyme A; ABCG5/G8, ATP-binding cassette (ABC) transporters

921 G5 and G8; ACAT2, acetyl CoA acetyltransferase 2; CETP, cholesteryl ester transfer protein;

922 CYP7A1, cholesterol 7 alpha-hydroxylase; IDL, intermediate density lipoprotein; HDL, high density

923 lipoprotein; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; HMGCR, HMG-CoA reductase; LCAT,

924 lecithin–cholesterol acyltransferase; LDL, low density lipoprotein; NPC1L1, Niemann-Pick C1-Like

925 1; PCSK9, proprotein convertase subtilisin/kexin type 9; SCAP, sterol regulatory element-binding

926 protein cleavage-activating protein; SREBP-2, sterol regulatory element-binding protein 2; scavenger

927 receptor, class B type 1(SR-B1); VLDL, very low density lipoprotein.

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929

930 **Figure 2:** Conceptual model of obesity/ageing induced changes to hepatic cholesterol metabolism

931 which could result in low levels of LDL-C. A metabolic feature which has been observed in a number

932 of studies involving the oldest old. Abbreviations: Acetyl-CoA, acetyl coenzyme A; ACAT2, acetyl

933 CoA acetyltransferase 2; CETP, cholesteryl ester transfer protein; CYP7A1, Cholesterol 7 alpha-

934 hydroxylase; IDL, intermediate density lipoprotein; HDL, high density lipoprotein; HMG-CoA, 3-

935 hydroxy-3-methylglutaryl-CoA; HMGCR, HMG-CoA reductase; LCAT, lecithin–cholesterol

936 acyltransferase; LDL, low density lipoprotein; MUHO, metabolically unhealthy obese; ROS, reactive

937 oxygen species; SREBP-2,scavenger receptor, class B type 1(SR-B1); VLDL, very low density

938 lipoprotein.