Proceedings of the Nutrition Society (2022), **81**, 146–161 doi:10.1017/S0029665121003815

First published online 22 November 2021

The Nutrition Society Summer Conference 2021 was held virtually on 6–8 July 2021

Conference on 'Nutrition in a changing world'
Symposium 4: Changing nutrition and non-alcoholic fatty liver disease (NAFLD)

Non-alcoholic fatty liver disease: a multi-system disease influenced by ageing and sex, and affected by adipose tissue and intestinal function

Josh Bilson[1,2], Jaswinder K. Sethi[1,2,3] and Christopher D. Byrne[1,2]*
[1]*Human Development and Health, Faculty of Medicine, University of Southampton, Southampton, UK*
[2]*National Institute for Health Research Southampton Biomedical Research Centre, University of Southampton and University Hospital Southampton National Health Service Foundation Trust, Southampton, UK*
[3]*Institute for Life Sciences, University of Southampton, Southampton, UK*

In recent years, a wealth of factors are associated with increased risk of developing non-alcoholic fatty liver disease (NAFLD) and NAFLD is now thought to increase the risk of multiple extra-hepatic diseases. The aim of this review is first to focus on the role of ageing and sex as key, poorly understood risk factors in the development and progression of NAFLD. Secondly, we aim to discuss the roles of white adipose tissue (WAT) and intestinal dysfunction, as producers of extra-hepatic factors known to further contribute to the pathogenesis of NAFLD. Finally, we aim to summarise the role of NAFLD as a multi-system disease affecting other organ systems beyond the liver. Both increased age and male sex increase the risk of NAFLD and this may be partly driven by alterations in the distribution and function of WAT. Similarly, changes in gut microbiota composition and intestinal function with ageing and chronic overnutrition are likely to contribute to the development of NAFLD both directly (i.e. by affecting hepatic function) and indirectly via exacerbating WAT dysfunction. Consequently, the presence of NAFLD significantly increases the risk of various extra-hepatic diseases including CVD, type 2 diabetes mellitus, chronic kidney disease and certain extra-hepatic cancers. Thus changes in WAT and intestinal function with ageing and chronic overnutrition contribute to the development of NAFLD – a multi-system disease that subsequently contributes to the development of other chronic cardiometabolic diseases.

Non-alcoholic fatty liver disease: Adipose tissue dysfunction: Gut microbiota: Diabetes: CVD: Age: Sex

Current estimates indicate that about 30 % of the global adult population are affected by non-alcoholic fatty liver disease (NAFLD) and the increasing prevalence of this disease has occurred in parallel with the global epidemic of obesity and type 2 diabetes mellitus (T2DM)[1,2]. Considered to be the predominant cause of chronic

Abbreviations: CKD, chronic kidney disease, GM, gut microbiota, HCC, hepatocellular carcinoma, LPS, lipopolysaccharides, MetS, metabolic syndrome, NAFLD, non-alcoholic fatty liver disease, NASH, non-alcoholic steatohepatitis; NEFA, non-esterified fatty acids, SAT, subcutaneous WAT, T2DM, type 2 diabetes mellitus, VAT, visceral WAT, WAT, white adipose tissue.

***Corresponding author:** Christopher D. Byrne, email C.D.Byrne@soton.ac.uk

liver disease in many parts of the world, NAFLD represents a spectrum of progressive hepatic disease phenotypes extending from hepatic steatosis to non-alcoholic steatohepatitis (NASH), liver fibrosis and cirrhosis[1,3,4]. Evidence now shows that NAFLD increases the risk of liver-related complications and is also a multi-system disease that increases the risk of CVD and cardiac disease[5,6], chronic kidney disease (CKD)[7], T2DM[8,9] and some extra-hepatic cancers[1]. Therefore, it is no surprise that the presence of NAFLD is strongly associated with an increased risk of all-cause mortality[3,10]. Indeed, CVD is the main cause of mortality in patients with NAFLD, followed by extra-hepatic cancers and liver-related complications[10]. Additionally, recent evidence suggests that there may be an even greater cardiometabolic risk with the more advanced stages of liver disease, such as liver fibrosis, which is also a strong predictor of all-cause and disease-specific mortality[11–13].

In recent years, a wealth of factors have been shown to be associated with an increased risk of developing NAFLD. The aim of this review is first to focus on the role of ageing and sex as key, poorly understood risk factors in the development and progression of NAFLD. Secondly, we will discuss the roles of white adipose tissue (WAT) and intestinal dysfunction, as producers of extra-hepatic factors known to further contribute to the pathogenesis of NAFLD. Finally, we will summarise the role of NAFLD as a multi-system disease affecting other organ systems beyond the liver.

Sex and age as risk factors for non-alcoholic fatty liver disease

The involvement of age and sex in the development of NAFLD has received increased attention in recent years yet the reasons why these are risk factors for NAFLD remain poorly understood. The prevalence of NAFLD is higher in men and is thought to increase into middle age and then decline after the age of 50–60 years[14]. In contrast, pre-menopausal women appear to be relatively protected from NAFLD; however, this protective capacity is lost after the fifth decade of life when the prevalence of NAFLD is thought to be similar in both sexes[14,15]. The incidence of NASH and cirrhosis is also thought to be greater in both men and women who are ≥50 years of age compared to younger age groups[2]. Recent meta-analysis suggests that whilst pre-menopausal women may have a lower risk of NAFLD, women ≥50 years of age may be at an increased risk of NAFLD progression, compared to men of a similar age[16]. Specifically, among older age groups (≥50 years of age), the relative risk of NASH and advanced liver fibrosis was found to be 17 and 56 % higher respectively, in women compared to men[16]. Conversely, the risk of NAFLD progression was not significantly different between men and women in populations with an average age of ≤50 years[16]. Further work is required to elucidate potential mechanisms underlying the apparent increased risk of NAFLD progression in older women. For example, studies exploring sexual dimorphism in liver metabolism have recently linked hepatic actions of oestrogens to lipid metabolism and female reproductive functions[17]. Whether these or other sexually dimorphic metabolic or endocrine factors are important in NAFLD remains to be investigated[18].

Advancing age also increases the risk of hepatic and extra-hepatic complications of NAFLD[14]. Thus it is expected that older patients with NAFLD will have a higher likelihood of overall and disease-specific mortality[19,20]. Whether the association between NAFLD and all-cause mortality is modified by sex is currently unclear. Previous studies suggest a worse outcome in men[20,21], whilst others have found trends suggesting that NAFLD is associated with an increased risk of all-cause mortality in women but not men[22]. Thus, further large prospective cohort studies should explore whether the direction and magnitude of the association between NAFLD and mortality are modified by sex.

White adipose tissue mass and distribution in non-alcoholic fatty liver disease

A wealth of evidence indicates that obesity increases the risk of NAFLD[23–26]. Obesity is defined as excess body fat and results from chronic overnutrition. For adults, it is most frequently classified as a weight for height index or BMI and includes underweight or 'wasting' (<18·5 kg/m2), overweight (≥25 kg/m^2), obesity (≥30 kg/m^2) and morbid obesity (≥40 kg/m^2)[27]. In contrast, waist circumference provides a simpler anthropometric measurement to diagnose central obesity which is an important independent risk factor for NAFLD and an important component of the metabolic syndrome (MetS). As previously described[28], MetS is defined as the presence of three or more of the following criteria; increased waist circumference, hypertriglyceridemia, reduced HDL-cholesterol, hypertension and hyperglycaemia. It is worth highlighting that neither BMI nor waist circumference is considered reliable indicators of adiposity *per se* since they do not provide an assessment of WAT mass nor volume[29]. Nonetheless, BMI and waist circumference have proved to be extremely useful measures for population-based studies and firmly established the importance of obesity as a risk factor for NAFLD. Despite this, it is an oversimplification to consider NAFLD solely as a consequence of obesity given the growing evidence indicating that NAFLD can also occur in individuals with a non-obese BMI, or low WAT mass[30,31]. It has been proposed that an increase in the accumulation of central WAT and a reduction in the functional capacity of WAT (particularly subcutaneous WAT (SAT)) to store excess energy as TAG are crucial factors that underpin the relationship between obesity, systemic metabolic disease and NAFLD[31].

Studies utilising adipose tissue-targeted technologies coupled with histological assessment have suggested that the hypertrophic expansion of adipocytes within visceral WAT (VAT) rather than SAT is particularly associated with NAFLD. After approximately 4 years of follow up, a larger VAT area was found to be associated

https://doi.org/10.1017/S0029665121003815 Published online by Cambridge University Press

with a higher risk of incident NAFLD, whereas larger areas of SAT were associated with regression of NAFLD[32]. Moreover, several recent studies have demonstrated that increased VAT, as opposed to SAT, increases the risk of, and predicts advanced liver fibrosis in patients with NAFLD[33–35]. Similarly, evidence also indicates that VAT accumulation is an independent risk factor for hepatocellular carcinoma (HCC) recurrence in patients with suspected NASH[36]. Thus, this evidence supports a fundamental hypothesis that 'the risk of developing metabolic disease associated with obesity is governed by the regional distribution of WAT within the individual, with the expansion of certain fat depots being more strongly associated with metabolic dysfunction than others'[37]. Collectively, it is likely that the distribution and capacity of SAT to effectively expand and store lipid, rather than the obesity *per se*, is a pivotal factor in the relationship between increased adiposity and NAFLD risk.

The distribution of WAT is known to differ significantly between sexes, changes with increasing age and has been hypothesised to be partly responsible for the increased prevalence of NAFLD in men and older age groups, particularly post-menopausal women (Fig. 1)[38–40]. Whilst the mechanisms regulating the distribution of WAT remain largely elusive, evidence indicates that ageing and male sex are associated with a restricted capacity to effectively expand so-called 'metabolically protective' SAT depots[41]. Whilst pre-menopausal women typically have greater total adiposity, men tend to accumulate greater amounts of VAT with ageing and pre-menopausal women accumulate gluteal femoral SAT which is associated with a lower risk of metabolic disease and NAFLD[42]. In both men and women, older age (i.e. post-menopausal women and men >50 years) is associated with a reduction in the capacity of SAT to expand and an increase in VAT[43–45]. The limited capacity of SAT to store TAG in men and with increasing age is likely to re-direct lipid accumulation ectopically in non-adipose tissues, including the liver, leading to lipotoxicity, a chronic local and systemic pro-inflammatory environment and eventually NAFLD development[46]. The importance of effective SAT expansion can be seen in individuals with certain genetic or acquired lipodystrophies that are characterised by the complete or partial absence of SAT[47]. In spite of their often lean appearance, these individuals appear to exhibit much higher rates of NAFLD/NASH progression and other cardiometabolic complications than would be expected based on their BMI alone[31,47]. Given this, it is likely that differences in WAT distribution between sexes and changes occurring with increasing age are both important in the increased risk of NAFLD associated with ageing and with male sex.

Adipose tissue dysfunction and non-alcoholic fatty liver disease

WAT is composed of mature unilocular adipocyte fraction and a stromal vascular fraction, comprised of numerous cell types such as vascular, mesenchymal and immune cells. At a cellular level, WAT expansion can be mediated by an enlargement of individual adipocytes (hypertrophy), an increase in the number of adipocytes (hyperplasia) or a mixture of both. Adipocyte hypertrophy, rather than hyperplasia, is more closely associated with WAT dysfunction and metabolic disease[48]. Factors including hypoxia, low-grade chronic inflammation (i.e. metaflammation) and improper extracellular matrix remodelling are thought to limit adipocyte differentiation and the healthy expansion of adipose tissue (hyperplasia)[49,50]. This limit can result in adipocyte hypertrophy, dysfunction, stress and eventually death[51,52]. In this context, WAT dysfunction refers to a reduction in the tissues ability to effectively sense and respond to dynamic changes in nutrient availability (i.e. metabolic inflexibility) and can coexist with adipose insulin resistance and metaflammation. Specifically, this dysfunction is thought to affect WAT metabolism and in particular its ability to handle lipids and increase the lipolytic rate of WAT due to a reduction in tissue insulin sensitivity, increasing the flux of non-esterified fatty acids (NEFA) to the liver and consequently increasing the risk of NAFLD[31,53–55].

Accompanying the changes in the distribution of WAT, ageing is associated with a marked reduction in insulin, lipolytic and NEFA responsiveness in WAT. This metabolic inflexibility may underly the known association between ageing and increased risk of NAFLD[43–45]. The reduction in SAT with ageing in both men and women may in part be driven by a reduction in the adipogenic potential of progenitor cells and the accumulation of senescent adipocytes in aged WAT. Preadipocytes isolated from peripheral SAT in elderly individuals were found to have a reduced rate of replication compared to those isolated from younger individuals[56]. Additionally, ageing is associated with an accumulation of senescent adipocyte-derived stem cells within SAT which lack the ability to differentiate into adipocytes in response to metabolic stress, consequently affecting the tissue's capacity to store TAG[57]. Through their senescence-associated secretory phenotype, senescent adipocyte progenitor cells within WAT are also likely to contribute to WAT inflammation and subsequent metabolic complications[58,59].

In addition to ageing, there are also sexually dimorphic differences in WAT function whereby WAT in females is generally more insulin-sensitive, more lipogenic and less susceptible to inflammation than WAT from males. This phenomenon is also strongly associated with differences in sex hormone concentrations[60,61]. Menopause appears to associate with a preferential increase in VAT (rather than SAT) in both obese and non-obese women[62–65], further supporting a role for sex hormones, such as oestrogen, in regulating the beneficial distribution and function of WAT. Circulating concentrations of oestrogen decrease markedly after menopause which is thought to lead to the redistribution of lipids into VAT and the liver which, in combination with overnutrition, increases the risk of VAT accumulation and NAFLD in post-menopausal women[66].

https://doi.org/10.1017/S0029665121003815 Published online by Cambridge University Press

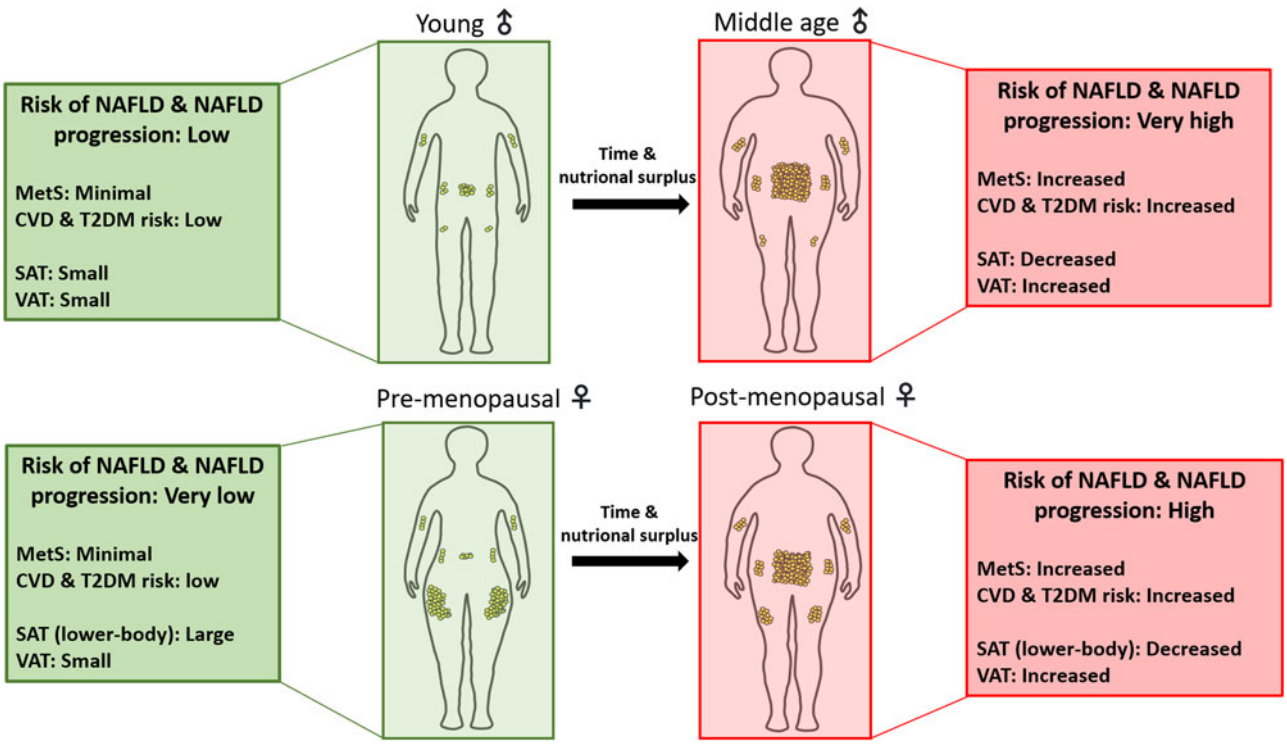

Fig. 1. Age-related changes in WAT distribution in men and women are associated with increased risk NAFLD, MetS, T2DM and CVD. Sex and age are key factors that modify the risk of NAFLD and NAFLD progression. NAFLD risk is lower in younger women compared to younger men whereas the risk of NAFLD is similar in older men and women (i.e. post-menopausal). Younger women have an increased capacity to preferentially expand gluteal femoral SAT consequently protecting them from NAFLD. Age-associated changes in WAT leads to the redistribution of WAT which is typically characterised by a marked reduction in SAT and increased central metabolically-unfavourable VAT which may partly explain the increased risk of NAFLD associated with ageing in both men and women. WAT distribution is different between men and women, is heavily influenced by ageing and is strongly associated with NAFLD risk. T2DM, type 2 diabetes; MetS, metabolic syndrome; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; NAFLD, non-alcoholic fatty liver disease; WAT, white adipose tissue.

Pre-clinical studies utilising ovariectomised murine models also support a causative relationship between reduced oestrogen production, increased VAT mass and the development of NASH[42,66–68]. Whilst an in-depth discussion of the role of oestrogen within WAT is beyond the scope of this review (see other relevant reviews[42,69,70]), it is thought that the increased expression of oestrogen receptor α in the gluteal femoral SAT of premenopausal women promotes lipoprotein lipase activity and accumulation of TAG in adipocytes within this depot[71]. Thus, it is likely that differences in WAT function (partly driven by differences in sex hormone concentrations and the expression of functional target receptors) is an important factor underlying the observed differences in NAFLD risk between men and women. Furthermore, changes in WAT with ageing are likely to exacerbate WAT dysfunction associated with a state of chronic energy surplus and are likely to have an important role in the increased risk of NAFLD associated with older age.

Adipokines and non-alcoholic fatty liver disease

WAT is an endocrine tissue capable of secreting a wide range of adipokines which have various roles in the regulation of whole-body energy homeostasis and inter-organ communication[72]. The aberrant production of these adipokines has been linked to multiple obesity-related metabolic diseases. Amongst these adipokines, leptin and adiponectin are predominately produced by adipocytes. In addition to its well-established role in regulating appetite and energy homeostasis[49,73], leptin exerts a dual action on hepatic function and NAFLD severity. Recent meta-analyses including an analysis of over thirty studies indicated that circulating concentrations of leptin are elevated in patients with NAFLD compared to healthy controls and supports a positive relationship between leptin and NAFLD[74]. As recently highlighted[75,76], under normoleptinemia conditions, leptin is thought to suppress hepatic glucose production and hepatic lipogenesis thus providing an insulin-sensitising anti-steatotic effect. Conversely, in the context of chronic hyperleptinemia as is common in obesity, a state of leptin resistance can result, which may also contribute to the NASH phenotype. It is suggested that in the liver, high concentrations of leptin can increase the expression of matrix remodelling enzymes via interacting with leptin receptors on Kupffer and sinusoidal endothelial cells, in turn activating hepatic stellate cells, and possibly contributing to liver fibrosis[77].

https://doi.org/10.1017/S0029665121003815 Published online by Cambridge University Press

Sexual dimorphism has also been reported for leptin expression[78]. Despite their lower risk of NAFLD, circulating concentrations of leptin are higher in pre-menopausal women compared to age-matched men and higher leptin levels are thought to be driven by both greater adiposity and an increased production rate of leptin per unit mass of WAT in women compared to men[79]. In both men and women, circulating concentrations of leptin are thought to gradually decline with ageing, with reductions being most noticeable in women compared to men whilst appearing to be independent of menopausal status[79,80]. Despite these findings, it is currently unknown whether differences in circulating concentrations of leptin between sexes and age groups have an impact on the risk of NAFLD.

Similar to leptin, a wealth of studies indicate that the circulating concentrations of adiponectin, the most systemically abundant adipokine, are altered in patients with NAFLD (as reviewed in[81]). Adiponectin is a hepatoprotective adipokine that has well-established anti-inflammatory[82–84] and insulin-sensitising effects[85] both systemically and within the liver. Meta-analysis indicates that adiponectin concentrations are significantly lower in patients with NAFLD compared to healthy controls; furthermore, NASH is associated with lower adiponectin when compared to simple steatosis[86]. Conversely, adiponectin concentrations are thought to increase in patients with NAFLD-cirrhosis potentially due to a reduction in the hepatic clearance of adiponectin and/or an increase in its production as a result of the tissue repair process associated with NAFLD-cirrhosis[87–89]. Along with its well-established role in promoting hepatic insulin sensitivity[90,91], evidence indicates that adiponectin also has antifibrogenic effects via inhibiting the proliferation of hepatic stellate cells[92]. Whilst the role of adiponectin in ageing remains uncertain, it is thought that circulating concentrations of adiponectin are paradoxically increased in older age and are positively associated with physical disability and mortality in elderly individuals[93]. Furthermore, some evidence suggests that the association between adiponectin and ageing may be modified by sex[94]. In addition to leptin and adiponectin, a wealth of other studies have demonstrated that numerous other adipokines may be involved in the development and progression of NAFLD (Table 1). It should be noted that there is a substantial amount of conflicting evidence regarding the changes in circulating concentrations of other adipokines in the context of NAFLD and little is known about the potential pathological role of these adipokines in NAFLD (Table 1). Moreover, further studies are required to elucidate whether the effects of age and sex on adipokine production influences NAFLD risk.

WAT dysfunction and changes in adipokine secretion are also strongly associated with increased low-grade chronic inflammation in WAT (metaflammation); characterised by the infiltration of various leucocytes, an increase in the ratio of proinflammatory/anti-inflammatory macrophages and leucocytes and the increased presence of crown-like structures (dying adipocytes surrounded by pro-inflammatory macrophages)[95]. Consequently, metaflammation in WAT (particularly VAT inflammation) is associated with an increased expression of pro-inflammatory cytokines such as IL-6, IL-1β, TNF-α and monocyte chemoattractant protein-1[96–98]. Some of these have been shown to contribute to local insulin resistance, elevated fatty acid lipolysis, anti-adipogenesis and pro-inflammatory macrophage infiltration[49,97–101]. This in turn can impact both metabolic and endocrine functions of WAT. Evidence from murine diet-induced obesity studies indicates that WAT inflammation and reductions in protective anti-inflammatory lipokines such as palmitoleic acid may be important in the development of NASH[102–104].

By virtue of its anatomical links, via the portal vein, increased VAT inflammation is of particular importance in NAFLD/NASH since VAT-derived inflammatory cytokines (other adipokines, lipokines and metabolites (e.g. NEFA)) are initially transported to the liver and therefore may exacerbate NAFLD severity. Consequently, this may in turn increase the associated risk of T2DM and CVD[105,106]. Collectively, findings indicate that changes in the production of adipokines and increased WAT inflammation may contribute to NAFLD via modulating local and hepatic function, inducing insulin resistance and modulating the local and systemic pro-inflammatory conditions. Whether these changes in WAT function contribute to the increased risk of extra-hepatic diseases associated with NAFLD independently requires further investigation.

Intestinal dysfunction, dysbiosis and non-alcoholic fatty liver disease

Emerging evidence now suggests that changes in gut microbiota (GM) (i.e. dysbiosis) and intestinal function may exacerbate WAT dysfunction which may indirectly contribute to metabolic dysfunction and NAFLD[107]. The gastrointestinal tract is the first point of contact for ingested nutrients where it has an integral role in nutrient breakdown and absorption, regulation of whole-body energy homeostasis and is an important host defence barrier. Occupying the gastrointestinal tract is an extensive number of microorganisms, collectively known as the GM which are thought to modulate local and distal tissue function via a range of complex mechanisms[108,109]. The microbial organisms occupying the gastrointestinal tract mainly include bacteria, archaea, fungi and viruses (predominantly bacteriophages); however, studies exploring the role of the GM in NAFLD have predominantly focused on bacteria[110,111].

A plethora of studies have revealed that GM dysbiosis is associated with and is a contributing factor to NAFLD[112–115]. The dominating phyla within human GM are Bacteroidetes and Firmicutes with a significant inter-individual variation in the GM at lower taxonomical levels[116,117]. Previous evidence indicates that the relative abundance of Bacteroidetes is lower in patients with NASH compared to those with hepatic steatosis and healthy controls[117]. More recently, *Bacteroides* abundance was found to be significantly increased in

https://doi.org/10.1017/S0029665121003815 Published online by Cambridge University Press

Table 1. Changes in circulating concentrations of adipokines and their potential roles in NAFLD

Adipokine	Association with NAFLD	Potential role in NAFLD
Leptin	Increased[74]	Anti-steatotic during normoleptinemia[77] Pro-fibrogenic during hyperleptinemia via increasing the expression of fibrogenic factors from activated HSC[176,177]
Adiponectin	Decreased[86]	Hypoadiponectinemia is thought to contribute to: increased hepatic steatosis, increased hepatic insulin resistance and an increased pro-inflammatory state[81,178]
Resistin	No association[179–181], increased[182–184]	Largely unknown, increased concentrations potentially promotes a pro-inflammatory environment[185]
RBP-4	Increased[186,187]	Largely unknown, potentially induces hepatic mitochondrial dysfunction and promotes hepatic steatosis[188,189]
Adipsin	Decreased[190], no association[191], increased[192]	Largely unknown, low concentrations may impact hepatic function via a reduction in insulin production[193]
Chemerin	Increased[191,194,195]	Largely unknown, increased concentrations potentially protective via the suppression of pro-inflammatory cytokines[196]
Apelin	Not associated[197], increased[198]	Largely unknown, increased concentrations are potentially profibrogenic via increasing the expression of profibrotic factors from HSC[199,200]

HSC, hepatic stellate cells; NAFLD, non-alcoholic fatty liver disease; RBP-4, retinol binding protein-4.
In contrast to classic adipocyte-derived adipokines leptin and adiponectin, studies investigating changes in circulating concentrations of other adipokines in patients with NAFLD are largely inconsistent. Similarly, whilst the role of leptin and adiponectin in the development and progression of NAFLD remains somewhat debated, there is currently very little known about the potential roles of other adipokines on hepatic function and NAFLD. It should be noted that the expression of many adipokines (e.g. chemerin and RBP-4) is not restricted to WAT and also occurs within other tissues including the liver. Consequently, whilst changes in the secretion of WAT-derived adipokines may contribute to altered circulating concentrations, other sources (particularly hepatic) may also influence circulating concentrations, hepatic function and NAFLD.

patients with NASH and the abundance of *Ruminococcus* was increased in patients with liver fibrosis[118]. As recently reviewed[113], this shifting in GM in relation to NAFLD severity is supported by numerous other studies. Indeed, the presence of bacteria belonging to the Proteobacteria phylum was increased significantly in patients with ≥F3 when compared to patients with F0–F2 liver fibrosis (Table 2)[119]. Emerging evidence also supports a strong link between the GM and NAFLD-cirrhosis indicating that the composition of the GM may be a useful tool for the identification and staging of NAFLD. Utilising a unique twin and family study design, one study identified a specific GM signature that had a robust diagnostic accuracy, with an area under the receiver operating characteristic of 0·92, for the detection of NAFLD-cirrhosis[120]. Further work demonstrated the robustness and potential universal applicability of this microbiome signature of NAFLD-cirrhosis in two independent cohorts across geographically and culturally distinct populations[121]. However, given the impact of host genetics and environmental factors on the composition of GM[122], it is unlikely that a single GM signature will be able to distinguish between NAFLD phenotypes at an individual level.

Miele *et al.* were the first to identify that patients with NAFLD generally have increased intestinal permeability and alterations in intestinal tight junction integrity (observed as a reduction in zonula occludens-1 within intestinal crypt cells), compared to healthy subjects[123]. Recent meta-analysis found that 39·1 % of NAFLD patients had evidence of increased intestinal permeability compared to 6·8 % of healthy controls (OR 5·08, 95 % CI 1·98, 13·05)[124]. Furthermore, subgroup analysis indicated that there was a higher incidence of increased intestinal permeability in patients with NASH compared to patients with simple steatosis[124]. It is generally well-accepted that the increased intestinal permeability commonly seen in NAFLD facilitates the translocation of GM-derived metabolites and bacterial products (such as lipopolysaccharides (LPS) and ethanol) which may in turn contribute to metaflammation and the pathogenesis of NAFLD[125].

In addition to altered GM and intestinal permeability, the abundance of GM-dependent metabolites is thought to be altered in NAFLD, many of which may be detected

Table 2. Histological definitions of liver fibrosis stages and corresponding liver-biopsy validated liver VCTE cut-off values

Liver fibrosis stage	Histological definition	Liver VTCE cut-off (kPa)
F0	None	
F1	Perisinusoidal or periportal	
F1A	Mild, zone 3 perisinusoidal	
F1B	Moderate, zone 3, perisinusoidal	
F1C	Portal/periportal	
F2	Perisinusoidal and portal/periportal	8·2
F3	Bridging fibrosis	9·7
F4	Cirrhosis	13·6

VCTE, vibration-controlled transient elastography; kPa, kilopascal; PPV, positive predictive value; NPV, negative predictive value; NASH, non-alcoholic steatohepatitis.
Liver fibrosis stages and corresponding histological definitions are based on the NASH clinical scoring network scoring system[201]. Liver VCTE cut-off values are based on the findings from a recent large validation study[202]. The liver VCTE threshold of 8·2 kPa was found to have a: sensitivity of 0·71 (0·64–0·77), specificity of 0·70 (0·62–0·77), PPV of 0·78 (0·71–0·83) and NPV of 0·61 (0·54–0·69) for the identification of ≥F2 liver fibrosis. For the prediction of ≥F3 liver fibrosis, 9·7 kPa was found to have a sensitivity of 0·71 (0·62–0·78), specificity of 0·75 (0·69–0·80), PPV of 0·63 (0·55–0·71) and NPV of 0·81 (0·74–0·85). For the prediction of ≥F4 fibrosis, 13·6 kPa was found to have a sensitivity of 0·85 (0·69–0·95), specificity of 0·79 (0·74–0·83), PPV of 0·29 (0·24–0·57) and NPV of 0·98 (0·95–0·99).

https://doi.org/10.1017/S0029665121003815 Published online by Cambridge University Press

in stool samples and may offer a tool for the assessment of disease severity. For example, work comparing the abundance of distinct stool metabolites in patients with NAFLD-cirrhosis *v.* healthy subjects revealed 17 metabolites which, in combination, were able to accurately detect the presence of NAFLD-cirrhosis (AUROC 0·91, 95 % CI 0·89, 0·93)[121]. Thus, evidence is accumulating to suggest that accumulation of certain microbial species, changes in intestinal function and increased intestinal permeability are likely to contribute not only to the pathogenesis of NAFLD but also to increased liver disease severity. Further studies are required to elucidate the potential role of non-bacterial species within the GM on the development and progression of NAFLD.

Intestinal dysfunction, dysbiosis and links with white adipose tissue function in non-alcoholic fatty liver disease

Associated with WAT dysfunction are changes in intestinal function and GM dysbiosis, which have also been proposed to be key factors contributing to NAFLD. Receiving about 70 % of its blood supply from intestinal vascularisation, the liver is constantly exposed to the metabolic products, toxins and nutrients produced by the GM[126]. It has been suggested that when in a dysbiotic state, GM may contribute to the development and progression of NAFLD via a range of pathways; including changes in dietary energy harvest[127,128], alterations in SCFA production (particularly butyrate)[129,130], increased bacterial LPS translocation[125,131], alternations in bile acid profiles[132] and increased endogenous ethanol production[133]. Indeed, the potential effects of these factors on hepatic function and NAFLD have been discussed in various recent reviews[110,112–114,125,134]; furthermore, alterations in appetite-regulating gut hormones are also likely to have an important role in the development and progression of NAFLD, as recently reviewed[135–137].

Disruptions in intestinal permeability associated with obesity and NAFLD are likely to be accompanied by a reduction in the integrity of intestinal tight junctions[123,138]. Increased intestinal permeability in the presence of GM dysbiosis is thought to facilitate the translocation of bacterial products including pro-inflammatory endotoxins such as LPS. Circulating concentrations of LPS were found to be significantly higher in patients with NAFLD compared to healthy controls[139,140] and have been shown to be positively associated with the expression of pro-inflammatory genes within both VAT and SAT in individuals with obesity[141]. This is supported by evidence from pre-clinical murine studies indicating that increased LPS may directly contribute to WAT inflammation and increase the release of WAT-derived pro-inflammatory cytokines[142]. Accompanying these findings, various other studies have proposed additional mechanisms by which changes in intestinal function and GM dysbiosis may impact NAFLD development both directly and in-directly via detrimentally impacting WAT function (Table 3 and Fig. 2).

Evidence also suggests that the composition of the GM and intestinal function can differ between sexes and such differences may partly explain differences in the risk of metabolic disease between sexes[143–145]. Similarly, changes in GM composition and intestinal function are strongly associated with ageing and are likely to contribute to the increased risk of NAFLD associated with older age both directly and indirectly via exacerbating WAT dysfunction[146–148]. Similar to obesity, ageing is also associated with disruptions in intestinal permeability subsequently facilitating the translocation of bacterial products such as LPS which are known to contribute to both hepatic and WAT dysfunction (Fig. 2)[149,150]. Collectively, existing studies demonstrate the existence of a gut-WAT axis which, in addition to the well-established gut-liver axis, may indirectly contribute to NAFLD pathogenesis. Furthermore, differences in GM composition and intestinal function between men and women and with ageing may contribute to both hepatic and WAT dysfunction and subsequently drive the development of NAFLD.

Non-alcoholic fatty liver disease and extra-hepatic complications

Non-alcoholic fatty liver disease, type 2 diabetes mellitus and metabolic syndrome

Type 2 diabetes is both a risk factor for NAFLD and an extra-hepatic complication of NAFLD. The association between T2DM and NAFLD is well-established and T2DM is considered to be one of the most important risk factors for NAFLD. A meta-analysis of twenty-four studies found that the pooled prevalence of NAFLD in patients with T2DM was 59·7 % (95 % CI 54·3, 64·9 %), with the prevalence of NAFLD being slightly higher in men (60·1 %, 95 % CI 53·6, 66·4 %), compared to women (59·35 %, 95 % CI 53·3, 65·3 %)[151]. Furthermore, the presence of obesity, hypertension and dyslipidaemia, as features of the MetS, were associated with an increased prevalence of NAFLD in patients with T2DM, suggesting that these factors may act with T2DM to further increase the risk of NAFLD[151]. The presence of T2DM increases the risk of liver fibrosis by approximately 2–6-fold[1]. The mechanism by which T2DM increases the risk of liver fibrosis is uncertain. However, numerous factors have been proposed that could mediate the increase in the risk of liver fibrosis in patients with T2DM and these include insulin resistance, hyperglycaemia, hypoadiponectinemia, mitochondrial dysfunction, increased reactive oxygen species, excess free cholesterol, increased proinflammatory cytokines and endoplasmic reticulum stress[152]. Recently we have shown in patients with NAFLD that increased circulating concentrations of growth-differentiation factor-15, a stress-inducible cytokine, are independently associated with the presence of ≥F3 and ≥F2 liver fibrosis (Table 2)[153]. We also showed in this work that growth-differentiation factor-15 may be an important

https://doi.org/10.1017/S0029665121003815 Published online by Cambridge University Press

Table 3. Changes in GM-derived factors/metabolites in NAFLD and their proposed effects in WAT and the liver

Factor/ metabolite	Association with NAFLD	Proposed effect on WAT	Proposed effect on liver
LPS	Increased[139,140,203]	Increased inflammation and decreased insulin sensitivity[131,204,205]	Increased liver inflammation via the activation of hepatic macrophages and platelets[203]. Pro-fibrogenic via the activation of HSC[206]
Endogenous ethanol	Increased[133,207]	Largely unknown. Potentially induces oxidative stress and inflammation[208]	Increased hepatic mitochondrial dysfunction[209], increased hepatic steatosis and inflammation[133]
Butyrate	Decreased[210]	Decreased production is thought to contribute to increased inflammation and decreased fatty acid oxidation[211,212]	Decreased production is thought to contribute to hepatic mitochondrial dysfunction[213], increased hepatic steatosis and inflammation[214]
TMAO	Increased[215,216]	Increased inflammation and impaired expression of insulin signalling-related genes[217,218]	Reduced insulin sensitivity and increased hepatic steatosis and inflammation[217,219]
Indole	Decreased[220]	Reduced regulation of microRNA expression (specifically miR-181) leading to increased inflammation and decreased insulin sensitivity[221]	Decreased production is thought to contribute to increased hepatic steatosis and inflammation[220]

LPS, lipopolysaccharide; HSCs, hepatic stellate cells; TMAO, trimethylamine *N*-oxide; NAFLD, non-alcoholic fatty liver disease; WAT, white adipose tissue. Changes in the production of various GM-derived metabolites/factors may contribute to the development of NAFLD both directly (i.e. via a direct action within the liver) and indirectly via affecting the function of WAT. Whilst evidence of altered circulating concentrations of certain factors (namely LPS, endogenous ethanol and TMAO) is well-reported in patients with NAFLD, changes in circulating concentrations of other factors (particularly SCFA) require further investigation. Furthermore, more research is required to elucidate the potential contribution of endogenously produced ethanol on WAT dysfunction in the context of NAFLD.

factor contributing to the increased risk of liver fibrosis associated with T2DM, and that HbA1c levels explained about 30 % of the variance in growth-differentiation factor-15 concentrations[153]. However, further work is required to fully elucidate the role of growth-differentiation factor-15 in the development and progression of NAFLD in patients with T2DM.

The estimated global prevalence of NAFLD among patients with T2DM is 55·5 % (95 % CI 47·3, 63·7 %) with prevalence estimates varying between geographical regions[154]. This study also found that the estimated global prevalence of NASH and advanced fibrosis in patients with T2DM was 37·3 % (95 % CI 24·7, 50·0 %) and 4·8 % (95 % CI 0·0, 17·5 %) respectively[154]. The presence of T2DM is also an important risk factor for the faster progression of NAFLD towards NASH, cirrhosis or HCC[1,155,156]. Patients with NAFLD and coexisting T2DM are thought to have between a 2 and 6-fold increased risk of developing advanced fibrosis compared to patients with only NAFLD[1]. In addition to T2DM, the presence of MetS is also recognised as an important NAFLD risk factor. The presence of MetS in patients with NAFLD but without diabetes is associated with more severe NAFLD compared to patients without MetS[157]. Furthermore, this study suggested that a higher number of MetS features was associated with a greater probability of NASH, with 70 % of patients diagnosed with NASH having three or more features of MetS. The presence of MetS has also recently been shown to be associated with progression to advanced fibrosis in patients with NAFLD[158]. These findings support those of others which also show that NAFLD severity is positively associated with the presence of MetS features, particularly the level of hypertension, hyperglycaemia and hypertriglyceridemia[159].

It is important to highlight that the link between T2DM, MetS and NAFLD is complex and bi-directional. Evidence from a recent large meta-analysis of over 500 000 individuals found that NAFLD was associated with an about 2·2-fold increased risk of incident diabetes independently of age, sex, adiposity and other common metabolic risk factors[160]. Interestingly, in this study, the risk of incident diabetes was found to increase in relation to the underlying severity of NAFLD with a particularly noticeable increase in risk according to the severity of liver fibrosis (*n* 5 studies; random-effects HR 3·42, 95 % CI 2·3, 5·1)[160]. These findings support other evidence from meta-analyses and observational studies which demonstrate that individuals with NAFLD had a higher risk for incident T2DM than individuals without NAFLD[8,161]. Evidence collated from eight studies with a median follow-up period of 4·5 years indicated that NAFLD was associated with an increased risk of incident MetS with a pooled relative risk of 3·2 (95 % CI 3·1, 3·4) when NAFLD was diagnosed via ultrasonography[162]. Collectively, this evidence suggests that a vicious cycle of worsening disease states is likely to exist between T2DM, MetS and NAFLD[1].

Non-alcoholic fatty liver disease and CVD

Evidence indicates that NAFLD is an important risk factor for various extra-hepatic diseases and the detrimental relationship between T2DM and NAFLD likely exacerbates this risk. Furthermore, given the strong associations with NAFLD and other cardiometabolic risk factors, including central obesity, atherogenic dyslipidaemia and hypertension, it is no surprise that NAFLD is also associated with an increased risk of CVD[6,163]. Recent evidence suggests that CVD is one of the most important causes of death among people with NAFLD[164], and patients with NAFLD are more likely to experience CVD-related death than a

https://doi.org/10.1017/S0029665121003815 Published online by Cambridge University Press

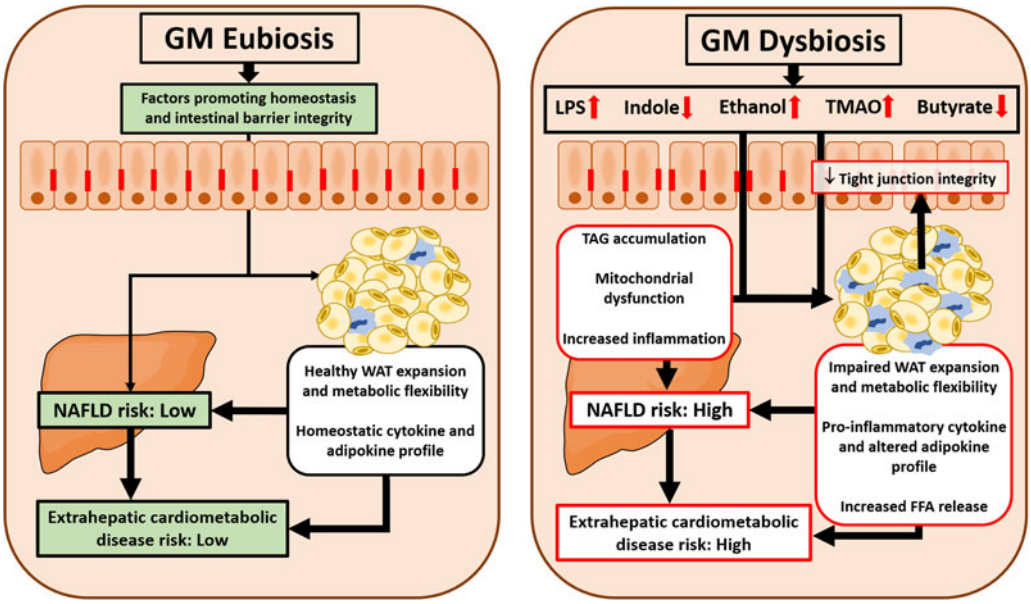

Fig. 2. NAFLD is associated with changes in gut microbiota-derived factors that can alter hepatic and WAT function Changes in GM in NAFLD result in alterations in the production of various metabolites/factors that are thought to contribute to NAFLD both directly (i.e. by directly impacting hepatic function) and indirectly through detrimentally influencing WAT function. As highlighted on the left, intestinal eubiosis and healthy gut function (such as that typically found in young individuals) promotes intestinal barrier integrity and homeostasis whilst restricting the production and dissemination of metabolically detrimental factors (such as LPS and endogenous ethanol) into circulation, the liver and WAT. Conversely, as highlighted on the right, intestinal dysbiosis (such as that often associated with older age) leads to alterations in various GM-derived factors/metabolites that impair the function of tight junction-associated proteins located within the intestinal epithelium. Consequently, these changes are thought to contribute to an increased risk of NAFLD both directly (via inducing hepatic mitochondrial function, inflammation and steatosis) and indirectly through detrimentally impacting WAT function (impairing WAT expansion, metabolic flexibility and increasing the production of pro-inflammatory cytokines). The increased production of inflammatory cytokines is thought to lead to a state of chronic low-grade inflammation which is likely to further disrupt the function of tight junction-associated proteins, thus forming a vicious cycle of worsening metabolic dysfunction and NAFLD disease severity. GM, gut microbiota; LPS, lipopolysaccharide; TMAO, trimethylamine *N*-oxide; NAFLD, non-alcoholic fatty liver disease; WAT, white adipose tissue.

liver-related death[26,163,165]. Recent meta-analysis incorporating a total of sixteen observational studies and over 34 000 individuals with a median follow-up of about 7 years concluded that NAFLD conferred an OR of 1·6 for fatal and/or non-fatal CVD events (random-effects OR of 1·6, 95 % CI 1·3, 2·1)[166]. This is consistent with findings from others that suggest that the risk of incident CVD events increases further with greater severity of NAFLD even after adjusting for other established CVD risk factors[13]. Emerging data also support the evolving notion that sex is an important modifier of NAFLD outcomes and suggest that the occurrence and prevalence of CVD-related events and mortality are likely to differ between sexes. One study found that in about 108 000 individuals with NAFLD, cardiovascular events were two times higher in women compared to men (OR 2·1, 95 % CI 1·7, 2·7)[167]. Women also had higher cardiovascular mortality with advancing age starting at age 42 years further highlighting the importance of both age and sex as important risk factors for both NAFLD and CVD[167].

Non-alcoholic fatty liver disease and chronic kidney disease

The risk of CKD is also increased in patients with NAFLD. CKD is a complex, progressive chronic condition that is defined by an abnormality in either the structure and/or function of the kidneys for ≥3 months with serious implications for health[7,168]. Evidence from three meta-analyses demonstrates a higher incidence of CKD in patients with NAFLD[169–171]. The first of these studies, which included thirty-three observational (twenty cross-sectional and thirteen longitudinal) studies concluded that NAFLD was associated with a 2-fold increased prevalence of CKD (random-effects OR 2·1, 95 % CI 1·7, 2·7) and that NAFLD was associated with a nearly 80 % increased risk of incident CKD (random-effects HR 1·8, 95 % CI 1·7, 2·0)[7,169]. Similarly, the second more recent meta-analysis confirmed that NAFLD was associated with an about 40 % increase in the long-term risk of incident CKD (random-effects HR 1·4, 95 % CI 1·2, 1·5)[170]. Most recently, findings from a large updated meta-analysis

https://doi.org/10.1017/S0029665121003815 Published online by Cambridge University Press

indicate that NAFLD was significantly associated with an about 1·45-fold increased long-term risk of incident CKD and this association was independent of age, sex and conventional CKD risk factors[171]. Interestingly, these studies also support an association between increased NAFLD severity (particularly the presence of advanced fibrosis) and increased risk of CKD[169–171]. Another large database study in Germany also supports a strong link between NAFLD and increased risk of CKD that is independent of age, sex and the presence of additional cardiometabolic risk factors such as diabetes, obesity and hypertension[172].

Non-alcoholic fatty liver disease and non-hepatic cancers

In addition to increasing the risk of HCC, recent evidence suggests that NAFLD may also increase the risk of various non-hepatic cancers. Findings from a recent large population-based cohort study concluded that, compared to healthy controls, patients with biopsy-confirmed NAFLD had significantly increased overall cancer incidence over a median 13·8 years follow-up period (adjusted HR 1·3, 95 % CI 1·2, 1·4)[173]. Whilst this increase was mostly driven by a higher HCC incidence, the presence of NAFLD was also associated with modestly increased rates of melanoma, pancreatic and kidney/bladder cancers[173]. In support of these findings, a meta-analysis of ten cohort studies (>180 000 individuals, 24·8 % with NAFLD) found that NAFLD was significantly associated with a nearly 1·5–2-fold increased risk of developing gastrointestinal cancers (oesophagus, stomach, pancreas or colorectal cancers) independently of confounding factors such as age, sex, obesity, diabetes and smoking status[174]. There is currently very limited data on the severity of NAFLD (particularly the severity of liver fibrosis) and the risk of developing extra-hepatic cancers. One recent study found that more severe NAFLD was associated with significantly increased overall mortality with most of the excess mortality observed being driven by extrahepatic cancer and liver cirrhosis[175]. Whilst it is reasonable to assume that the risk of developing extra-hepatic cancers is increased in relation to NAFLD severity, further large prospective studies are needed to confirm this link. Such studies should account for the potential modifying effect of important genetic variants, age, sex and obesity along with other NAFLD-associated comorbidities when considering the relationship between NAFLD severity and risk of specific extra-hepatic cancers. This latter consideration is particularly important since it is not yet clear whether NAFLD is associated with an increased risk of certain extra-hepatic cancers simply as a consequence of shared metabolic risk factors or whether NAFLD itself directly contributes to an increased risk of developing extrahepatic cancers[174].

Conclusions

The risk of developing NAFLD differs between sexes, changes with age and is likely to be modulated by complex interactions between genetic and environmental factors. Differences in WAT mass, its distribution (VAT *v.* SAT) and functionality (metabolic and endocrine), are likely to be key drivers of hepatic steatosis and NAFLD development. Similarly, differences in the regional distribution and function of WAT between men and women and between age groups are likely to contribute to the increased risk of NAFLD progression associated with sex and age. The development of GM dysbiosis and intestinal dysfunction is likely to contribute to NAFLD both directly and indirectly via the exacerbation of WAT inflammation and dysfunction through a range of GM-derived factors. Collectively, in the presence of chronic nutritional surplus, both WAT and intestinal dysfunction act in a synergistic manner to drive systemic metabolic dysfunction and the development of NAFLD and are further influenced by sex and age. In turn, NAFLD increases the risk of chronic hepatic and extra-hepatic metabolic diseases including T2DM, CVD, CKD, HCC and certain extra-hepatic cancers.

Acknowledgements

The authors would like to thank the National Institute for Health Research Southampton Biomedical Research Centre for their funding and support.

Financial Support

J. B., J. K. S. and C. B. are supported by the National Institution for Health Research through the NIHR Southampton Biomedical Research Centre; J. K. S. is funded by the Wellcome Trust (grant number 206453/Z/17/Z).

Conflict of Interest

None.

Authorship

The authors had sole responsibility for all aspects of preparation of this paper.

References

1. Targher G, Tilg H & Byrne CD (2021) Non-alcoholic fatty liver disease: a multisystem disease requiring a multidisciplinary and holistic approach. *Lancet Gastroenterol Hepatol* **6**, 578–588.
2. Sayiner M, Koenig A, Henry L *et al.* (2016) Epidemiology of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis in the United States and the rest of the world. *Clin Liver Dis* **20**, 205–214.
3. Mantovani A, Scorletti E, Mosca A *et al.* (2020) Complications, morbidity and mortality of nonalcoholic fatty liver disease. *Metabolism* **111s**, 154170.
4. Paik JM, Golabi P, Younossi Y *et al.* (2020) Changes in the global burden of chronic liver diseases from 2012 to

https://doi.org/10.1017/S0029665121003815 Published online by Cambridge University Press

2017: the growing impact of NAFLD. *Hepatology* **72**, 1605–1616.
5. Byrne CD & Targher G (2015) NAFLD: a multisystem disease. *J Hepatol* **62**, S47–S64.
6. Targher G, Corey KE & Byrne CD (2021) NAFLD, and cardiovascular and cardiac diseases: factors influencing risk, prediction and treatment. *Diabetes Metab* **47**, 101215.
7. Byrne CD & Targher G (2020) NAFLD as a driver of chronic kidney disease. *J Hepatol* **72**, 785–801.
8. Morrison AE, Zaccardi F, Khunti K *et al.* (2019) Causality between non-alcoholic fatty liver disease and risk of cardiovascular disease and type 2 diabetes: a meta-analysis with bias analysis. *Liver Int* **39**, 557–567.
9. Mantovani A, Byrne CD, Bonora E *et al.* (2018) Nonalcoholic fatty liver disease and risk of incident type 2 diabetes: a meta-analysis. *Diabetes Care* **41**, 372.
10. Tsochatzis EA & Newsome PN (2018) Non-alcoholic fatty liver disease and the interface between primary and secondary care. *Lancet Gastroenterol Hepatol* **3**, 509–517.
11. Ekstedt M, Hagström H, Nasr P *et al.* (2015) Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. *Hepatology* **61**, 1547–1554.
12. Angulo P, Kleiner DE, Dam-Larsen S *et al.* (2015) Liver fibrosis, but no other histologic features, is associated with long-term outcomes of patients with nonalcoholic fatty liver disease. *Gastroenterology* **149**, 389–397.e310.
13. Taylor RS, Taylor RJ, Bayliss S *et al.* (2020) Association between fibrosis stage and outcomes of patients with nonalcoholic fatty liver disease: a systematic review and meta-analysis. *Gastroenterology* **158**, 1611–1625.e1612.
14. Lonardo A, Bellentani S, Argo CK *et al.* (2015) Epidemiological modifiers of non-alcoholic fatty liver disease: focus on high-risk groups. *Dig Liver Dis* **47**, 997–1006.
15. Lonardo A, Nascimbeni F, Ballestri S *et al.* (2019) Sex differences in nonalcoholic fatty liver disease: state of the art and identification of research gaps. *Hepatology* **70**, 1457–1469.
16. Balakrishnan M, Patel P, Dunn-Valadez S *et al.* (2021) Women have a lower risk of nonalcoholic fatty liver disease but a higher risk of progression vs men: a systematic review and meta-analysis. *Clin Gastroenterol Hepatol* **19**, 61–71.e15.
17. Maggi A & Della Torre S (2018) Sex, metabolism and health. *Mol Metab* **15**, 3–7.
18. Lefebvre P & Staels B (2021) Hepatic sexual dimorphism – implications for non-alcoholic fatty liver disease. *Nature Reviews Endocrinology* **11**, 662–670.
19. Bertolotti M, Lonardo A, Mussi C *et al.* (2014) Nonalcoholic fatty liver disease and aging: epidemiology to management. *World J Gastroenterol* **20**, 14185–14204.
20. Ong JP, Pitts A & Younossi ZM (2008) Increased overall mortality and liver-related mortality in non-alcoholic fatty liver disease. *J Hepatol* **49**, 608–612.
21. Bedogni G, Miglioli L, Masutti F *et al.* (2007) Incidence and natural course of fatty liver in the general population: the Dionysos study. *Hepatology* **46**, 1387–1391.
22. Liu Y, Zhong G-C, Tan H-Y *et al.* (2019) Nonalcoholic fatty liver disease and mortality from all causes, cardiovascular disease, and cancer: a meta-analysis. *Sci Rep* **9**, 11124.
23. Younossi ZM (2019) Non-alcoholic fatty liver disease – a global public health perspective. *J Hepatol* **70**, 531–544.
24. Fan J-G, Kim S-U & Wong VW-S (2017) New trends on obesity and NAFLD in Asia. *J Hepatol* **67**, 862–873.
25. Younossi Z, Anstee QM, Marietti M *et al.* (2018) Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol* **15**, 11–20.
26. Younossi ZM, Koenig AB, Abdelatif D *et al.* (2016) Global epidemiology of nonalcoholic fatty liver disease-meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* **64**, 73–84.
27. Kelly T, Yang W, Chen CS *et al.* (2008) Global burden of obesity in 2005 and projections to 2030. *Int J Obes* **32**, 1431–1437.
28. Alberti KG, Eckel RH, Grundy SM *et al.* (2009) Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation* **120**, 1640–1645.
29. WHO (2000) Obesity: preventing and managing the global epidemic. Report of a WHO consultation. *World Health Organization technical report series* 894, i-xii, 1–253.
30. Ye Q, Zou B, Yeo YH *et al.* (2020) Global prevalence, incidence, and outcomes of non-obese or lean non-alcoholic fatty liver disease: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol* **8**, 739–752.
31. Azzu V, Vacca M, Virtue S *et al.* (2020) Adipose tissue-liver cross talk in the control of whole-body metabolism: implications in nonalcoholic fatty liver disease. *Gastroenterology* **158**, 1899–1912.
32. Kim D, Chung GE, Kwak MS *et al.* (2016) Body fat distribution and risk of incident and regressed nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* **14**, 132–138.e134.
33. Eguchi Y, Eguchi T, Mizuta T *et al.* (2006) Visceral fat accumulation and insulin resistance are important factors in nonalcoholic fatty liver disease. *J Gastroenterol* **41**, 462–469.
34. Motamed N, Khonsari MR, Rabiee B *et al.* (2017) Discriminatory ability of visceral adiposity index (VAI) in diagnosis of metabolic syndrome: a population based study. Exp *Clin Endocrinol Diabetes* **125**, 202–207.
35. Yu SJ, Kim W, Kim D *et al.* (2015) Visceral obesity predicts significant fibrosis in patients with nonalcoholic fatty liver disease. *Medicine* **94**, e2159–e2159.
36. Ohki T, Tateishi R, Shiina S *et al.* (2009) Visceral fat accumulation is an independent risk factor for hepatocellular carcinoma recurrence after curative treatment in patients with suspected NASH. *Gut* **58**, 839.
37. Jensen MD (2008) Role of body fat distribution and the metabolic complications of obesity. *J Clin Endocrinol Metab* **93**, S57–S63.
38. De Carvalho FG, Justice JN, Freitas ECD *et al.* (2019) Adipose tissue quality in aging: how structural and functional aspects of adipose tissue impact skeletal muscle quality. *Nutrients* **11**, 2553.
39. Vlassopoulos A, Combet E & Lean ME (2014) Changing distributions of body size and adiposity with age. *Int J Obes* **38**, 857–864.
40. Karastergiou K, Smith SR, Greenberg AS *et al.* (2012) Sex differences in human adipose tissues – the biology of pear shape. *Biol Sex Differ* **3**, 13.
41. Mancuso P & Bouchard B (2019) The impact of aging on adipose function and adipokine synthesis. *Front Endocrinol* **10**, 137–137.
42. Eaton SA & Sethi JK (2019) Immunometabolic links between estrogen, adipose tissue and female reproductive metabolism. *Biology* **8**, 1.

https://doi.org/10.1017/S0029665121003815 Published online by Cambridge University Press

43. Hughes VA, Roubenoff R, Wood M *et al.* (2004) Anthropometric assessment of 10-y changes in body composition in the elderly. *Am J Clin Nutr* **80**, 475–482.
44. Tchkonia T, Morbeck DE, Von Zglinicki T *et al.* (2010) Fat tissue, aging, and cellular senescence. *Aging Cell* **9**, 667–684.
45. Kuk JL, Saunders TJ, Davidson LE *et al.* (2009) Age-related changes in total and regional fat distribution. *Ageing Res Rev* **8**, 339–348.
46. Godoy-Matos AF, Silva Júnior WS & Valerio CM (2020) NAFLD as a continuum: from obesity to metabolic syndrome and diabetes. *Diabetol Metab Syndr* **12**, 60.
47. Polyzos SA, Perakakis N & Mantzoros CS (2019) Fatty liver in lipodystrophy: a review with a focus on therapeutic perspectives of adiponectin and/or leptin replacement. *Metabolism* **96**, 66–82.
48. Longo M, Zatterale F, Naderi J *et al.* (2019) Adipose tissue dysfunction as determinant of obesity-associated metabolic complications. *Int J Mol Sci* **20**, 2358.
49. Sethi JK & Vidal-Puig AJ (2007) Thematic review series: adipocyte biology. Adipose tissue function and plasticity orchestrate nutritional adaptation. *J Lipid Res* **48**, 1253–1262.
50. Cawthorn WP, Heyd F, Hegyi K *et al.* (2007) Tumour necrosis factor-alpha inhibits adipogenesis via a beta-catenin/TCF4(TCF7L2)-dependent pathway. *Cell Death Differ* **14**, 1361–1373.
51. Rutkowski JM, Stern JH & Scherer PE (2015) The cell biology of fat expansion. *J Cell Biol* **208**, 501–512.
52. Halberg N, Khan T, Trujillo ME *et al.* (2009) Hypoxia-inducible factor 1alpha induces fibrosis and insulin resistance in white adipose tissue. *Mol Cell Biol* **29**, 4467–4483.
53. Blüher M (2009) Adipose tissue dysfunction in obesity. *Exp Clin Endocrinol Diabetes* **117**, 241–250.
54. Gastaldelli A (2017) Insulin resistance and reduced metabolic flexibility: cause or consequence of NAFLD? *Clin Sci* **131**, 2701–2704.
55. Byrne CD (2013) Ectopic fat, insulin resistance and non-alcoholic fatty liver disease. *Proc Nutr Soc* **72**, 412–419.
56. Caso G, McNurlan MA, Mileva I *et al.* (2013) Peripheral fat loss and decline in adipogenesis in older humans. *Metabolism* **62**, 337–340.
57. Schipper BM, Marra KG, Zhang W *et al.* (2008) Regional anatomic and age effects on cell function of human adipose-derived stem cells. *Ann Plast Surg* **60**, 538–544.
58. Palmer AK & Kirkland JL (2016) Aging and adipose tissue: potential interventions for diabetes and regenerative medicine. *Exp Gerontol* **86**, 97–105.
59. Xu M, Palmer AK, Ding H *et al.* (2015) Targeting senescent cells enhances adipogenesis and metabolic function in old age. *eLife* **4**, e12997.
60. Grove KL, Fried SK, Greenberg AS *et al.* (2010) A microarray analysis of sexual dimorphism of adipose tissues in high-fat-diet-induced obese mice. *Int J Obes* **34**, 989–1000.
61. Macotela Y, Boucher J, Tran TT *et al.* (2009) Sex and depot differences in adipocyte insulin sensitivity and glucose metabolism. *Diabetes* **58**, 803–812.
62. Phillips GB, Jing T & Heymsfield SB (2008) Does insulin resistance, visceral adiposity, or a sex hormone alteration underlie the metabolic syndrome? Studies in women. *Metabolism* **57**, 838–844.
63. Lovejoy JC, Champagne CM, de Jonge L *et al.* (2008) Increased visceral fat and decreased energy expenditure during the menopausal transition. *Int J Obes* **32**, 949–958.
64. Abdulnour J, Doucet E, Brochu M *et al.* (2012) The effect of the menopausal transition on body composition and cardiometabolic risk factors: a Montreal-Ottawa new emerging team group study. *Menopause* **19**, 760–767.
65. Leeners B, Geary N, Tobler PN *et al.* (2017) Ovarian hormones and obesity. *Hum Reprod Update* **23**, 300–321.
66. DiStefano JK (2020) NAFLD and NASH in postmenopausal women: implications for diagnosis and treatment. *Endocrinology* **161**.
67. Babaei P, Mehdizadeh R, Ansar MM *et al.* (2010) Effects of ovariectomy and estrogen replacement therapy on visceral adipose tissue and serum adiponectin levels in rats. *Menopause Int* **16**, 100–104.
68. Rogers NH, Perfield JW, III, Strissel KJ *et al.* (2009) Reduced energy expenditure and increased inflammation are early events in the development of ovariectomy-induced obesity. *Endocrinology* **150**, 2161–2168.
69. Lizcano F & Guzmán G (2014) Estrogen deficiency and the origin of obesity during menopause. *Biomed Res Int* **2014**, 757461.
70. Cooke PS & Naaz A (2004) Role of estrogens in adipocyte development and function. *Exp Biol Med* **229**, 1127–1135.
71. Frank AP, de Souza Santos R, Palmer BF *et al.* (2019) Determinants of body fat distribution in humans may provide insight about obesity-related health risks. *J Lipid Res* **60**, 1710–1719.
72. Funcke JB & Scherer PE (2019) Beyond adiponectin and leptin: adipose tissue-derived mediators of inter-organ communication. *J Lipid Res* **10**, 1648–1684.
73. Montague CT, Farooqi IS, Whitehead JP *et al.* (1997) Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature* **387**, 903–908.
74. Polyzos SA, Aronis KN, Kountouras J *et al.* (2016) Circulating leptin in non-alcoholic fatty liver disease: a systematic review and meta-analysis. *Diabetologia* **59**, 30–43.
75. Polyzos SA, Kountouras J & Mantzoros CS (2015) Leptin in nonalcoholic fatty liver disease: a narrative review. *Metabolism* **64**, 60–78.
76. Jiménez-Cortegana C, García-Galey A, Tami M *et al.* (2021) Role of leptin in non-alcoholic fatty liver disease. *Biomedicines* **9**, 762.
77. Polyzos SA, Kountouras J, Zavos C *et al.* (2011) The potential adverse role of leptin resistance in nonalcoholic fatty liver disease: a hypothesis based on critical review of the literature. *J Clin Gastroenterol* **45**, 50–54.
78. Saad MF, Damani S, Gingerich RL *et al.* (1997) Sexual dimorphism in plasma leptin concentration. *J Clin Endocrinol Metab* **82**, 579–584.
79. Castracane VD, Kraemer RR, Franken MA *et al.* (1998) Serum leptin concentration in women: effect of age, obesity, and estrogen administration. *Fertil Steril* **70**, 472–477.
80. Isidori AM, Strollo F, Morè M *et al.* (2000) Leptin and aging: correlation with endocrine changes in male and female healthy adult populations of different body weights. *J Clin Endocrinol Metab* **85**, 1954–1962.
81. Boutari C & Mantzoros CS (2020) Adiponectin and leptin in the diagnosis and therapy of NAFLD. *Metabolism* **103**, 154028.
82. Maeda N, Shimomura I, Kishida K *et al.* (2002) Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. *Nat Med* **8**, 731–737.
83. Ouchi N, Kihara S, Arita Y *et al.* (1999) Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. *Circulation* **100**, 2473–2476.
84. Tilg H & Hotamisligil GS (2006) Nonalcoholic fatty liver disease: cytokine-adipokine interplay and regulation of insulin resistance. *Gastroenterology* **131**, 934–945.

https://doi.org/10.1017/S0029665121003815 Published online by Cambridge University Press

85. Yamauchi T, Kamon J, Minokoshi Y *et al.* (2002) Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med* **8**, 1288–1295.
86. Polyzos SA, Toulis KA, Goulis DG *et al.* (2011) Serum total adiponectin in nonalcoholic fatty liver disease: a systematic review and meta-analysis. *Metabolism* **60**, 313–326.
87. Sohara N, Takagi H, Kakizaki S *et al.* (2005) Elevated plasma adiponectin concentrations in patients with liver cirrhosis correlate with plasma insulin levels. *Liver Intr* **25**, 28–32.
88. Tietge UJ, Böker KH, Manns MP *et al.* (2004) Elevated circulating adiponectin levels in liver cirrhosis are associated with reduced liver function and altered hepatic hemodynamics. *Am. J Physiol Endocrinol Metab* **287**, E82–E89.
89. Polyzos SA, Kountouras J & Zavos C (2010) Nonlinear distribution of adiponectin in patients with nonalcoholic fatty liver disease limits its use in linear regression analysis. *J Clin Gastroenterol* **44**, 229–230; author reply 230–221.
90. Buechler C, Wanninger J & Neumeier M (2011) Adiponectin, a key adipokine in obesity related liver diseases. *World J Gastroenterol* **17**, 2801–2811.
91. Polyzos SA, Kountouras J, Zavos C *et al.* (2010) The role of adiponectin in the pathogenesis and treatment of nonalcoholic fatty liver disease. Diabetes, *Obes Metab* **12**, 365–383.
92. Adachi M & Brenner DA (2008) High molecular weight adiponectin inhibits proliferation of hepatic stellate cells via activation of adenosine monophosphate-activated protein kinase. *Hepatology* **47**, 677–685.
93. Kizer JR, Arnold AM, Strotmeyer ES *et al.* (2010) Change in circulating adiponectin in advanced old age: determinants and impact on physical function and mortality. The cardiovascular health study all stars study. *J Gerontol* A, *Biol Sci Med Sci* **65**, 1208–1214.
94. Adamczak M, Rzepka E, Chudek J *et al.* (2005) Ageing and plasma adiponectin concentration in apparently healthy males and females. *Clin Endocrinol* **62**, 114–118.
95. Reilly SM & Saltiel AR (2017) Adapting to obesity with adipose tissue inflammation. *Nat Rev Endocrinol* **13**, 633–643.
96. Mohamed-Ali V, Flower L, Sethi J *et al.* (2001) beta-Adrenergic regulation of IL-6 release from adipose tissue: in vivo and in vitro studies. *J Clin Endocrinol Metab* **86**, 5864–5869.
97. Cawthorn WP & Sethi JK (2008) TNF-alpha and adipocyte biology. *FEBS Lett* **582**, 117–131.
98. Sethi JK & Hotamisiligil GS (2021) Metabolic messengers: tumour necrosis factor. *Nat Metab* **3**, 1302–1312.
99. Hotamisligil GS, Arner P, Caro JF *et al.* (1995) Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *J Clin Invest* **95**, 2409–2415.
100. Hotamisligil G, Shargill N & Spiegelman B (1993) Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Sci* **259**, 87–91.
101. Ota T (2013) Chemokine systems link obesity to insulin resistance. *Diabetes Metab J* **37**, 165–172.
102. Duval C, Thissen U, Keshtkar S *et al.* (2010) Adipose tissue dysfunction signals progression of hepatic steatosis towards nonalcoholic steatohepatitis in C57Bl/6 mice. *Diabetes* **59**, 3181–3191.
103. Cao H, Gerhold K, Mayers JR *et al.* (2008) Identification of a lipokine, a lipid hormone linking adipose tissue to systemic metabolism. *Cell* **134**, 933–944.
104. Souza CO, Teixeira AAS, Biondo LA *et al.* (2020) Palmitoleic acid reduces high fat diet-induced liver inflammation by promoting PPAR-γ-independent M2a polarization of myeloid cells. *Biochim Biophys Acta Mol Cell Biol Lipids* **1865**, 158776.
105. Item F & Konrad D (2012) Visceral fat and metabolic inflammation: the portal theory revisited. *Obes Rev* **13** (Suppl. 2), 30–39.
106. Kabir M, Catalano KJ, Ananthnarayan S *et al.* (2005) Molecular evidence supporting the portal theory: a causative link between visceral adiposity and hepatic insulin resistance. *Am J Physiol Endocrinol Metab* **288**, E454–E461.
107. Lundgren P & Thaiss CA (2020) The microbiome-adipose tissue axis in systemic metabolism. *Am J Physiol-Gastrointest Liver Physiol* **318**, G717–G724.
108. Valdes AM, Walter J, Segal E *et al.* (2018) Role of the gut microbiota in nutrition and health. *Br Med J* **361**, k2179.
109. Zmora N, Suez J & Elinav E (2019) You are what you eat: diet, health and the gut microbiota. *Nat Rev Gastroenterol Hepat* **16**, 35–56.
110. Hu H, Lin A, Kong M *et al.* (2020) Intestinal microbiome and NAFLD: molecular insights and therapeutic perspectives. *J Gastroenterol* **55**, 142–158.
111. Camarillo-Guerrero LF, Almeida A, Rangel-Pineros G *et al.* (2021) Massive expansion of human gut bacteriophage diversity. *Cell* **184**, 1098–1109.e1099.
112. Canfora EE, Meex RCR, Venema K *et al.* (2019) Gut microbial metabolites in obesity, NAFLD and T2DM. *Nat Rev Endocrinol* **15**, 261–273.
113. Aron-Wisnewsky J, Vigliotti C, Witjes J *et al.* (2020) Gut microbiota and human NAFLD: disentangling microbial signatures from metabolic disorders. *Nat Rev Gastroenterol Hepat* **17**, 279–297.
114. Jiang X, Zheng J, Zhang S *et al.* (2020) Advances in the involvement of gut microbiota in pathophysiology of NAFLD. *Front Med* **7**.
115. Le Roy T, Llopis M, Lepage P *et al.* (2013) Intestinal microbiota determines development of non-alcoholic fatty liver disease in mice. *Gut* **62**, 1787–1794.
116. Bäckhed F, Ley RE, Sonnenburg JL *et al.* (2005) Host-bacterial mutualism in the human intestine. *Science* **307**, 1915–1920.
117. Mouzaki M, Comelli EM, Arendt BM *et al.* (2013) Intestinal microbiota in patients with nonalcoholic fatty liver disease. *Hepatology* **58**, 120–127.
118. Boursier J, Mueller O, Barret M *et al.* (2016) The severity of nonalcoholic fatty liver disease is associated with gut dysbiosis and shift in the metabolic function of the gut microbiota. *Hepatology* **63**, 764–775.
119. Loomba R, Seguritan V, Li W *et al.* (2017) Gut microbiome-based metagenomic signature for non-invasive detection of advanced fibrosis in human nonalcoholic fatty liver disease. *Cell Metab* **25**, 1054–1062. e1055.
120. Caussy C, Tripathi A, Humphrey G *et al.* (2019) A gut microbiome signature for cirrhosis due to nonalcoholic fatty liver disease. *Nat Commun* **10**, 1406.
121. Oh TG, Kim SM, Caussy C *et al.* (2020) A universal gut-microbiome-derived signature predicts cirrhosis. *Cell Metab* **32**, 878–888.
122. Li K, Peng W, Zhou Y *et al.* (2020) Host genetic and environmental factors shape the composition and function

https://doi.org/10.1017/S0029665121003815 Published online by Cambridge University Press

of gut microbiota in populations living at high altitude. *Biomed Res Int* **2020**, 1482109.
123. Miele L, Valenza V, La Torre G *et al.* (2009) Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. *Hepatology* **49**, 1877–1887.
124. Luther J, Garber JJ, Khalili H *et al.* (2015) Hepatic injury in nonalcoholic steatohepatitis contributes to altered intestinal permeability. *Cell Mol Gastroenterol Hepatol* **1**, 222–232.
125. Kolodziejczyk AA, Zheng D, Shibolet O *et al.* (2019) The role of the microbiome in NAFLD and NASH. *EMBO Mol Med* **11**, e9302.
126. Ridlon JM, Kang DJ, Hylemon PB *et al.* (2014) Bile acids and the gut microbiome. *Curr Opin Gastroenterol* **30**, 332–338.
127. Bäckhed F, Manchester JK, Semenkovich CF *et al.* (2007) Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. *Proc Natl Acad Sci USA* **104**, 979–984.
128. Bäckhed F, Ding H, Wang T *et al.* (2004) The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci USA* **101**, 15718–15723.
129. Rau M, Rehman A, Dittrich M *et al.* (2018) Fecal SCFAs and SCFA-producing bacteria in gut microbiome of human NAFLD as a putative link to systemic T-cell activation and advanced disease. *United European Gastroenterol J* **6**, 1496–1507.
130. Zhou D, Pan Q, Xin F-Z *et al.* (2017) Sodium butyrate attenuates high-fat diet-induced steatohepatitis in mice by improving gut microbiota and gastrointestinal barrier. *World J Gastroenterol* **23**, 60–75.
131. Cani PD, Amar J, Iglesias MA *et al.* (2007) Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* **56**, 1761–1772.
132. Ferslew BC, Xie G, Johnston CK *et al.* (2015) Altered bile acid metabolome in patients with nonalcoholic steatohepatitis. *Dig Dis Sci* **60**, 3318–3328.
133. Zhu L, Baker SS, Gill C *et al.* (2013) Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: a connection between endogenous alcohol and NASH. *Hepatology* **57**, 601–609.
134. Grabherr F, Grander C, Effenberger M *et al.* (2019) Gut dysfunction and non-alcoholic fatty liver disease. *Front Endocrinol* **10**, 611–611.
135. Mells JE & Anania FA (2013) The role of gastrointestinal hormones in hepatic lipid metabolism. *Semin Liver Dis* **33**, 343–357.
136. Mok JKW, Makaronidis JM & Batterham RL (2019) The role of gut hormones in obesity. *Curr Opin Endocr Metab Res* **4**, 4–13.
137. Koukias N, Buzzetti E & Tsochatzis EA (2017) Intestinal hormones, gut microbiota and non-alcoholic fatty liver disease. *Minerva Endocrinol* **42**, 184–194.
138. Durkin LA, Childs CE & Calder PC (2021) Omega-3 polyunsaturated fatty acids and the intestinal epithelium – a review. *Foods* **10**, 1.
139. Harte AL, da Silva NF, Creely SJ *et al.* (2010) Elevated endotoxin levels in non-alcoholic fatty liver disease. *J Inflamm* **7**, 15–15.
140. Kitabatake H, Tanaka N, Fujimori N *et al.* (2017) Association between endotoxemia and histological features of nonalcoholic fatty liver disease. *World J Gastroenterol* **23**, 712–722.
141. Clemente-Postigo M, Oliva-Olivera W, Coin-Aragüez L *et al.* (2019) Metabolic endotoxemia promotes adipose dysfunction and inflammation in human obesity. *Am J Physiol-Endocrinol Metab* **316**, E319–E332.
142. Chang C-C, Sia K-C, Chang J-F *et al.* (2019) Lipopolysaccharide promoted proliferation and adipogenesis of preadipocytes through JAK/STAT and AMPK-regulated cPLA2 expression. *Int J Med Sci* **16**, 167–179.
143. Kim YS, Unno T, Kim BY *et al.* (2020) Sex differences in gut microbiota. *World J Mens Health* **38**, 48–60.
144. Valeri F & Endres K (2021) How biological sex of the host shapes its gut microbiota. *Front Neuroendocrinol* **61**, 100912.
145. Sheng L, Jena PK, Liu HX *et al.* (2017) Gender differences in bile acids and microbiota in relationship with gender dissimilarity in steatosis induced by diet and FXR inactivation. *Sci Rep* **7**, 1748.
146. Rea MC, O'Sullivan O, Shanahan F *et al.* (2012) Clostridium difficile carriage in elderly subjects and associated changes in the intestinal microbiota. *J Clin Microbiol* **50**, 867–875.
147. O'Toole PW & Jeffery IB (2015) Gut microbiota and aging. *Science* **350**, 1214–1215.
148. Nagpal R, Mainali R, Ahmadi S *et al.* (2018) Gut microbiome and aging: physiological and mechanistic insights. *Nutr Healthy Aging* **4**, 267–285.
149. Tran L & Greenwood-Van Meerveld B (2013) Age-associated remodeling of the intestinal epithelial barrier. *J Gerontol A* **68**, 1045–1056.
150. Man AL, Bertelli E, Rentini S *et al.* (2015) Age-associated modifications of intestinal permeability and innate immunity in human small intestine. *Clin Sci* **129**, 515–527.
151. Dai W, Ye L, Liu A *et al.* (2017) Prevalence of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus: a meta-analysis. *Medicine* **96**, e8179–e8179.
152. Cariou B, Byrne CD, Loomba R *et al.* (2021) Nonalcoholic fatty liver disease as a metabolic disease in humans: a literature review. *Diabetes Obes Metab* **23**, 1069–1083.
153. Bilson J, Scorletti E, Bindels LB *et al.* (2021) Growth differentiation factor-15 and the association between type 2 diabetes and liver fibrosis in NAFLD. *Nutr Diabetes* **11**.
154. Younossi ZM, Golabi P, de Avila L *et al.* (2019) The global epidemiology of NAFLD and NASH in patients with type 2 diabetes: a systematic review and meta-analysis. *J Hepatol* **71**, 793–801.
155. Targher G, Corey KE, Byrne CD *et al.* (2021) The complex link between NAFLD and type 2 diabetes mellitus – mechanisms and treatments. *Nat Rev Gastroenterol Hepat* **18**, 599–612.
156. Jarvis H, Craig D, Barker R *et al.* (2020) Metabolic risk factors and incident advanced liver disease in non-alcoholic fatty liver disease (NAFLD): a systematic review and meta-analysis of population-based observational studies. *PLoS Med* **17**, e1003100.
157. Kanwar P, Nelson JE, Yates K *et al.* (2016) Association between metabolic syndrome and liver histology among NAFLD patients without diabetes. *BMJ Open Gastroenterol* **3**, e000114.
158. Kleiner DE, Brunt EM, Wilson LA *et al.* (2019) Association of histologic disease activity with progression of nonalcoholic fatty liver disease. *JAMA Network Open* **2**, e1912565.
159. Hsiao PJ, Kuo KK, Shin SJ *et al.* (2007) Significant correlations between severe fatty liver and risk factors for metabolic syndrome. *J Gastroenterol Hepatol* **22**, 2118–2123.
160. Mantovani A, Petracca G, Beatrice G *et al.* (2020) Nonalcoholic fatty liver disease and risk of incident diabetes mellitus: an updated meta-analysis of 501 022 adult individuals. *Gut* **70**, 962–969.

https://doi.org/10.1017/S0029665121003815 Published online by Cambridge University Press

161. Li Y, Wang J, Tang Y *et al.* (2017) Bidirectional association between nonalcoholic fatty liver disease and type 2 diabetes in Chinese population: evidence from the Dongfeng-Tongji cohort study. *PLoS ONE* **12**, e0174291.
162. Ballestri S, Zona S, Targher G *et al.* (2016) Nonalcoholic fatty liver disease is associated with an almost twofold increased risk of incident type 2 diabetes and metabolic syndrome. Evidence from a systematic review and meta-analysis. *J Gastroenterol Hepatol* **31**, 936–944.
163. Targher G, Byrne CD & Tilg H (2020) NAFLD and increased risk of cardiovascular disease: clinical associations, pathophysiological mechanisms and pharmacological implications. *Gut* **69**, 1691–1705.
164. Paik JM, Henry L, De Avila L *et al.* (2019) Mortality related to nonalcoholic fatty liver disease is increasing in the United States. *Hepatol Commun* **3**, 1459–1471.
165. Przybyszewski EM, Targher G, Roden M *et al.* (2021) Nonalcoholic fatty liver disease and cardiovascular disease. *Clin Liver Dis* **17**, 19–22.
166. Targher G, Byrne CD, Lonardo A *et al.* (2016) Non-alcoholic fatty liver disease and risk of incident cardiovascular disease: a meta-analysis. *J Hepatol* **65**, 589–600.
167. Khalid YS, Dasu NR, Suga H *et al.* (2020) Increased cardiovascular events and mortality in females with NAFLD: a meta-analysis. *Am J Cardiovasc Dis* **10**, 258–271.
168. Stevens PE & Levin A (2013) Evaluation and management of chronic kidney disease: synopsis of the kidney disease: improving global outcomes 2012 clinical practice guideline. *Ann Intern Med* **158**, 825–830.
169. Musso G, Gambino R, Tabibian JH *et al.* (2014) Association of non-alcoholic fatty liver disease with chronic kidney disease: a systematic review and meta-analysis. *PLoS Med* **11**, e1001680.
170. Mantovani A, Zaza G, Byrne CD *et al.* (2018) Nonalcoholic fatty liver disease increases risk of incident chronic kidney disease: a systematic review and meta-analysis. *Metabolism* **79**, 64–76.
171. Mantovani A, Petracca G, Beatrice G *et al.* (2020) Non-alcoholic fatty liver disease and risk of incident chronic kidney disease: an updated meta-analysis. *Gut* **71**, 156–162.
172. Kaps L, Labenz C, Galle PR *et al.* (2020) Non-alcoholic fatty liver disease increases the risk of incident chronic kidney disease. *United European Gastroenterol J* **8**, 942–948.
173. Simon TG, Roelstraete B, Sharma R *et al.* (2021) Cancer risk in patients with biopsy-confirmed nonalcoholic fatty liver disease: a population-based cohort study. *Hepatology* **74**, 2410–2423.
174. Mantovani A, Petracca G, Beatrice G *et al.* (2021) Non-alcoholic fatty liver disease and increased risk of incident extrahepatic cancers: a meta-analysis of observational cohort studies. *Gut*, Epub ahead of print.
175. Simon TG, Roelstraete B, Khalili H *et al.* (2021) Mortality in biopsy-confirmed nonalcoholic fatty liver disease: results from a nationwide cohort. *Gut* **70**, 1375.
176. Cao Q, Mak KM & Lieber CS (2007) Leptin represses matrix metalloproteinase-1 gene expression in LX2 human hepatic stellate cells. *J Hepatol* **46**, 124–133.
177. Cao Q, Mak KM, Ren C *et al.* (2004) Leptin stimulates tissue inhibitor of metalloproteinase-1 in human hepatic stellate cells: respective roles of the JAK/STAT and JAK-mediated H2O2-dependant MAPK pathways. *J Biol Chem* **279**, 4292–4304.
178. Polyzos SA, Kountouras J, Zavos C *et al.* (2010) The role of adiponectin in the pathogenesis and treatment of non-alcoholic fatty liver disease. *Diabetes Obes Metab* **12**, 365–383.
179. Baranova A, Gowder SJ, Schlauch K *et al.* (2006) Gene expression of leptin, resistin, and adiponectin in the white adipose tissue of obese patients with non-alcoholic fatty liver disease and insulin resistance. *Obes Surg* **16**, 1118–1125.
180. Musso G, Gambino R, Durazzo M *et al.* (2005) Adipokines in NASH: postprandial lipid metabolism as a link between adiponectin and liver disease. *Hepatology* **42**, 1175–1183.
181. Wong VW, Hui AY, Tsang SW *et al.* (2006) Metabolic and adipokine profile of Chinese patients with non-alcoholic fatty liver disease. *Clin Gastroenterol Hepatol* **4**, 1154–1161.
182. Ajmera V, Perito ER, Bass NM *et al.* (2017) Novel plasma biomarkers associated with liver disease severity in adults with nonalcoholic fatty liver disease. *Hepatology* **65**, 65–77.
183. Jamali R, Razavizade M, Arj A *et al.* (2016) Serum adipokines might predict liver histology findings in non-alcoholic fatty liver disease. *World J Gastroenterol* **22**, 5096–5103.
184. Dendi VSR, Aloor S, Runkana A *et al.* (2015) Elevated serum resistin in non-alcoholic fatty liver disease and steatohepatitis: a meta-analysis: 2149. *Am Coll Gastroenterol* **110**, S896.
185. Silswal N, Singh AK, Aruna B *et al.* (2005) Human resistin stimulates the pro-inflammatory cytokines TNF-alpha and IL-12 in macrophages by NF-kappaB-dependent pathway. *Biochem Biophys Res Commun* **334**, 1092–1101.
186. Chen X, Shen T, Li Q *et al.* (2017) Retinol binding protein-4 levels and non-alcoholic fatty liver disease: a community-based cross-sectional study. *Sci Rep* **7**, 45100.
187. Wang X, Chen X, Zhang H *et al.* (2020) Circulating retinol-binding protein 4 is associated with the development and regression of non-alcoholic fatty liver disease. *Diabetes Metab* **46**, 119–128.
188. Liu Y, Mu D, Chen H *et al.* (2016) Retinol-binding protein 4 induces hepatic mitochondrial dysfunction and promotes hepatic steatosis. *J Clin Endocrinol Metab* **101**, 4338–4348.
189. Xia M, Liu Y, Guo H *et al.* (2013) Retinol binding protein 4 stimulates hepatic sterol regulatory element-binding protein 1 and increases lipogenesis through the peroxisome proliferator-activated receptor-γ coactivator 1β-dependent pathway. *Hepatology* **58**, 564–575.
190. Zhang J, Li K, Pan L *et al.* (2021) Association of circulating adipsin with nonalcoholic fatty liver disease in obese adults: a cross-sectional study. *BMC Gastroenterol* **21**, 131.
191. Yilmaz Y, Yonal O, Kurt R *et al.* (2011) Serum levels of omentin, chemerin and adipsin in patients with biopsy-proven nonalcoholic fatty liver disease. *Scand J Gastroenterol* **46**, 91–97.
192. Qiu Y, Wang SF, Yu C *et al.* (2019) Association of circulating adipsin, visfatin, and adiponectin with nonalcoholic fatty liver disease in adults: a case-control study. *Ann Nutr Metab* **74**, 44–52.
193. Lo JC, Ljubicic S, Leibiger B *et al.* (2014) Adipsin is an adipokine that improves β cell function in diabetes. *Cell* **158**, 41–53.
194. Kukla M, Zwirska-Korczala K, Hartleb M *et al.* (2010) Serum chemerin and vaspin in non-alcoholic fatty liver disease. *Scand J Gastroenterol* **45**, 235–242.
195. Bekaert M, Verhelst X, Geerts A *et al.* (2016) Association of recently described adipokines with liver histology in

https://doi.org/10.1017/S0029665121003815 Published online by Cambridge University Press

biopsy-proven non-alcoholic fatty liver disease: a systematic review. *Obes Rev* **17**, 68–80.
196. Cash JL, Hart R, Russ A *et al.* (2008) Synthetic chemerin-derived peptides suppress inflammation through ChemR23. *J Exp Med* **205**, 767–775.
197. Aktas B, Yilmaz Y, Eren F *et al.* (2011) Serum levels of vaspin, obestatin, and apelin-36 in patients with non-alcoholic fatty liver disease. *Metabolism* **60**, 544–549.
198. Montazerifar F, Bakhshipour AR, Karajibani M *et al.* (2017) Serum omentin-1, vaspin, and apelin levels and central obesity in patients with nonalcoholic fatty liver disease. *J Res Med Sci* **22**, 70.
199. Wang Y, Song J, Bian H *et al.* (2019) Apelin promotes hepatic fibrosis through ERK signaling in LX-2 cells. *Mol Cell Biochem* **460**, 205–215.
200. Lv S-Y, Cui B, Chen W-D *et al.* (2017) Apelin/APJ system: a key therapeutic target for liver disease. *Oncotarget* **8**, 112145–112151.
201. Kleiner DE, Brunt EM, Van Natta M *et al.* (2005) Design and validation of a histological scoring system for non-alcoholic fatty liver disease. *Hepatology* **41**, 1313–1321.
202. Eddowes PJ, Sasso M, Allison M *et al.* (2019) Accuracy of FibroScan controlled attenuation parameter and liver stiffness measurement in assessing steatosis and fibrosis in patients with nonalcoholic fatty liver disease. *Gastroenterology* **156**, 1717–1730.
203. Carpino G, Del Ben M, Pastori D *et al.* (2020) Increased liver localization of lipopolysaccharides in human and experimental NAFLD. *Hepatology* **72**, 470–485.
204. Song MJ, Kim KH, Yoon JM *et al.* (2006) Activation of toll-like receptor 4 is associated with insulin resistance in adipocytes. *Biochem Biophys Res Commun* **346**, 739–745.
205. Mehta NN, McGillicuddy FC, Anderson PD *et al.* (2010) Experimental endotoxemia induces adipose inflammation and insulin resistance in humans. *Diabetes* **59**, 172.
206. Douhara A, Moriya K, Yoshiji H *et al.* (2015) Reduction of endotoxin attenuates liver fibrosis through suppression of hepatic stellate cell activation and remission of intestinal permeability in a rat non-alcoholic steatohepatitis model. *Mol Med Rep* **11**, 1693–1700.
207. Volynets V, Küper MA, Strahl S *et al.* (2012) Nutrition, intestinal permeability, and blood ethanol levels are altered in patients with nonalcoholic fatty liver disease (NAFLD). *Dig Dis Sci* **57**, 1932–1941.
208. Kema VH, Mojerla NR, Khan I *et al.* (2015) Effect of alcohol on adipose tissue: a review on ethanol mediated adipose tissue injury. *Adipocyte* **4**, 225–231.
209. Chen X, Zhang Z, Li H *et al.* (2020) Endogenous ethanol produced by intestinal bacteria induces mitochondrial dysfunction in non-alcoholic fatty liver disease. *J Gastroenterol Hepatol* **35**, 2009–2019.
210. Chen J & Vitetta L (2020) Gut Microbiota metabolites in NAFLD pathogenesis and therapeutic implications. *Int J Mol Sci* **21**, 5214.
211. Wang X, He G, Peng Y *et al.* (2015) Sodium butyrate alleviates adipocyte inflammation by inhibiting NLRP3 pathway. *Sci Rep* **5**, 12676.
212. Ohira H, Fujioka Y, Katagiri C *et al.* (2013) Butyrate attenuates inflammation and lipolysis generated by the interaction of adipocytes and macrophages. *J Atheroscler Thromb* **20**, 425–442.
213. Mollica MP, Mattace Raso G, Cavaliere G *et al.* (2017) Butyrate regulates liver mitochondrial function, efficiency, and dynamics in insulin-resistant obese mice. *Diabetes* **66**, 1405–1418.
214. Zhou D, Chen Y-W, Zhao Z-H *et al.* (2018) Sodium butyrate reduces high-fat diet-induced non-alcoholic steatohepatitis through upregulation of hepatic GLP-1R expression. *Exp Mol Med* **50**, 1–12.
215. Chen Y-M, Liu Y, Zhou R-F *et al.* (2016) Associations of gut-flora-dependent metabolite trimethylamine-N-oxide, betaine and choline with non-alcoholic fatty liver disease in adults. *Sci Rep* **6**, 19076.
216. León-Mimila P, Villamil-Ramírez H, Li XS *et al.* (2021) Trimethylamine N-oxide levels are associated with NASH in obese subjects with type 2 diabetes. *Diabetes Metab* **47**, 101183.
217. Gao X, Liu X, Xu J *et al.* (2014) Dietary trimethylamine N-oxide exacerbates impaired glucose tolerance in mice fed a high fat diet. *J Biosci Bioeng* **118**, 476–481.
218. Gao X, Xu J, Jiang C *et al.* (2015) Fish oil ameliorates trimethylamine N-oxide-exacerbated glucose intolerance in high-fat diet-fed mice. *Food Funct* **6**, 1117–1125.
219. Tan X, Liu Y, Long J *et al.* (2019) Trimethylamine N-oxide aggravates liver steatosis through modulation of bile acid metabolism and inhibition of farnesoid X receptor signaling in nonalcoholic fatty liver disease. *Mol Nutr Food Res* **63**, e1900257.
220. Ma L, Li H, Hu J *et al.* (2020) Indole alleviates diet-induced hepatic steatosis and inflammation in a manner involving myeloid cell 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3. *Hepatology* **72**, 1191–1203.
221. Virtue AT, McCright SJ, Wright JM *et al.* (2019) The gut microbiota regulates white adipose tissue inflammation and obesity via a family of microRNAs. *Sci Transl Med* **11**, eaav1892.

https://doi.org/10.1017/S0029665121003815 Published online by Cambridge University Press