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Hepatic fatty acid synthesis and partitioning: the effect of metabolic and nutritional state

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When we consume dietary fat, a series of complex metabolic processes ensures that fatty acids are absorbed, transported around the body and used/stored appropriately. The liver is a central metabolic organ within the human body and has a major role in regulating fat and carbohydrate metabolism. Studying hepatic metabolism in human subjects is challenging; the use of stable isotope tracers and measurement of particles or molecules secreted by the liver such as VLDL-TAG and 3-hydroxybutyrate offers the best insight into postprandial hepatic fatty acid metabolism in human subjects. Diet derived fatty acids are taken up by the liver and mix with fatty acids coming from the lipolysis of adipose tissue, and those already present in the liver (cytosolic TAG) and fatty acids synthesised *de novo* within the liver from non-lipid precursors (known as *de novo* lipogenesis). Fatty acids are removed from the liver by secretion as VLDL-TAG and oxidation. Perturbations in these processes have the potential to impact on metabolic health. Whether fatty acids are partitioned towards oxidation or esterification pathways appears to be dependent on a number of metabolic factors; not least ambient insulin concentrations. Moreover, along with the phenotype and lifestyle factors (e.g. habitual diet) of an individual, it is becoming apparent that the composition of the diet (macronutrient and fatty acid composition) may play pivotal roles in determining if intra-hepatic fat accumulates, although what remains to be elucidated is the influence these nutrients have on intra-hepatic fatty acid synthesis and partitioning.

Liver: VLDL-TAG: Fatty acid partitioning: Fatty acid oxidation

The liver has a major role in regulating metabolic homeostasis; it is a central cross-road for fatty acid and glucose metabolism. It serves as an intermediary organ between dietary (exogenous) and endogenous energy sources and other extra-hepatic organs/tissues that consume energy; perturbations in its metabolism have the potential to impact widely on metabolic disease risk. In health, the liver rapidly adapts to alterations in nutritional state and the nutrient fluxes that occur from a fasted (postabsorptive) to fed (postprandial) state. However, the deposition of fat (ectopic fat) in non-adipose tissues such as the liver has been suggested to be an important factor in the development of obesity related metabolic abnormalities[1,2]. The net retention of intra-hepatic fat is a prerequisite for the development of non-alcoholic fatty liver disease (NAFLD)[3], which is now recognised as the hepatic manifestation of the metabolic syndrome[2]. A well-recognised risk factor for NAFLD is obesity, and both are risk factors for more severe metabolic diseases such as type-2 diabetes and CVD; the prevalence of NAFLD parallels that of type-2 diabetes[4]. Why the liver starts to accumulate fat is not well understood but is likely to occur when fatty acid input and synthesis exceed the liver's capacity for removal (e.g. for secretion or oxidation)[5–7]. The intra-hepatic fatty acid pool represents fatty acids from multiple sources, including NEFA that are derived from hydrolysis of peripheral or visceral adipose tissue,

Abbreviations: DNL, *de novo* lipogenesis; NAFLD, non-alcoholic fatty liver disease; 3-OHB, 3-hydroxybutyrate; TE, total energy.

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hepatic fatty acids that are produced by *de novo* lipogenesis (DNL) or stored TAG in the cytosol of hepatocytes, and in the postprandial period, this will also include dietary fatty acids (Fig. 1a). The delivery of fatty acids to the liver and synthesis and partitioning (into esterification or oxidation pathways) within the liver appear to be dependent on a number of metabolic factors; not least ambient and postprandial insulin concentrations. The aim of the present paper is to summarise the findings from *in vivo* human metabolic studies performed by ourselves and others that have helped to understand the way in which fatty acids are synthesised and partitioned within the human liver.

Studying *in vivo* human hepatic metabolism

The liver by virtue of its anatomical position makes studying hepatic processes directly *in vivo* in human subjects challenging. Direct assessment of the liver can only be achieved by arterio-venous difference measurements, which is impractical in human subjects due to the inaccessibility of the portal vein. However, surrogate markers, such as VLDL-TAG and the ketone body, 3-hydroxybutyrate (3-OHB) which are produced and secreted by the liver offer the opportunity to gain insight into hepatic processes such as hepatic fatty acid esterification and oxidation pathways[7]. It has been suggested that the TAG composition in VLDL reflects that of liver TAG[8,9] although differentiating the contribution of different fatty acid sources (dietary, adipose tissue derived and DNL derived) cannot be determined. The use of metabolic tracers, such as stable isotopes offers the opportunity to trace the fate of specific fatty acid sources through hepatic esterification and/or oxidation pathways (Fig. 1a (based on Hodson and Frayn[7] and Pramfalk *et al.*[10])). Moreover, as human subjects spend a large proportion of a 24 h period in the postprandial state[11,12] studies in the postprandial state, in combination with stable-isotope methodologies offer the best insight into human hepatic fatty acid metabolism.

Fatty acid sources: adipose tissue

In the fasting state, plasma NEFA arise predominantly from the hydrolysis of adipose tissue TAG and are a major contributor to the intra-hepatic fatty acid pool[8]. NEFA release (per unit mass) from subcutaneous abdominal (upper-body) adipose tissue has been inversely associated with fasting plasma insulin concentrations[13]. Studies assessing whole-body adipose tissue lipolysis have reported insulin-resistant subjects with NAFLD have an elevated lipolytic rate when compared with insulin-sensitive subjects without NAFLD[14–16]. Whether the higher lipolytic rate observed in insulin-resistant individuals with NAFLD is a cause or a consequence of NAFLD remains unclear. Although often suggested to be the major contributor to liver TAG, Donnelly *et al.*[8] using stable-isotope tracer methodology, reported that plasma NEFA contributed between 45 and 74 % to liver TAG (after a 4 d stable-isotope labelling procedure); thus it is plausible that some, but not all, individuals develop NAFLD due to an increased fatty acid flux from adipose tissue to the liver. Positron emission tomography/computed tomography in combination with labelled palmitate (^{11}C) or a palmitate analogue fluoro-6-thia-heptadecanoic acid have been used to assess hepatic fatty acid uptake[17,18]. It was found that hepatic fatty acid uptake was higher, although not significantly different, in obese compared with overweight control subjects[17] whilst in morbidly obese individuals hepatic fatty acid uptake is significantly higher before and 6 months after bariatric surgery than in lean controls, despite liver fat content being normalised[18]. The authors suggested that the persistence of high hepatic fatty acid uptake compared with the lean control subjects, in the combination with the reduction in liver fat content indicates fatty acids may be preferentially directed into oxidation rather than esterification pathways[18].

The relative contribution of systemic NEFA (that is fatty acids predominantly from subcutaneous adipose tissue) to VLDL-TAG in lean/insulin-sensitive individuals has been reported to be between 75 and 84 %[15,19,20–22] whilst for obese/insulin-resistant individuals and those with NAFLD, the contribution is between 42 and 72 %[8,15,20–22]. In the fasting state, the remaining fatty acids come from non-systemic NEFA, some of which would include fatty acids derived from the lipolysis of visceral adipose tissue. As liver fat content is strongly associated with visceral adiposity[23], it is plausible that increasing amounts of visceral fat would contribute to a greater extent to the fatty acid supply being released into the portal vein[24]. Indeed, Meek *et al.*[25] have previously demonstrated that moderate hyperinsulinaemia appeared to increase the proportions of fatty acids reaching the liver from visceral fat compared with systemic sources.

In the transition to the postprandial state, and after consumption of a mixed meal, the antilipolytic action of insulin will suppress the hydrolysis of adipose tissue TAG, resulting in decreased plasma NEFA concentrations[26]. This is reflected in the relative contribution of plasma NEFA to VLDL-TAG in the postprandial state where it is between 28 and 57 % for lean/insulin-sensitive individuals[19,22,27] and 28–30 % for obese/insulin-resistant individuals and those with NAFLD[8,22]. The degree to which adipose tissue hydrolysis is suppressed may be influenced by insulin resistance as some[12,28] but not all[29] studies have reported postprandial NEFA suppression occurs to a lesser degree in insulin-resistant compared with insulin-sensitive individuals. One plausible explanation for the apparent lower degree of NEFA suppression reported in insulin-resistant individuals is that spillover fatty acids, derived from the peripheral lipoprotein lipase mediated lipolysis of chylomicron-TAG may somewhat reduce this effect. We have recently demonstrated that the contribution of spillover fatty acids to the systemic NEFA pool is lower in obese/insulin-resistant compared with lean insulin-sensitive individuals[30]. In the situation where suppression of adipose tissue hydrolysis is attenuated,

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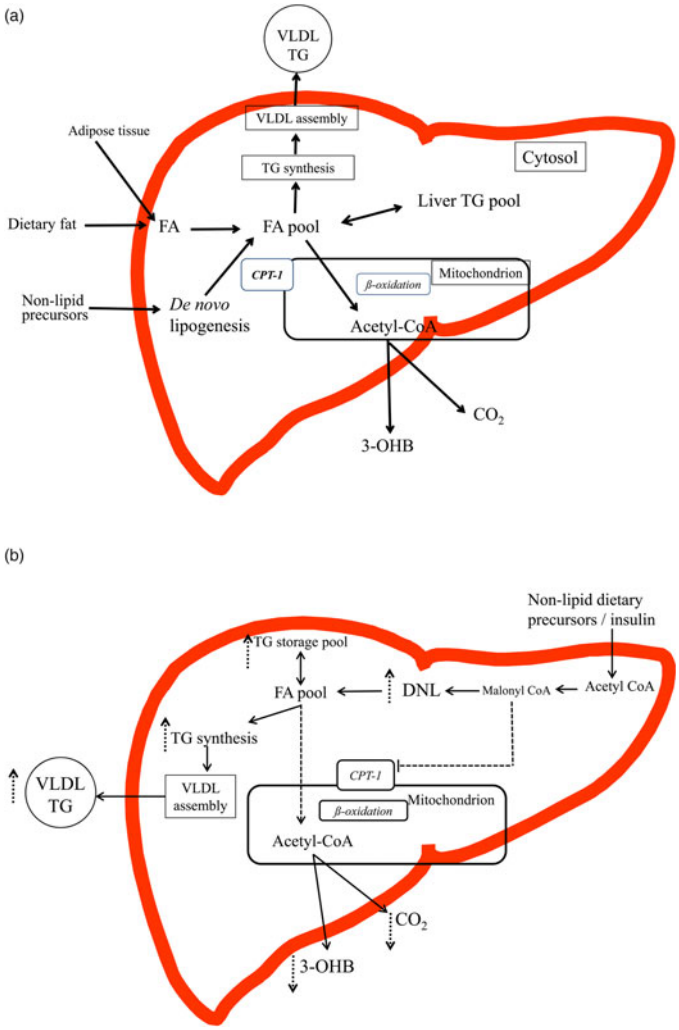

Fig. 1. (Colour online) (a) and (b) An overview of the intra-hepatic pathways of hepatic fatty acid (FA) metabolism (figures are based on previous schematics overviews in Hodson and Frayn[7] and Pramfalk *et al.*[10]). (a) In the fasting state FA from the lipolysis of subcutaneous and visceral adipose tissue enter the liver and mix with FA from the cytosolic TAG (TG) pool and those from *de novo* lipogenesis (DNL). FA are then preferentially partitioned towards the oxidative pathway where the acetyl-CoA produced can enter the tricarboxylic acid cycle or ketogenic pathway to produce CO_2 or 3-hydroxybutyrate (3-OHB). FA are also esterified to TAG and enter a cytosolic storage pool that is constantly turning over. TAG is then hydrolysed to release FA which are then re-esterified to TAG and utilised in the production of VLDL particles. In the transition to the postprandial state, FA from the diet also enter the liver and mix with endogenous sources. The postprandial increase in plasma insulin concentrations suppresses adipose tissue lipolysis and up-regulates the DNL, which would shift the cellular metabolism of FA away from oxidative pathways towards esterification (TAG synthesis). (b) In individuals with an 'unhealthy' phenotype (e.g. insulin resistance, hepatic fat accumulation) in both the fasting and the postprandial state it is proposed that the DNL pathway will be up-regulated and FA from the intra-hepatic FA pool will be partitioned towards esterification (TAG production) pathways and utilised in the production of VLDL particles or stored in the cytosolic TAG pool rather than entering oxidative pathways. The increase in DNL, will increase malonyl-CoA, which will inhibit carnitine-palmitoyl-transferase 1, located on the outer mitochondrial membrane, therefore decreasing the flux of FA into the mitochondria for oxidation, leading to decreases in hepatic CO_2 production and plasma 3-OHB concentrations.

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such as insulin resistance, it is plausible there is a greater delivery of adipose tissue derived fatty acids to the liver in the postprandial state compared with more insulin-sensitive individuals. We have previously reported the relative contribution of endogenous systemic NEFA to VLDL-TAG was lower in the insulin-resistant when compared with the insulin-sensitive subjects[27]. This finding suggests other fatty acid sources were contributing to a greater extent to VLDL-TAG production in the insulin-resistant group; however when expressed as a concentration, the contribution from endogenous systemic NEFA was similar in the groups[27]. We did not observe a difference in either the relative or absolute contribution of systemic NEFA to VLDL-TAG in abdominally obese insulin-resistant males, compared with lean insulin-sensitive controls when fed three sequential meals, 5 h apart over a 24 h period[22].

Fatty acid sources: *de novo* synthesised

DNL is the process where excess non-lipid precursors (e.g. sugars and protein) are synthesised to fat; in human subjects this primarily occurs in the liver[31]. DNL is a complex process: it takes place in the cellular cytoplasm, and acetyl-CoA is required as the precursor and the principal building blocks, with the major quantitative product being palmitoyl-CoA[31,32]. The synthesis of one molecule of palmitoyl-CoA from acetyl-CoA is an energy inefficient process costing seven molecules of ATP and requiring the conversion of fourteen high-energy NADPH molecules to NADP+[33]. Despite being energetically inefficient and likely a minor route for storing excess energy, the contribution of *de novo* fatty acids to VLDL-TAG has been reported to be up to about 10 % in healthy, insulin-sensitive adults[10,19,34–38] and 14–22 % in individuals with insulin resistance or NAFLD[8,34,36–38]. As individuals with insulin resistance/NAFLD have notably higher hepatic DNL compared with those without, it is often speculated, although unsubstantiated, that enhanced DNL is a causal factor in the development of insulin resistance/NAFLD; thus it remains unclear if insulin resistance/NAFLD causes enhanced hepatic DNL or *vice versa*.

The consumption of a mixed meal exposes hepatic lipogenic enzymes to higher concentrations of insulin and this has been shown to stimulate enzyme activity by increasing the transcription of the genes for fatty acid synthase and acetyl-CoA carboxylase[1]. In the postprandial state the contribution of DNL fatty acids to VLDL-TAG has been reported to be between 8 and 13 % in insulin-sensitive individuals[19,35,38] and higher, between 12 and 26 %, in individuals who are insulin resistant or with NAFLD[8,38].

The amount of dietary carbohydrate and/or fat consumed may influence the contribution of DNL fatty acids to VLDL-TAG. Wilke *et al.*[32] compared the effects of a short-term (3 d) high-fat (about 37 % total energy (TE), carbohydrate 48 % TE) diet to a lower-fat (about 23 % TE, carbohydrate 59 % TE) diet on hepatic DNL in obese individuals with and without type-2 diabetes. In both groups hepatic DNL was notably up-regulated on the low-fat diet with palmitate accounting for >70 % of the newly synthesised fatty acids in VLDL-TAG; whilst on the higher-fat diet the synthesis of fatty acids were notably reduced[32]. Thus, when investigating the effect of phenotype on the contribution of DNL derived fatty acids to VLDL-TAG, it would be important that dietary intakes are standardised prior to measuring hepatic DNL.

Fatty acid sources: dietary

The contribution of dietary fat to liver fat accumulation and the appearance in VLDL-TAG is dependent on the amount and frequency of fat intake. Dietary fat can enter the liver either as chylomicron remnants or chylomicron-derived spillover NEFA[39]. By feeding a single high-fat meal, labelled with ^{13}C fatty acids and tracing the trafficking of the labelled fatty acids with ^{13}C-MRI/spectroscopy, it was demonstrated that there was a transient increase in liver fat content over the postprandial period[40,41] which is in line with the liver being a dynamic organ that responds rapidly to changes in nutritional status. Dietary fatty acids have been reported to contribute 15 % of liver-TAG, in individuals with NAFLD[8]; as individuals consumed stable-isotope tracers for 4 d prior to the biopsy it is unclear how reflective this is as there would have been 'recycling' of the tracer from previous meals, which may suggest a higher contribution.

The relative contribution of dietary fatty acids to VLDL-TAG has been reported to be 19–39 % for lean/insulin-sensitive individuals[19,22,27], and between 12 and 28 % in obese/insulin-resistant individuals and those with NAFLD[8,22,27]. Few studies have compared differences in contribution between individuals with varying degrees of insulin resistance. We have previously compared the contribution of dietary fatty acids to VLDL-TAG between insulin-sensitive and insulin-resistant individuals and found no difference in the relative or absolute contribution of dietary fatty acids to VLDL-TAG at the end of a 6 h postprandial period, after consumption of a mixed-test meal[27]. After feeding three sequential test meals, 5 h apart, we found the relative contribution of dietary fatty acids to VLDL-TAG was lower in abdominally obese insulin-resistant males compared with lean insulin-sensitive males; however there was no difference in the absolute contribution[22]. Differences in the reported contributions of dietary fatty acids to VLDL-TAG (and potentially liver TAG) can in part be explained by differences in the amount and type of fat consumed in the test meal, the frequency of meals, the amount of time after meal consumption the measurement was made, and the phenotype of the individual; all of which differ/vary across studies. The contribution of dietary fat to VLDL-TAG or liver TAG is not often measured and is rarely reported in intervention studies where the amount and composition of dietary fat has been altered. This would be of interest to do as the type of fat consumed appears to influence liver fat content as previously reviewed[42–44]. Taken together it has been found that diets overfeeding SFA increase

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liver fat content to a greater extent than diets overfeeding unsaturated (primarily monounsaturated and *n*-6 PUFA) fatty acids[45–47]. Supplementing the diet with *n*-3 fatty acids (namely EPA and DHA) at 4 g/d (as ethyl esters) for periods between 8 weeks and 16 months has also been reported to decrease liver fat[48–50].

Although these studies have investigated the effect of dietary fat quality on liver fat content, there is limited work investigating the effect on intra-hepatic fatty acid synthesis and partitioning. In the study by Luukkonen *et al.*[47] it was found that fasting hepatic DNL was unchanged after 3 weeks overconsumption of SFA, whilst fasting hepatic DNL significantly increased after overconsumption of the carbohydrate-enriched diet. Rosqvist *et al.*[46] over fed participants with SFA or *n*-6 PUFA and found no change in fasting plasma 3-OHB concentrations.

We have previously found no difference in the contribution of systemic NEFA and dietary fatty acids to VLDL-TAG, when participants consumed a mixed-test meal after short-term (3 d) consumption of a high-fat (40 % TE) or high-carbohydrate (75 % TE) diet[51]; however there was a higher contribution of splanchnic fatty acids (which would include fatty acids from DNL, liver TAG and visceral fat) after the high-carbohydrate diet. Indeed, the lipogenic index in VLDL-TAG (a surrogate marker of hepatic DNL) was significantly increased after the high-carbohydrate diet[51]. As this was a short-term intervention, it would be of interest to investigate how the composition of dietary intake may influence the contribution of fatty acid sources to VLDL-TAG (and presumably liver TAG) over a longer period of time. We have undertaken a pilot study to investigate the effect of EPA and DHA supplementation on hepatic fatty acid partitioning[50]. We found that long-term (16–18 months) supplementation with EPA and DHA (total 4 g/d as ethyl esters) significantly decreased the contribution of DNL fatty acids to VLDL-TAG in the postprandial state, in individuals who showed an increase of >2 % in erythrocyte DHA compared with those who did not[50]. In the individuals with an increased erythrocyte DHA, we found a significantly higher incorporation of ^{13}C (which was given in a test meal) appear in plasma 3-OHB over the postprandial period, suggesting that a greater proportion of recently ingested fatty acids were undergoing oxidation to produce 3-OHB[50]. Given the effect that different fatty acids are reported to have on liver fat content, it would be of interest to study the effects they have on hepatic fatty acid synthesis and partitioning to understand their role in liver fat metabolism.

Intra-hepatic partitioning of fatty acids: esterification

Fatty acids within the hepatocyte are broadly partitioned between two pathways: (i) esterification to form mainly TAG that will enter the hepatic TAG pool that may be secreted as VLDL-TAG or stored as cytosolic TAG and (ii) oxidation (Fig. 1a). Typically the rate of VLDL-TAG secretion has been investigated in the fasting state[21,52–55] and has not often been reported in the postprandial period[19]. In the fasting state, secretion of TAG has been reported to be higher in individuals with NAFLD, who are insulin resistant compared with those without NAFLD, who are by comparison insulin sensitive[52]. In the postprandial state, Barrows and Parks[19] reported a decrease in rate of appearance of VLDL-TAG upon consumption of a meal; a comparison of the rate of VLDL-TAG secretion in the postprandial state has not been undertaken between insulin-sensitive and insulin-resistant individuals.

Increases in insulin, either induced experimentally using a euglycaemic/hyperinsulaemic clamp protocol or during the transition to the postprandial state, suppress VLDL production in insulin-sensitive individuals[56]. However, this suppressive effect of insulin on VLDL production is blunted in individuals with NAFLD[53]. During the postprandial period, there are approximately ten times more VLDL than chylomicron particles[57]; thus for individuals who do not suppress VLDL production during the postprandial period this would result in a more exaggerated postprandial lipaemia. It is suggested that as ApoB-100 secretion is not increased in NAFLD patients[15] the particles secreted are more TAG-rich and thus they have an increased particle size[58]. Evidence of the effect of diet on VLDL-TAG secretion is lacking. Given the pronounced effect that not only the nutrient, but fatty acid composition of the diet has on liver fat accumulation and potentially intra-hepatic fatty acid synthesis and partitioning it is important to determine the effect of dietary components on VLDL-TAG secretion; VLDL-TAG secretion is a pathway of disposal for intra-hepatic TAG (Fig. 1a).

Intra-hepatic partitioning of fatty acids: oxidation

Fatty acids entering the β-oxidation pathway generate intra-mitochondrial acetyl-CoA and this may then enter either the tricarboxylic acid cycle (Krebs cycle) for oxidation to CO_2, or undergo ketogenesis to form ketone bodies (Fig. 1a). In the ketogenic pathway, two molecules of acetyl-CoA are combined within the mitochondrion to form acetoacetic acid, from which the other major ketone body, 3-OHB is produced. Systemic concentrations of 3-OHB are often used as a surrogate marker of hepatic fatty acid oxidation whilst direct assessment of hepatic CO_2 production, in human subjects *in vivo* is challenging. A major regulator of hepatic fatty acid oxidation, particularly ketogenesis is undoubtedly the rate of supply of fatty acids from adipose tissue.

A number of studies have investigated hepatic fatty acid partitioning and its hormonal regulation in human subjects. By using a radio-tracer, in individuals who had undergone an 18 h fast it was demonstrated that of the fatty acids entering the liver, approximately 2-fold more fatty acids were converted to ketone bodies than to VLDL-TAG and there are twice as many fatty acids converted to VLDL-TAG than CO_2, demonstrating that ketogenesis is considerably greater than complete oxidation[59]. In hyperlipidaemic subjects fatty acids

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were converted in similar proportions to ketone bodies, VLDL-TAG and CO_2[59].

By using a combination of stable-isotope labelled tracers (^2H and ^{13}C) Sunny *et al.*[60] found mitochondrial oxidation, which represented the tricarboxylic acid cycle to be twice as high in subjects with NAFLD (17 % liver fat content) compared with subjects without NAFLD (3 % liver fat content); with a strong direct association with flux through the cycle and liver fat content. They found no difference in ketone body production between the groups[60]. By using ^{13}C-acetate infusion in combination with a ^{13}C-MRS methodology Petersen *et al.*[61] reported similar rates of hepatic mitochondrial oxidation (based on a mathematical model using the appearance of ^{13}C in the tricarboxylic acid cycle intermediate glutamate) in subjects with high (about 9 %) and low (about 2 %) liver fat content. Aside from differences in methodologies used, the difference in findings between the studies of Sunny *et al.*[60] and Petersen *et al.*[61] may in part be explained by differences in participant age, BMI (the participants in the study by Petersen *et al.*[61] were notably younger and leaner), sex (Petersen *et al.*[61] studied only men), ethnicity (Sunny *et al.*[60] studied Hispanic and African American) and the degree of liver fat content; the high liver fat group in the study of Sunny *et al.*[60] has twice as much liver fat as the high liver fat group of Petersen *et al.*[61]. As it was not known how long the high liver fat individuals had their liver fat accumulation, it is also plausible that the difference in findings were due to metabolic adaptation; we have previously suggested that greater fatty acid oxidation may be an adaptive mechanism to prevent liver fat accumulation[22].

Studies measuring plasma 3-OHB concentrations in the fasting state as a marker of hepatic fatty acid oxidation have reported mixed findings, with concentrations being decreased[62], similar[22,27,38,63] or increased[14,64] in subjects with insulin resistance and/or NAFLD compared with those without insulin resistance and/or NAFLD.

There is a limited amount of evidence for differences in postprandial hepatic fatty acid β-oxidation in human subjects. No direct assessment of hepatic CO_2 production has been undertaken in human subjects over the postprandial period; however plasma concentrations of 3-OHB have been measured. We have previously found mixed results with some of our studies showing a significantly lower[27], similar[38] or higher[22] postprandial response of blood 3-OHB in insulin-resistant compared with insulin-sensitive individuals. We have also found that men have a notably lower postprandial 3-OHB concentration when compared with women[10]. This is in agreement with the work of Halkes *et al.*[65] who also found higher postprandial blood 3-OHB concentrations in females compared with males. The reported sexual dimorphism in postprandial plasma 3-OHB concentrations may be due to males having a more prolonged pattern of hepatic DNL than females[10].

In the postprandial period ketogenesis is suppressed, compared with the fasting state, as adipose tissue lipolysis is suppressed[10,22,27,38]. Using stable-isotope tracers we have demonstrated that dietary fatty acids in the form of chylomicron derived 'spillover' fatty acids and chylomicron remnant fatty acids are still utilised for ketogenesis[10,22]. Using this methodology we have demonstrated that incorporation of ^{13}C from recently ingested dietary fat into plasma 3-OHB was higher in insulin-resistant compared with insulin-sensitive individuals over 24 h[22]. More recently and in contrast to our previous finding, we found greater incorporation of ^{13}C from dietary fat, in insulin-sensitive compared with insulin-resistant individuals[38] and in women compared with men[10]. These findings demonstrate that recently ingested dietary fatty acids enter the ketogenic pathway; however the regulation of this process still remains to be elucidated.

It remains unclear if insulin regulates intra-hepatic fatty acid partitioning into the ketogenic pathway. The transport of fatty acyl-CoA into the mitochondrion for oxidation is achieved via the 'carnitine shuttle', and the key regulated enzyme is carnitine-palmitoyl-transferase 1, located on the outer mitochondrial membrane. Malonyl-CoA, an intermediate in the pathway of DNL is a potent inhibitor of carnitine-palmitoyl-transferase 1[66], thus when DNL is stimulated under conditions of carbohydrate excess both directly via the carbohydrate response element binding protein, and via insulin, fat oxidation is consequently suppressed via inhibition of carnitine-palmitoyl-transferase 1 (Fig. 1b (based on Hodson and Frayn[7] and Pramfalk *et al.*[10])). This will complement the suppression of fatty acid release from adipose tissue which is under the influence of insulin. Indeed, we have previously found strong inverse associations between hepatic DNL and plasma 3-OHB concentrations in the postprandial period in some, but not all, of our participants[10,38] suggesting a dissociation between the pathways. It could be speculated that some individuals have hepatic insulin resistance (which is not obvious at a systemic level) and this leads to a lack of suppression in ketone body production in the postprandial period. Alternatively, the difference in results may be explained by the fact that postprandial studies typically feed a standard test meal and it is possible that there may be variations from true energy balance of the individual and this could result in either suppression or enhancement of fatty acid oxidation, along with perturbations of other plasma metabolites, such as insulin.

Discrepancy in findings regarding fatty acid oxidation between studies is most likely explained by differences in phenotypes, methodology to assess fatty acid oxidation and duration/severity of disease. It is also plausible that the higher oxidation reported in insulin resistance, compared with insulin-sensitive individuals is a compensatory response to try and normalise liver (ectopic) fat content in insulin-resistant compared with insulin-sensitive males[22]. However, this was an observation study and it would be of interest to undertake studies where dietary fatty acid oxidation was measured regularly over time, in individuals who were on a trajectory to become insulin resistant or get NAFLD (e.g. increasing body fat, particularly abdominal fat) to see if fatty acid oxidation changed.

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Conclusions

When we consume dietary fat, a series of complex metabolic processes ensures that fatty acids are absorbed, transported around the body and used/stored appropriately. The liver is a central metabolic organ within the human body and has a major role in regulating fat and carbohydrate metabolism; it serves as an intermediary organ between dietary (exogenous) and endogenous energy sources and other extra-hepatic organs/tissues that consume energy. Hepatic fat accumulation represents an imbalance between pathways of fatty acid delivery and fatty acid removal. Why hepatic fat starts to accumulate is, surprisingly, not well understand but must involve alterations in hepatic fatty acid input, synthesis and removal. Whether fatty acids are partitioned towards oxidation or esterification pathways appears to be dependent on a number of metabolic factors; not least ambient insulin concentrations. Moreover, along with the phenotype of an individual (i.e. BMI, plasma insulin concentrations), it is apparent that lifestyle factors (e.g. habitual diet) of an individual may also play a role (and explain some of the variation and discrepancies between studies). It is becoming increasingly clearer that dietary composition (macronutrient and fatty acid composition) may play pivotal roles in determining if intra-hepatic fat accumulates, although what remains to be elucidated is the influence these nutrients have on intra-hepatic fatty acid synthesis and partitioning.

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Conflict of Interest

None.

Authorship

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

References

1. Lewis GF, Carpentier A, Adeli K *et al.* (2002) Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes. *Endocr Rev* **23**, 201–229.
2. Yki-Jarvinen H (2010) Liver fat in the pathogenesis of insulin resistance and type 2 diabetes. *Dig Dis* **28**, 203–209.
3. Angulo P (2002) Nonalcoholic fatty liver disease. *N Engl J Med* **346**, 1221–1231.
4. Vernon G, Baranova A & Younossi ZM (2011) Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Aliment Pharmacol Ther* **34**, 274–285.
5. Diraison F & Beylot M (1998) Role of human liver lipogenesis and reesterification in triglycerides secretion and in FFA reesterification. *Am J Physiol* **274**, E321–E327.
6. Babin PJ & Gibbons GF (2009) The evolution of plasma cholesterol: direct utility or a 'spandrel' of hepatic lipid metabolism? *Prog Lipid Res* **48**, 73–91.
7. Hodson L & Frayn KN (2011) Hepatic fatty acid partitioning. *Curr Opin Lipidol* **22**, 216–224.
8. Donnelly KL, Smith CI, Schwarzenberg SJ *et al.* (2005) Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. *J Clin Invest* **115**, 1343–1351.
9. Peter A, Cegan A, Wagner S *et al.* (2009) Hepatic lipid composition and stearoyl-coenzyme A desaturase 1 mRNA expression can be estimated from plasma VLDL fatty acid ratios. *Clin Chem* **55**, 2113–2120.
10. Pramfalk C, Pavlides M, Banerjee R *et al.* (2015) Sex-specific differences in hepatic fat oxidation and synthesis may explain the higher propensity for NAFLD in men. *J Clin Endocrinol Metab* **100**, 4425–4433.
11. Ruge T, Hodson L, Cheeseman J *et al.* (2009) Fasted to fed trafficking of Fatty acids in human adipose tissue reveals a novel regulatory step for enhanced fat storage. *J Clin Endocrinol Metab* **94**, 1781–1788.
12. McQuaid SE, Hodson L, Neville MJ *et al.* (2011) Downregulation of adipose tissue fatty acid trafficking in obesity: a driver for ectopic fat deposition? *Diabetes* **60**, 47–55.
13. Karpe F, Dickmann JR & Frayn KN (2011) Fatty acids, obesity, and insulin resistance: time for a reevaluation. *Diabetes* **60**, 2441–2449.
14. Bugianesi E, Gastaldelli A, Vanni E *et al.* (2005) Insulin resistance in non-diabetic patients with non-alcoholic fatty liver disease: sites and mechanisms. *Diabetologia* **48**, 634–642.
15. Fabbrini E, Mohammed BS, Magkos F *et al.* (2008) Alterations in adipose tissue and hepatic lipid kinetics in obese men and women with nonalcoholic fatty liver disease. *Gastroenterology* **134**, 424–431.
16. Korenblat KM, Fabbrini E, Mohammed BS *et al.* (2008) Liver, muscle, and adipose tissue insulin action is directly related to intrahepatic triglyceride content in obese subjects. *Gastroenterology* **134**, 1369–1375.
17. Iozzo P, Bucci M, Roivainen A *et al.* (2010) Fatty acid metabolism in the liver, measured by positron emission

https://doi.org/10.1017/S0029665118002653 Published online by Cambridge University Press

tomography, is increased in obese individuals. *Gastroenterology* **139**, 846–856, 856 e1–6.
18. Immonen H, Hannukainen JC, Kudomi N *et al.* (2018) Increased liver fatty acid uptake is partly reversed and liver fat content normalized after bariatric surgery. *Diabetes Care* **41**, 368–371.
19. Barrows BR & Parks EJ (2006) Contributions of different fatty acid sources to very low-density lipoprotein-triacylglycerol in the fasted and fed states. *J Clin Endocrinol Metab* **91**, 1446–1452.
20. Vedala A, Wang W, Neese RA *et al.* (2006) Delayed secretory pathway contributions to VLDL-triglycerides from plasma NEFA, diet, and *de novo* lipogenesis in humans. *J Lipid Res* **47**, 2562–2574.
21. Fabbrini E, Magkos F, Mohammed BS *et al.* (2009) Intrahepatic fat, not visceral fat, is linked with metabolic complications of obesity. *Proc Natl Acad Sci USA* **106**, 15430–5.
22. Hodson L, McQuaid SE, Humphreys SM *et al.* (2010) Greater dietary fat oxidation in obese compared with lean men: an adaptive mechanism to prevent liver fat accumulation? *Am J Physiol Endocrinol Metab* **299**, E584–E592.
23. Kotronen A, Westerbacka J, Bergholm R *et al.* (2007) Liver fat in the metabolic syndrome. *J Clin Endocrinol Metab* **92**, 3490–3497.
24. Nielsen S, Guo ZK, Johnson CM *et al.* (2004) Splanchnic lipolysis in human obesity. *J Clin Invest* **113**, 1582–1588.
25. Meek SE, Nair KS & Jensen MD (1999) Insulin regulation of regional free fatty acid metabolism. *Diabetes* **48**, 10–14.
26. Frayn KN, Arner P & Yki-Jarvinen H (2006) Fatty acid metabolism in adipose tissue, muscle and liver in health and disease. *Essays Biochem* **42**, 89–103.
27. Hodson L, Bickerton AS, McQuaid SE *et al.* (2007) The contribution of splanchnic fat to VLDL triglyceride is greater in insulin-resistant than insulin-sensitive men and women: studies in the postprandial state. *Diabetes* **56**, 2433–2441.
28. Coppack SW, Evans RD, Fisher RM *et al.* (1992) Adipose tissue metabolism in obesity: lipase action in vivo before and after a mixed meal. *Metabolism* **41**, 264–272.
29. Bickerton AS, Roberts R, Fielding BA *et al.* (2008) Adipose tissue fatty acid metabolism in insulin-resistant men. *Diabetologia* **51**, 1466–1474.
30. Piche ME, Parry SA, Karpe F *et al.* (2018) Chylomicron-derived fatty acid spillover in adipose tissue: a signature of metabolic health? *J Clin Endocrinol Metab* **103**, 25–34.
31. Hellerstein MK, Schwarz JM & Neese RA (1996) Regulation of hepatic *de novo* lipogenesis in humans. *Annu Rev Nutr* **16**, 523–557.
32. Wilke MS, French MA, Goh YK *et al.* (2009) Synthesis of specific fatty acids contributes to VLDL-triacylglycerol composition in humans with and without type 2 diabetes. *Diabetologia* **52**, 1628–1637.
33. Solinas G, Boren J & Dulloo AG (2015) *De novo* lipogenesis in metabolic homeostasis: more friend than foe? *Mol Metab* **4**, 367–377.
34. Diraison F, Moulin P & Beylot M (2003) Contribution of hepatic *de novo* lipogenesis and reesterification of plasma non esterified fatty acids to plasma triglyceride synthesis during non-alcoholic fatty liver disease. *Diabetes Metab* **29**, 478–485.
35. Timlin MT & Parks EJ (2005) Temporal pattern of *de novo* lipogenesis in the postprandial state in healthy men. *Am J Clin Nutr* **81**, 35–42.
36. Lambert JE, Ramos-Roman MA, Browning JD *et al.* (2014) Increased *de novo* lipogenesis is a distinct characteristic of individuals with nonalcoholic Fatty liver disease. *Gastroenterology* **146**, 726–735.
37. Lee JJ, Lambert JE, Hovhannisyan Y *et al.* (2015) Palmitoleic acid is elevated in fatty liver disease and reflects hepatic lipogenesis. *Am J Clin Nutr* **101**, 34–43.
38. Pramfalk C, Pavlides M, Banerjee R *et al.* (2016) Fasting plasma insulin concentrations are associated with changes in hepatic fatty acid synthesis and partitioning prior to changes in liver fat content in healthy adults. *Diabetes* **65**, 1858–1867.
39. Hodson L & Fielding BA (2010) Trafficking and partitioning of fatty acids: the transition from fasted to fed state. *Clin Lipidol* **5**, 131–144.
40. Ravikumar B, Carey PE, Snaar JE *et al.* (2005) Real-time assessment of postprandial fat storage in liver and skeletal muscle in health and type 2 diabetes. *Am J Physiol Endocrinol Metab* **288**, E789–E797.
41. Lindeboom L, Nabuurs CI, Hesselink MK *et al.* (2015) Proton magnetic resonance spectroscopy reveals increased hepatic lipid content after a single high-fat meal with no additional modulation by added protein. *Am J Clin Nutr* **101**, 65–71.
42. Green CJ & Hodson L (2014) The influence of dietary fat on liver fat accumulation. *Nutrients* **6**, 5018–5033.
43. Yki-Jarvinen H (2015) Nutritional modulation of non-alcoholic fatty liver disease and insulin resistance. *Nutrients* **7**, 9127–9138.
44. Parry SA & Hodson L (2017) Influence of dietary macronutrients on liver fat accumulation and metabolism. *J Investig Med* **65**, 1102–1115.
45. Bjermo H, Iggman D, Kullberg J *et al.* (2012) Effects of n-6 PUFAs compared with SFAs on liver fat, lipoproteins, and inflammation in abdominal obesity: a randomized controlled trial. *Am J Clin Nutr* **95**, 1003–1012.
46. Rosqvist F, Iggman D, Kullberg J *et al.* (2014) Overfeeding polyunsaturated and saturated fat causes distinct effects on liver and visceral fat accumulation in humans. *Diabetes* **63**, 2356–2368.
47. Luukkonen PK, Sadevirta S, Zhou Y *et al.* (2018) Saturated fat is more metabolically harmful for the human liver than unsaturated fat or simple sugars. *Diabetes Care* **41**, 1732–1739.
48. Cussons AJ, Watts GF, Mori TA *et al.* (2009) Omega-3 fatty acid supplementation decreases liver fat content in polycystic ovary syndrome: a randomized controlled trial employing proton magnetic resonance spectroscopy. *J Clin Endocrinol Metab* **94**, 3842–3848.
49. Scorletti E, Bhatia L, McCormick KG *et al.* (2014) Effects of purified eicosapentaenoic and docosahexaenoic acids in nonalcoholic fatty liver disease: results from the Welcome* study. *Hepatology* **60**, 1211–1221.
50. Hodson L, Bhatia L, Scorletti E *et al.* (2017) Docosahexaenoic acid enrichment in NAFLD is associated with improvements in hepatic metabolism and hepatic insulin sensitivity: a pilot study. *Eur J Clin Nutr* **71**, 973–979.
51. Chong MF, Hodson L, Bickerton AS *et al.* (2008) Parallel activation of *de novo* lipogenesis and stearoyl-CoA desaturase activity after 3 d of high-carbohydrate feeding. *Am J Clin Nutr* **87**, 817–823.
52. Adiels M, Taskinen MR, Packard C *et al.* (2006) Overproduction of large VLDL particles is driven by increased liver fat content in man. *Diabetologia* **49**, 755–765.
53. Adiels M, Westerbacka J, Soro-Paavonen A *et al.* (2007) Acute suppression of VLDL1 secretion rate by insulin is

https://doi.org/10.1017/S0029665118002653 Published online by Cambridge University Press

associated with hepatic fat content and insulin resistance. *Diabetologia* **50**, 2356–2365.
54. Fabbrini E, Mohammed BS, Korenblat KM *et al.* (2010) Effect of fenofibrate and niacin on intrahepatic triglyceride content, very low-density lipoprotein kinetics, and insulin action in obese subjects with nonalcoholic fatty liver disease. *J Clin Endocrinol Metab* **95**, 2727–2735.
55. Hodson L, Banerjee R, Rial B *et al.* (2015) Menopausal status and abdominal obesity are significant determinants of hepatic lipid metabolism in women. *J Am Heart Assoc* **4**, e002258.
56. Malmstrom R, Packard CJ, Caslake M *et al.* (1998) Effects of insulin and acipimox on VLDL1 and VLDL2 apolipoprotein B production in normal subjects. *Diabetes* **47**, 779–787.
57. Schneeman BO, Kotite L, Todd KM *et al.* (1993) Relationships between the responses of triglyceride-rich lipoproteins in blood plasma containing apolipoproteins B-48 and B-100 to a fat-containing meal in normolipidemic humans. *Proc Natl Acad Sci USA* **90**, 2069–2073.
58. Adiels M, Olofsson SO, Taskinen MR *et al.* (2008) Overproduction of very low-density lipoproteins is the hallmark of the dyslipidemia in the metabolic syndrome. *Arterioscler Thromb Vasc Biol* **28**, 1225–1236.
59. Havel RJ, Kane JP, Balasse EO *et al.* (1970) Splanchnic metabolism of free fatty acids and production of triglycerides of very low density lipoproteins in normotriglyceridemic and hypertriglyceridemic humans. *J Clin Invest* **49**, 2017–2035.
60. Sunny NE, Parks EJ, Browning JD *et al.* (2011) Excessive hepatic mitochondrial TCA cycle and gluconeogenesis in humans with nonalcoholic fatty liver disease. *Cell Metab* **14**, 804–810.
61. Petersen KF, Befroy DE, Dufour S *et al.* (2016) Assessment of hepatic mitochondrial oxidation and pyruvate cycling in NAFLD by (13)C magnetic resonance spectroscopy. *Cell Metab* **24**, 167–171.
62. Croci I, Byrne NM, Choquette S *et al.* (2013) Whole-body substrate metabolism is associated with disease severity in patients with non-alcoholic fatty liver disease. *Gut* **62**, 1625–1633.
63. Kotronen A, Seppala-Lindroos A, Vehkavaara S *et al.* (2009) Liver fat and lipid oxidation in humans. *Liver Int* **29**, 1439–1446.
64. Sanyal AJ, Campbell-Sargent C, Mirshahi F *et al.* (2001) Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. *Gastroenterology* **120**, 1183–1192.
65. Halkes CJ, van Dijk H, Verseyden C *et al.* (2003) Gender differences in postprandial ketone bodies in normolipidemic subjects and in untreated patients with familial combined hyperlipidemia. *Arterioscler Thromb Vasc Biol* **23**, 1875–1880.
66. McGarry JD & Foster DW (1977) Hormonal control of ketogenesis. Biochemical considerations. *Arch Intern Med* **137**, 495–501.

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