COMMENTARY

Hypoxia and non-alcoholic fatty liver disease

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ABSTRACT

NAFLD (non-alcoholic fatty liver disease) represents a spectrum of fatty liver diseases associated with an increased risk of Type 2 diabetes and cardiovascular disease. The spectrum of fatty liver diseases comprises simple steatosis, steatosis with inflammation [i.e. NASH (non-alcoholic steatohepatitis)], fatty liver disease with inflammation and fibrosis (severe NASH) and cirrhosis. The molecular mechanisms contributing to NASH are the subject of considerable investigation, as a better understanding of the pathogenesis of NASH will lead to novel therapies for a condition that hitherto remains difficult to treat. In the present issue of Clinical Science, Piguet and co-workers have investigated the effects of hypoxia in the PTEN (phosphatase and tensin homologue deleted on chromosome 10)-deficient mouse, a mouse model that develops NAFLD. The authors show that a short period (7 days) of exposure to hypoxia aggravates the NAFLD phenotype, causing changes in the liver that are in keeping with NASH with increased lipogenesis and inflammation.

Risk factors for NAFLD (non-alcoholic fatty liver disease) continue to increase with the epidemic of obesity and Type 2 diabetes. NAFLD is not a single disease, but represents a spectrum of fatty-liver-related diseases, extending from simple steatosis to steatosis with ballooned hepatocytes and lobular inflammation [NASH (non-alcoholic steatohepatitis)] to extensive hepatic fibrosis with regenerating nodules (cirrhosis). In some patients the liver condition may lead to the development of hepatocellular carcinoma. For researchers, clinicians and their patients, the key question is why do some people develop only simple steatosis without progression of the liver disease, whereas others develop a progressive liver condition with NASH developing and worsening over time to cirrhosis (for review see [1]).

In the present issue of Clinical Science, Piguet et al. [2] have investigated the effect of hypoxia on NAFLD. Specifically, the authors have tested the effect of relative hypoxia (10 % inspired oxygen) in a mouse model that is genetically susceptible to developing NAFLD to investigate the hypothesis that relative hypoxia worsens progression of NAFLD to NASH. Using mice with a specific hepatocellular deficiency in the PTEN (phosphatase and tensin homologue deleted on chromosome 10) gene, a tumour suppressor that is known to affect the insulin signalling pathway [3–5], the investigators have subjected the animals to 7 days of 10 % inspired oxygen tension, compared with 7 days of 21 % inspired oxygen in control PTEN-deficient animals. To prove that the animals were hypoxic, Piguet et al. [2] showed that there was a 10 % increase in the haematocrit. Affected animals were more insulin-resistant, lighter in weight, hypertriacylglycerolaemic, glucose-intolerant and had a marked increase in the Kleiner score (8.3 compared with 2.3 in control animals; \( P < 0.01 \)), a histopathological scoring system used to

Key words: \( \beta \)-oxidation, fatty liver, lipogenesis, non-alcoholic steatohepatitis (NASH), peroxisome-proliferator-activated receptor (PPAR), phosphatase and tensin homologue deleted on chromosome 10 (PTEN), sterol-regulatory-element-binding protein-1c (SREBP-1c).

Abbreviations: CYP2E1, cytochrome P450 2E1; HIF-2\( \alpha \), hypoxia-induced factor-2\( \alpha \); mTOR, mammalian target of rapamycin; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NEFA, non-esterified fatty acid (‘free fatty acid’); NF-\( \kappa \)B, nuclear factor \( \kappa \)B; PI3K, phosphoinositide 3-kinase; PPAR-\( \gamma \), peroxisome-proliferator-activated receptor-\( \gamma \); PTEN, phosphatase and tensin homologue deleted on chromosome 10; SREBP-1c, sterol-regulatory-element-binding protein-1c.

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quantify the severity of NAFLD, in keeping with NASH. In investigating the pathogenesis of hypoxia-induced NASH, they show that expression of key genes in the hepatic lipogenesis pathway were increased, whereas expression of a marker of β-oxidation [CPT-1 (carnitine palmitoyltransferase-1)] was decreased. The substrate proteins for the PTEN enzyme are PtdIns(3,4)P2 and PtdIns(3,4,5)P3, and the physiological function of PTEN is to dephosphorylate the second messengers generated by the activation of PI3K (phosphoinositide 3-kinase), thereby down-regulating or terminating insulin signalling downstream of PI3K [3]. Therefore the authors investigated the insulin signalling pathway to explore the effects of hypoxia on NAFLD.

Hypoxia induced an increase in phosphorylation status of the S6 ribosomal protein [2]. The S6 ribosomal protein belongs to the PI3K/Akt/mTOR (mammalian target of rapamycin) signalling pathway and is phosphorylated by p70 S6 kinase when this pathway is activated. It has been previously shown that a negative feedback loop operates from p70 S6 kinase to the upstream IRS (insulin receptor substrate)/PI3K/PDK1 (phosphoinositide-dependent kinase 1)/Akt insulin signalling pathway, suggesting a mechanism for the development of insulin resistance [6,7], and such a mechanism could operate in the model generated by Piguet et al. [2].

There is, however, still uncertainty as to the precise role of PTEN activity in regulating insulin sensitivity in insulin-responsive tissues. Overexpression of PTEN has been shown to have inhibitory effects on insulin signalling, including decreased Akt activation and GLUT4 (glucose transporter 4) translocation to the cell membrane [8,9]. Overexpression of PTEN in muscle from obese Fa/Fa Zucker rats had been shown to contribute to muscle insulin resistance in these animals [10]. In contrast, down-regulation of PTEN has the opposite effect with increased glucose uptake in fat and muscle in response to insulin [11]. In mice, liver-specific deletion of PTEN has been shown previously to increase insulin sensitivity yet, paradoxically, causes NAFLD and hepatocellular cancer [12,13]. The mechanism for this paradox is yet to be clarified, but there has been a number of hypotheses suggested, some of which highlight the lack of a negative regulation on the insulin signalling pathways by PTEN. In PTEN-deficient mice, there is increased synthesis and storage of triacylglycerol (triglyceride) in hepatocytes, perhaps due to up-regulation of PI3K/Akt activity [12,13] and, as a consequence of the lack of PTEN activity, there is increased hepatocyte fatty acid uptake, increased fatty acid synthesis and increased esterification of fatty acid to triacylglycerol.

The absent expression of liver-specific PTEN is associated with hepatic steatosis, inflammation, fibrosis and even tumours [5]. In one report, PTEN-deficient mice were shown to have biochemical and histological evidence of NASH, with fibrosis occurring at 40 weeks of age [5]. The mechanism for NASH in this animal model has been reported to be due to increased expression of PPAR-γ (peroxisome-proliferator-activated receptor-γ), SREBP-1c (sterol-regulatory-element-binding protein-1c) and downstream genes, including Akt and FOXO1 (forkhead box O1), resulting in increased lipogenesis, inflammation and fibrosis [5]. The reason for the increased fat deposition in the liver could be due partly to increased expression of SREBP-1c, as this is a key transcription factor for lipogenesis with increased ACC (acetyl-CoA carboxylase), FAS (fatty acid synthetase) and SCD-1 (sterol-CoA desaturase-1) enzyme activities, all of which act synergistically to promote fatty acid synthesis. Piguet et al. [2] show very similar effects in much younger 8-week-old female PTEN-deficient mice exposed to hypoxia for 7 days, suggesting that relative hypoxia accelerates many of the changes that have been observed in older PTEN-deficient mice developing NASH.

As a consequence of increased PPAR-γ expression, there is also a secondary induction of key enzymes involved in mitochondrial β-oxidation and, interestingly, Piguet et al. [2] show a hypoxia-induced increase in HIF-2α (hypoxia-induced factor-2α), with translocation of HIF-2α to the nucleus, that has been shown to inhibit β-oxidation. Whether this effect alters the known increase in ROS (reactive oxygen species) that occurs in PTEN-deficient mice is uncertain. PTEN-deficient mice have an increase in fat oxidation with a marked increase in the generation of oxidative free radicals, leading to inflammation and fibrosis, via activation of the NF-κB (nuclear factor κB) pathway [14,15]. It has been shown that there is a 7-fold increase in the hepatic concentration of H2O2 in PTEN-deficient mice compared with the wild-type [5] and, in their hypoxia model, Piguet et al. [2] suggest that the increase in CYP2E1 (cytochrome P450 2E1), which is known to increase oxidative stress, may contribute to the increase in inflammation observed in their model. Whether any change in CYP2E1 activity occurs as a consequence or cause of insulin resistance is not known. Moreover, the role of inflammation in vivo may influence the activity of PTEN to modulate insulin action further. In insulin-resistant subjects, there is increased plasma NEFA [non-esterified fatty acid (‘free fatty acid’)] concentrations with an increase in delivery and uptake of NEFAs by the hepatocytes. This results in the activation of the mTOR protein, which in turn leads to activation of NF-κB [4]. As both mTOR and NF-κB exist as a complex, there is a subsequent dissociation and then translocation of NF-κB into the nucleus, where it down-regulates the expression of PTEN at the level of transcription. The precise mechanism of how NF-κB transcriptionally down-regulates PTEN is not fully understood, but could be through the sequestration of the CBP (cAMP-response-element-binding protein-binding
Is hypoxia alone sufficient to cause NAFLD? To date, the evidence suggests that hypoxia aggravates the development of NAFLD only in susceptible animals, with worsening of NAFLD to a NASH-like phenotype. The last year has shown important advances in our understanding of factors affecting the aetiology and pathogenesis of NASH, such as the potential for the influence of early development to prime development of NASH [17]. Given the important clinical need to elucidate the mechanisms contributing to NASH and to subsequently develop treatments for NASH, the work of Piguet and co-workers [2] adds to our understanding of the potential interaction of exogenous fatty acids, PTEN deficiency and hypoxia in the pathogenesis of NASH (summarized in Figure 1). The challenge is now on to ascertain whether similar changes occur in people at risk of developing NAFLD.

REFERENCES


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Received 4 November 2009; accepted 10 November 2009
Published as Immediate Publication 10 November 2009, doi:10.1042/CS20090565