Title: Liver sympathetic nerve activity and steatosis

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Competing interests

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The liver and metabolic health

The liver plays a crucial regulatory role in the storage and distribution of lipids during fed and fasted states. With obesity, non-alcoholic fatty liver disease (NAFLD) often develops, a condition characterised by hepatic steatosis. Hepatic steatosis is the result of lipid input (from lipid uptake and *de novo* lipogenesis) exceeding lipid export (via *β*-oxidation, or VLDL and triglyceride secretion), resulting in net liver lipid accumulation. When present, hepatic steatosis can contribute to the development of other cardiometabolic diseases, such as type 2 diabetes and cardiovascular disease. It has been proposed that autonomic nervous system dysfunction is involved in the pathogenesis of obesity and its related co-morbidities. The liver is innervated by both afferent and efferent

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sympathetic and parasympathetic nerves. However, the importance of liver-brain signalling in the onset and/or the development of NAFLD has remained unclear.

Liver sympathetic nerve activity and steatosis

In the latest issue of the *Journal of Physiology*, Hurr *et al.* (2019) provided evidence to support a role for increased liver sympathetic nerve activity (SNA) in the development of hepatic steatosis in obesity. The authors used a 10 week high-fat diet (60% kcal from fat) to induce obesity and hepatic lipid accumulation in mice, which were compared to mice fed normal chow (with 5% kcal from fat). Multiunit recordings of liver SNA showed a ~2 fold increase in the obese mice with hepatic steatosis compared to normal chow-fed mice, which was mainly attributed to increased efferent nerve activity (the removal of afferent input was achieved by measuring nerve activity after sectioning the nerve distal to the recording site). The authors then utilised both whole-body (chemical denervation via the neurotoxin 6-OHDA) and liver specific methods (application of 10% phenol to the bundle of hepatic artery and portal vein) to inhibit liver SNA in the obese mice with hepatic steatosis. They showed that hepatic steatosis was alleviated in the treated *versus* the non-treated mice and this occurred despite no differential changes in body mass, energy or water intake, or energy expenditure. Interestingly, the inhibition of liver SNA in the chow-fed mice had no significant effect on liver lipid accumulation, suggesting that the increase in liver SNA following high-fat diet induced obesity is likely to be a homeostatic response to the energy and/or dietary lipid surplus.

Hurr *et al.* (2019) also investigated potential mechanisms by which liver SNA may alter hepatic steatosis, using quantitative real time PCR to show that obese mice with hepatic steatosis exhibited an increased mRNA expression of proteins and transcription factors involved in hepatic lipid uptake and *de novo* lipogenesis. This upregulation of lipogenic genes was however mitigated by the inhibition of liver SNA. Proteins involved in lipid oxidation were also upregulated in obese mice with hepatic steatosis (*versus* chow-fed controls) but were not significantly altered by subsequent liver sympathetic denervation. Taken together, these findings suggest that increased liver SNA develops alongside high-fat diet induced obesity and that the inhibition of liver SNA may alleviate hepatic steatosis via lipid acquisition pathways. Consequently, interventions that alleviate the increase in liver SNA activity with obesity could help to reduce lipid accumulation in the liver and therefore offer benefits for NAFLD and associated cardiometabolic diseases. Whilst sympathetic nerve activity is important for regulating physiological processes in many tissues in healthy humans, the findings of Hurr *et al.* (2019) are in line with suggestions that for some tissues (e.g. skeletal muscle and the kidneys), an abnormal increase in SNA can have a role in cardiometabolic disease pathogenesis (Guarino *et al.,* 2017).

Experimental considerations

In the work of Hurr *et al.* (2019), the increases in efferent nerve activity suggested that obesity-associated alterations in liver SNA could be mostly attributable to central nervous system

mechanisms, of which increased hypothalamic neuropeptide Y (NPY) expression is a likely candidate (Bruinstroop *et al.,* 2014). Alterations in SNA with diet-induced obesity could also be due to factors such as hyperinsulinemia, hyperleptinemia, increased angiotensin II levels, and/or baroreceptor dysfunction. These mechanisms could now also be explored to develop the research of Hurr *et al.* (2019). The authors also acknowledged that direct recordings of afferent liver SNA were needed to support their results. Indeed, greater fatty acid availability to the liver (due to the high-fat diet or heightened adipose tissue SNA and consequent increases in lipolysis) could have increased hepatic afferent signalling due to the lipid sensing role of the liver (Paolisso *et al.,* 2000). This response is however likely to be achieved via the parasympathetic branch of the liver, and thus its contribution to global hepatic SNA is currently unclear (Paolisso *et al.,* 2000). Measuring efferent and afferent liver SNA in combination with systemic lipid concentrations/hepatic lipid flux, and also investigating whether increases in SNA occur concomitantly across different tissues in obesity (or whether this response is first detected in specific tissues [e.g. in the adipose tissue before the liver]) would therefore be of interest. Another logical next step for this work would be to de-innervate the liver *prior* to the provision of a high-fat diet to further clarify the role of liver SNA in hepatic steatosis pathogenesis. This is important given the finding that hepatic steatosis in the obese mice was not completely reversed with either whole-body or liver-specific SNA inhibition, suggesting that hepatic steatosis may be a consequence of neural and non-neural mechanisms (although an intervention of >7 days may have resulted in a complete reversal of steatosis in the work of Hurr *et al.,* 2019).

Should we apply these results to humans?

The authors also discussed research in obese Zucker rats where the surgical removal of liver sympathetic nerves did not alleviate hepatic steatosis. This suggests that the role of liver SNA in the development of metabolic disease may be specific to the research model employed and/or species studied. The distribution of sympathetic and parasympathetic nerves in the liver differs across species, including in mice and humans (Yi *et al.,* 2010). Moreover, despite some genetic and physiological similarities between mice and humans, these species have evolved in different environments and differ in size, metabolic rate, sleep-wake cycles and feeding patterns. Whether the findings of Hurr *et al.* (2019) can be applied to humans is therefore unclear, but now warrants investigation. One potential next step would be to manipulate liver energy status in humans using diet (e.g. fructose and/or high-fat feeding) or exercise (to manipulate the liver glycogen content), which can be objectively measured using imaging techniques such as 13^{C} nuclear magnetic resonance. When combined with measures of liver SNA, such as hepatic noradrenaline spillover rate or gall bladder contractility (Bruinstroop *et al.,* 2014), this could provide some evidence that liver sympathetic signalling is associated with hepatic energy status in humans, at least in the short-term. Finally, although the increase in liver SNA in obesity may be locally maladaptive (i.e. manifesting as hepatic steatosis) this response may also help to protect other tissues. For example, an increased hepatic uptake of lipid could decrease systemic lipid concentrations, which may prevent ectopic storage in skeletal muscle (with benefits for insulin sensitivity) or cardiac tissue (which could help to preserve normal heart function) (Guarino *et al.,* 2017). This would be in line with suggestions that increases in sympathetic nerve activity in some peripheral tissues (e.g. in skeletal muscle) is an adaptive (albeit futile) mechanism to increase resting energy expenditure in an attempt to maintain energy balance (Guarino *et al.,* 2017).

Investigating the role of liver-brain signalling for metabolic diseases is certainly complex. Whilst autonomic nerve activity is altered in obesity, this involves tissue-specific changes in both afferent and efferent sympathetic and parasympathetic nerve activity. However, by combining different methods, the work of Hurr *et al.* (2019) provides novel insights into the role of liver SNA in the regulation of hepatic steatosis in mice with high-fat diet induced obesity and highlights a need to characterise liver autonomic nerve activity in the context of cardiometabolic diseases. Thus, whilst technically challenging, we should continue to pursue methods to measure and manipulate liver sympathetic signalling, as the research of Hurr *et al.* (2019) suggests that this may have implications for the development and/or treatment of cardiometabolic diseases.

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