Current Opinion in Clinical Nutrition and Metabolic Care

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--Manuscript Draft--

Manuscript Number:	
Full Title:	Nutritional regulation of hepatic de novo lipogenesis in humans
Article Type:	Review Article
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Nutritional regulation of hepatic *de novo* lipogenesis in humans

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Sources of support: LH is a British Heart Foundation Senior Research Fellow in Basic Science

Word count: 2499

Abstract

Purpose of Review:

De novo lipogenesis (DNL) is a metabolic process occurring mainly within the liver, in humans. Insulin is a primary signal for DNL; thus, nutritional state is a key determinant for upregulation of the pathway. However, the effects of dietary macronutrient composition on hepatic DNL remain unclear. Nor is it clear if a nutrition-induced increase in DNL results in accumulation of intra-hepatic TG (IHTG); a mechanism often proposed for pathological IHTG. Here, we review the latest evidence surrounding the nutritional regulation of hepatic DNL.

Recent findings:

The most studied dietary macronutrient on hepatic DNL in humans is carbohydrate, with only limited evidence for fats and proteins. Overall, it is found that increasing carbohydrate intake typically results in an upregulation of DNL, with fructose, appearing to have a higher lipogenic effect both alone and when combined with glucose (sucrose). For fat, it appears that increased n-3 polyunsaturated FA intakes downregulate DNL, whilst, in contrast, increased dietary protein intakes, are speculated to upregulate DNL.

Summary:

Although DNL is upregulated with mixed meal consumption, the effects of specific dietary macronutrients, namely fat and protein remains unclear. Moreover, the effect of different phenotypes and different dietary regimes on hepatic DNL requires elucidation.

Keywords: human, hepatic, fatty acids, *de novo* lipogenesis, nutrition

List of abbreviations:

DNL: *De novo* lipogenesis

IHTG: Intrahepatic triglyceride

LD: Lipid droplet

NAFLD: Non-alcoholic fatty liver disease

FA: Fatty acids

SFA: Saturated fatty acid

SSB: Sugar sweetened beverage

TE: Total energy

TG: Triglyceride

VLDL: Very low-density lipoprotein

Introduction

The liver plays a key role in the metabolic regulation of systemic lipid and glucose concentrations, with hepatocytes switching between anabolic and catabolic processes depending on nutritional state to achieve energy homeostasis. After consumption of a mixed-macronutrient meal, the liver moves from primarily fatty acid (FA) oxidation to esterification, where triglyceride (TG) is primarily, but not exclusively, synthesised. In turn, newly formed TG may be stored in lipid droplets (LDs) or secreted into systemic circulation within very low-density lipoprotein (VLDL). Although the liver receives FAs and TG (as TG-rich lipoprotein remnants) from systemic circulation, it is capable of synthesising FA *de novo* from non-lipid precursors, primarily carbohydrates. Owing to metabolic zonation (the segregation of processes based on oxygen availability to avoid futile cycling), DNL typically occurs within the reduced oxygen, pericentral regions [1, 2].

DNL is initiated by the committed step of ATP dependent carboxylation of acetyl-CoA to malonyl-CoA (Figure 1), which is primarily converted to the saturated FA (SFA), palmitate (16:0) by FA synthase (FAS). In metabolically healthy individuals, the total quantity of fat produced by DNL is suggested to be 1-2g per day [3]; substantially lower than typical dietary fat intake [4]. Although DNL may be considered a physiological mechanism to metabolise excess precursor substrates, when constitutively activated it has been speculated to underpin the pathological accumulation of intrahepatocellular-TG (IHTG) [5], which is the first stage of non-alcoholic fatty liver disease (NAFLD) with the pericentral region reportedly associated with NAFLD [1, 2, 6]. Insulin is the primary signal for activation of DNL and consequently, dietary intake, including total energy (TE) consumption and dietary macronutrient composition, is pivotal for determining the hepatic DNL rate. Here, we discuss recent evidence surrounding the nutritional regulation, and influence of specific macronutrients on hepatic DNL in humans.

Assessing hepatic DNL

Due to the inaccessibility of the human liver and the dynamic nature of the IHTG pool, hepatic DNL has been assessed using tracers (radio or stable isotopes) and/or changes in VLDL-TG composition.

Using VLDL-TG composition to measure hepatic DNL

TG synthesis is initiated within the liver by a series of acyltransferases esterifying FAs to glycerol 3-phosphate. Within hepatocytes, there are two diacylglycerol acyltransferases which catalyse the final TG synthesis step: DGAT1 which esterifies exogenous FAs and DGAT2 which utilises diacylglycerol (from DNL-derived FA) as substrates the latter being located near the endoplasmic reticulum (ER) [7]. Based on its location and function, it is plausible that TG from DGAT2 is preferentially partitioned to ER-associated pools for immediate secretion in VLDL rather than storage in LDs. This makes VLDL-TG a suitable lipid pool for assess changes in DNL.

To determine the usefulness of VLDL-TG as a proxy marker of hepatic DNL, Donnelly *et al.* [8] utilized stable-isotopes to compare the contribution of different FA sources (i.e., adipose-, dietary-, and DNL-derived) in IHTG and VLDL-TG in patients with NAFLD. Based on the strong positive correlations between FA sources in IHTG and VLDL-TG, the authors suggested that VLDL-TG was a good surrogate marker of IHTG composition [8]. Indeed, given the practical advantages, many studies have used VLDL-TG composition to measure DNL. However, when a TG-rich lipoprotein fraction is assessed (rather than purified VLDL-TG), small contributions from intestinal cells may be included, particularly if collected in the postprandial state, making inter-study comparisons challenging [9].

Assessing hepatic DNL using stable-isotope tracers

Stable-isotope tracers can be used to specifically measure hepatic DNL. Briefly, the stable-isotope tracers utilised include:

i) Deuterated water (also known as heavy water): participants consume deuterated water and then, typically following at least a 10-hour fast, the appearance of deuterium in the acyl chains of DNL-derived FA are measured;

ii) ^{13}*C acetate:* typically infused during an overnight fast, with the rate of inclusion of the label into DNL-derived FA measured;

iii) ^{13}*C sugars (e.g., glucose and fructose)*: incorporation into newly-derived FA is measured. However, this technique is not common as it only measures sugar-specific metabolism to DNL [10].

Consideration is required regarding which tracers are suitable for measuring DNL, with deuterated water accounting for all incorporated precursors. Another important factor is the phenotype being studied (e.g., IHTG content, elevated VLDL-TG etc.) as this may influence

the length of the labelling period required. For example, individuals with high IHTG content may require a longer labelling period to achieve equilibrium due to the slower turnover of the IHTG pool, than those with a low IHTG content [11]. Recently, it has been suggested, in individuals with a larger IHTG pool, a labelling period with deuterated water of more than 10 days is required to achieve a more accurate estimation of hepatic DNL [11]. Although with a longer labelling period, it is plausible that there is recirculation of labelled DNL-derived FA through hepatic VLDL-TG remnant uptake, which may occur to a greater extent in individuals with dysfunctional adipose tissue (e.g., where adipose does not sufficiently hydrolyse circulating VLDL-TG [12]. This may lead to individual specific overestimation of hepatic DNL [5, 13].

Assessing hepatic DNL using VLDL-TG FA composition

In the mid-1990s, the ratio of 16:0 (palmitate)/18:2 n-6 (linoleate) (the lipogenic index) in VLDL-TG was reported to reflect a carbohydrate-induced increase in hepatic DNL [14]. However, Rosqvist *et al.* [15] noted that the lipogenic index did not accurately discriminate between individuals with low and high hepatic DNL when consuming their habitual diet. Therefore, how well the lipogenic index reflects inter-individual diet induced variations remains unclear. The lipogenic index and tracer assessment methods are typically based on the use of palmitate, which is often considered the primary product of DNL. However, as other DNL-derived FAs can be synthesised (e.g., stearate, oleate) other FA species could be considered as the variation in species production may vary between phenotypes.

Hepatic DNL: the influence of nutritional state

The contribution of DNL-derived palmitate to VLDL-TG tends to be higher in the postprandial compared to the fasted state. In healthy, insulin-sensitive individuals, the contribution of DNL-derived FA, when quantified with stable-isotope methodologies, has been reported to range from ~1% following an overnight fast to 23% four hours post meal (Carbohydrate 54%-, Fat 32%-, protein 14% TE) consumption [16]. Whether the postprandial increase in hepatic DNL is as pronounced in individuals with insulin resistance or NAFLD where there appears to be constitutive activation [5] of DNL remains unclear.

The effect of dietary macronutrients on hepatic DNL

Dietary recommendations to lower the risk of metabolic disease typically focus on the quantity and quality of carbohydrate and fat, but the optimal proportions of these are highly debated. When the energy contribution of one macronutrient is altered, the contribution of other macronutrients is *de facto* altered. Given this, assessing the contribution of a single macronutrient *in vivo,* in humans is challenging.

Carbohydrates: High-carbohydrate or sugar enriched diets (with lower fat) have been the primary focus of studies investigating the relationship between nutrition and DNL. These have been shown to robustly shown to increase. Despite this robust increase, DNL-derived FA are demonstrated to contribute <5g of fat to VLDL-TG secretion in healthy adults [17].

Specifically, studies have focused on the effect of some individual sugars, mainly glucose and fructose, on hepatic DNL. Fructose is considered more lipogenic than glucose as it bypasses the initial rate-limiting step (phosphofructokinase activity) in glycolysis [18, 19]. As fructose is rarely consumed in isolation, it is important to consider its metabolic effects when combined with other sugars. To this end, Geidl-Flueck *et al.* [20] investigated the effect of individual monosaccharides consumed as sugar-sweetened beverages (SSBs) on hepatic DNL (using ^{13}C acetate) in 94 healthy males (age 18-30 y, BMI <24 kg/m^2). Subjects had the same amount of total sugar in SSB as either: glucose, fructose or sucrose for seven weeks. Consumption of the fructose and sucrose SSBs both caused a 2-fold increase in fasting and postprandial DNL while the glucose SSB had no effect compared to a sugar-free control [20]. The authors speculated that the similar effect of the sucrose and fructose SSBs, was due to a glucose-induced increased intestinal fructose uptake, as well as the fructose and glucose driving increased lipogenic gene expression [20]. More recently, the effect of SSB on plasma DNL-derived palmitate was investigated in overweight individuals [21]. Participants consumed 1L of either: SSB, semi-skimmed milk, aspartame sweetened soda or water daily for 25 weeks. Daily consumption of 1L of SSBs and milk resulted in a significant increase in phospholipid palmitate with no significant changes found in either TG or cholesteryl esters [21]. Despite measuring the FA composition of the respective plasma lipid fractions, the authors did not report the lipogenic index and therefore, given the ubiquitous nature of palmitate in foods, determining the source of palmitate and if it is solely DNL derived is challenging.

As hepatic DNL is often elevated in individuals with NAFLD, Cohen *et al.* [10] investigated the effect of dietary sugar restriction on hepatic DNL in adolescent (10-16 years) males with

NAFLD. Comparing a diet low in free sugars (3% of TE) with a habitual, control diet (free sugars 10% of TE) they observed a decrease in hepatic DNL (assessed using deuterated water) in the low-sugar-diet group (from 34.6% to 24.1%) with no change in the control group (from 33.9% to 34.6%). Furthermore, the authors found positive, significant associations between the change in hepatic DNL and free-sugar intakes, hepatic DNL and plasma insulin levels, but no association between hepatic DNL and IHTG content. Notably, when these associations were tested in the low-sugar group only, the significance disappeared [10]. Therefore, reconsideration may be needed for the previous assumptions that a high-sugar diet results in the accumulation of IHTG via DNL.

Fat: Most studies have investigated the effect of decreasing, rather than increasing total fat intakes and in this context, hepatic DNL typically increases. We recently compared the contribution of DNL-derived palmitate to VLDL-TG using deuterated water in the fasting and postprandial states following consumption of isocaloric diets enriched with either sugars or fat for 4 weeks and found no difference [15]. It is plausible that hepatic DNL was upregulated with the sugar-rich diet, but pre-diet measurements were not taken for comparison. When comparing the effects of excess energy (1000 kcal/day for 3 weeks) as dietary fat or carbohydrate in overweight adults, the contribution of DNL-derived palmitate to VLDL-TG (using deuterated water) in the fasting state only significantly increased in the carbohydrate group between the pre- and post-diet measurements [22].

Dietary fat contains a mixture of FAs and evidence on how FA composition influences hepatic DNL is sparse. Luukkonen *et al.* [22] compared the change in the contribution of DNL-derived FA to VLDL-TG (assessed using deuterated water) before and 3 weeks after overconsumption of either SFA or unsaturated (a mixture of mono- and polyunsaturated) FA and found no difference between the diets. SFA is often suggested to cause ER stress, resulting in increased DNL [23, 24], although the underlying mechanism(s) remains unclear and this has yet to be demonstrated in humans.

Recently, Costabile *et al.* [25] compared the effects of two diets: one enriched with monounsaturated FAs rich (18:1 n-9, oleic acid, from 30.2% to 33.9% TE), and the other enriched in unsaturated (monounsaturated, n-6 and n-3 polyunsaturated) FAs. They found a significant decrease in the lipogenic index after 8 weeks of consuming the unsaturated FA diet and no change with the monounsaturated FA diet [25]. It is plausible that although the ~4% increase in dietary monounsaturated FA was statistically significant, it was insufficient

to induce a change in DNL. However, the ubiquitous nature of monounsaturated FA in foods makes studying these effects challenging.

We have previously assessed the effect of the long-chain n-3 polyunsaturated FAs, 20:5 n-3 (eicosapentaenoic acid, EPA) and 22:6 n-3 (docosahexaenoic acid, DHA) (total 4 g/d as ethyl esters) on hepatic DNL [26, 27]. In a pilot study, we found after 16–18 months of supplementation with EPA and DHA there was a significant decrease in DNL-derived FA toy VLDL-TG [27]. More recently, we reported that 8 weeks of supplementation with EPA and DHA significantly decreased (by 30%) the contribution of DNL-derived FAs to VLDL-TG in the fasting and postprandial states [26]. We did not control for background diet and therefore cannot exclude the possibility that intake of other dietary FAs or carbohydrates were altered, rather than this being the result of supplementing with EPA and DHA alone. It is evident, from the limited data available, that an increased intake of n-3 (and n-6) polyunsaturated FAs attenuates hepatic DNL (Figure 1), potentially due to down-regulating DNL enzymes [28, 29].

Protein: Plasma amino acid levels reflect the balance between muscle protein catabolism, muscle protein synthesis and dietary protein intake. Although amino acids can be a potential carbon source for DNL (Figure 1) the amount they contribute remains unclear. To investigate, Charidemou *et al* [30] recruited nine healthy males who on separate days consumed an isoenergetic control meal (15% protein, 40% fat, 45% carbohydrate), a high-protein meal (32% protein, 33% fat and 35% carbohydrate), and a high-fat meal (14% protein, 62% fat, 24% carbohydrate) in random order. Blood was collected for 6-hours postprandially and lipodomics was used to detect short chain TGs (scTGs), with accumulation suggesting increased DNL [31]. There was a significant increase in postprandial plasma and LDL/VLDL scTGs in the high-protein compared to control and high-fat meals and the lipogenic index was significantly higher in the high-protein compared to control meal [30]. Complementary *in vitro* work using AML 12 hepatocytes found glutamate supplementation induced the transcription of multiple DNL associated genes [30]. However, given the limited data available, further work is required to determine the effects of protein on hepatic DNL.

Conclusion

Nutrition has a profound effect on hepatic DNL in humans, influenced by both dietary macronutrient quantity and quality. For example, decreasing total fat and increasing carbohydrate (particularly sugars) robustly upregulates DNL. However, it remains difficult to determine the effects of any single macronutrient in human experiments, owing to the *de facto* compensation caused in other macronutrients, particularly in an isocaloric setting. Additionally, the relative contribution to DNL from alternative substrates such as amino acids, requires elucidation.

Dietary induced exacerbation of DNL is suggested to be associated with increased risk of IHTG accumulation. Indeed, patients with NAFLD have a higher contribution of DNL-derived palmitate to VLDL-TG compared to non-NAFLD patients [5]. However, evidence for DNL as causative for the pathological IHTG accumulation is sparse, and it is likely pathological IHTG accumulation results from the perturbation of multiple metabolic pathways [32]. For example, it is plausible that pathways achieve a plateau when DNL increases beyond the capacity of VLDL secretion, thus predisposing an individual to IHTG accumulation.

Although it is suggested there is sexual dimorphism in hepatic DNL owing to oestrogen suppression of DNL genes [33], to date, most human work has studied male participants. Moreover, sexual dimorphism is demonstrated in the influence of BCAA levels on NAFLD severity [34] although the underlying mechanism for remains to be elucidated. Thus, future studies are warranted determining how factors including sex, age, menopause status, and ethnicity interact with dietary nutrients to influence hepatic DNL.

Key findings:

- Carbohydrates are well established to upregulate hepatic DNL in humans; recent evidence highlights the significant effect of fructose intakes on DNL.
- It remains unclear if an increase in sugar-induced hepatic DNL leads to an increase in IHTG content or whether compensatory mechanisms preclude this occurring.
- Although there is currently only limited evidence for the effect of specific dietary FA on hepatic DNL it is appears increased poly-unsaturated FA intakes downregulate DNL
- Evidence for the effect of increased dietary protein intakes on hepatic DNL in humans is sparse
- The majority of studies have studied males and it remains unclear if the effects would be similar in females.

Acknowledgements:

N/A

Financial support and sponsorship

LH is a British Heart Foundation Senior Research Fellow in Basic Science

Conflicts of interest

The authors have no conflicts of interest to declare.

Figure legends:

In the fed state, DNL acts as a way of maintaining energy homeostasis by metabolising extra macronutrients into FA for either storage as TG in LD or for secretion in VLDLs. **[1]** Glucose is taken up into the cell via GLUT2 receptor and metabolised via glycolysis. Fructose is also metabolised by glycolysis but skips the initial rate limiting steps. Glycolysis produces pyruvate which feeds into the citric acid (TCA) cycle within the mitochondria, to produce citrate. Which is then metabolised by ACLY (ATP citrate lyase), ACC1 (Acetyl CoA carboxylase) and FAS sequentially to yield palmitate. Palmitate is then esterified to TG and either stored in LDs or secreted in VLDLs. **[2]** Fats, specifically PUFA, inhibit the transcription of DNL associated genes. **[3]** Proteins feed into these pathways at various points depending on the amino acids. **[4]** Insulin upregulates DNL by upregulating the transcription of DNL associated genes. Glycolysis metabolites also upregulate DNL associated genes.

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* By undertaking a combination of human and cellular studies, this study provided insight into some of the potential mechanisms by which select amino acids may induce hepatic DNL.

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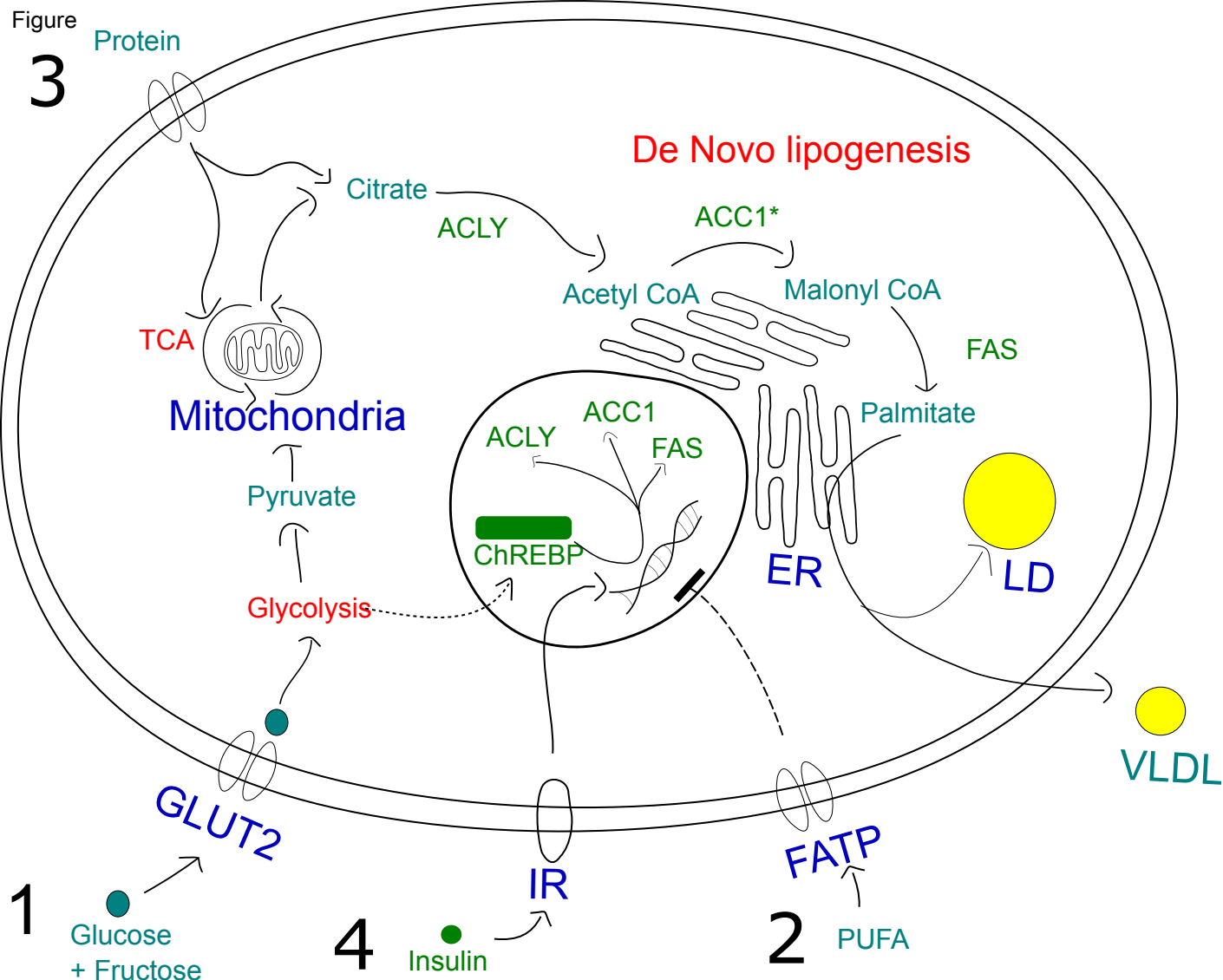
Figure 3


Protein
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Citrate
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