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Thyroid hormone receptor-β analogues for the treatment of metabolic dysfunction-associated steatohepatitis (MASH)

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Summary:

The association between suboptimal thyroid function ((sub)clinical hypothyroidism or low-normal thyroid function) and the metabolic syndrome and MASLD (metabolic dysfunction-associated steatotic liver disease) has been clearly established. Furthermore, in MASLD, intracellular thyroid hormone concentrations are low and the activation of the thyroid hormone receptor (THR) is reduced. Administration of thyroid hormone has been shown to reduce liver triglycerides by stimulating fatty acid disposal through lipophagy and beta-oxidation, and to lower LDL-cholesterol. As thyroid hormone exerts its effects in many different organs, including the heart and bone, several drug candidates have been developed as selective thyromimetics for the THR-β nuclear receptor with potent and liver-targeted activity. Importantly, these compounds have reduced affinity for the THR-α nuclear receptor and tissue distribution profiles that differ from endogenous thyroid hormones, thereby reducing unwanted cardiovascular side effects. The most advanced compound, resmetirom, is an oral drug that demonstrated, in a large phase III trial in patients with MASH (metabolic dysfunction-associated steatohepatitis), the ability to reduce liver fat, decrease aminotransferase levels and improve atherogenic dyslipidaemia with a good tolerability profile. This translated into histological improvement that led to accelerated approval of this drug for active fibrotic steatohepatitis, a milestone achievement as a first MASH drug.

Introduction

The complex pathophysiology of metabolic dysfunction-associated steatohepatitis (MASH) including intricate relationships with metabolic dysfunction, lipotoxicity, and insulin resistance has led to the exploration of multiple therapeutic pathways, with most drug candidates failing to secure approval despite solid biological rationale. The link between thyroid function and metabolic dysfunction-associated steatotic liver disease (MASLD), first described epidemiologically, then explored physiologically and, finally, exploited pharmacologically is one of the rare success stories that culminated in the first accelerated approval of a drug for MASH. Herein, we will review the level of epidemiological evidence that links low thyroid function to active fibrotic steatohepatitis, the physiological regulation of liver fat, inflammation and fibrosis by thyroid hormones, the pharmacological optimisation of selective thyromimetics and, finally, the clinical results when these compounds have been tested in patients with MASH. While data on longer-term benefit are yet to be determined and fully understood, thyroid hormone receptor (THR)-β agonists offer the first effective and safe therapeutic approach in this disease.

Epidemiological considerations of the link between thyroid dysfunction and MASLD

Thyroid function and the metabolic syndrome

The epidemiological association between thyroid function, the metabolic syndrome and MASLD covers three distinct conditions: overt hypothyroidism, subclinical hypothyroidism (SCH) and low thyroid function within the normal range.

Overt hypothyroidism is defined by an increased thyroid-stimulating hormone (TSH) level and a decreased free thyroxin (T4) level. Its prevalence in the general population is estimated at around 3%.[1] The association with weight gain, cardiovascular risk factors (such as increased weight circumference, arterial hypertension) and dyslipidaemia (high cholesterol and high LDL values) is well known[2] as is its impact on cardiovascular morbidity and mortality.[3]

SCH is defined by a serum TSH concentration above the upper limit of the reference range (>4.5 mIU/L) but with a normal serum free T4 concentration.[4] This well-recognised entity[5] is common in the general adult population with a prevalence ranging from 4.6% to 8.5%, which can be as high as 15% in the elderly.[6] People with SCH have a higher

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Keypoints

- Low thyroid function, which encompasses subclinical hypothyroidism (TSH 4.5 mIU/L, normal free T4) and low-normal thyroid function (TSH in the high-normal range, 2.5-4.5 mIU/L, normal free T4) is associated epidemiologically with components of the metabolic syndrome, insulin resistance, adiposity and MASLD. In patients with MASLD, there is an association with the severity of liver disease and, possibly, with cardiovascular mortality.
- Selective thyromimetics for the THR-β nuclear receptor with targeted liver actions are in development for MASH; the tissue distribution profile and reduced activity for the THR-α isoform ensure no or minimal cardiovascular side effects.
- Thyromimetics activate intracellular thyroid hormone signalling upon binding to the nuclear thyroid hormone receptor β which results in the stimulation of beta-oxidation and oxidative phosphorylation and, possibly, other direct pleiotropic effects on inflammation and fibrosis.
- Resmetirom, the first-in-class thyromimetic to be tested in MASH, improved histology (resolution of steatohepatitis and fibrosis reversal) in a large phase III trial which led to accelerated approval in this indication. The drug has antisteatotic properties and improved atherogenic dyslipidaemia without changes in weight or glycaemic control. A second phase III trial demonstrated a very good safety and an acceptable tolerability profile.

likelihood of progression to overt hypothyroidism than those with normal TSH levels <4.5 mIU/L. Based on cross-sectional studies, SCH has been associated with an increased risk of cardiovascular disease,[7] although this may be more substantial in younger individuals[8] or for higher TSH levels (>10 mIU/L), as shown in large individual patient data meta-analyses.[9] From the observation that both coronary heart disease (CHD) events and CHD mortality increase at TSH levels >10 mIU/L comes the recommendation to treat patients <70 years old, irrespective of symptoms, although a reduction of CHD has never been studied after thyroid hormone replacement. In the elderly, this has been investigated and thyroid hormone replacement has not been shown to improve wellbeing, while higher TSH has even been suggested to be protective in this population.[10] An increase in heart failure has also been described.[11] The association with the main components of the metabolic syndrome[12] is well documented:[13–15] obesity and visceral adiposity,[16] type 2 diabetes,[17] arterial hypertension[18,19] and hypercholesterolemia.[20] However, the directionality of the association has been debated,[12] as the association could be prone to residual confounders or reverse causality.

Low-normal thyroid function (LNTF) is defined by a normal serum free T4 concentration and a TSH concentration at a high value within the normal range (2.5-4.5 mIU/L). Even within the normal range, TSH is positively associated with BMI while free T4 displays a negative association,[21] thus confirming the relationship between adiposity and thyroid function. Moreover, rising TSH has been associated with increased weight gain over time.[22,23] It is hypothesised that the higher TSH concentrations that are found in obesity are due to an altered setpoint which is supported by Mendelian randomisation studies[24] and the fact that after bariatric surgery-induced weight loss, TSH decreases.[25] Higher TSH levels within the reference range have also been associated with worsening blood pressure, increases in lipid levels and an increased risk of metabolic syndrome.[15,26] Moreover, some studies have documented that higher TSH levels, even in the reference range, are predictive of higher mortality from coronary artery disease in females.[27] An association with changes in glomerular filtration rate and chronic kidney disease has also been described,[28] even after adjustment for multiple confounders.[29]

Thyroid function and MASLD

The association between low thyroid function, the metabolic syndrome and insulin resistance strongly suggested that MASLD may also be associated with (subclinical) hypothyroidism. Liangpunsakul and Chalasani were among the first to identify a possible relationship between hypothyroidism and MASLD.[30] Many studies, both in Western and Asian populations have since documented the association between low thyroid function and MASLD.[31] Importantly, these studies were also performed in the euthyroid general population thus highlighting that not only overt hypothyroidism but also SCH and LNTF are linked with MASLD.[32] The association was still significant in patients with type 2 diabetes[33] and after adjusting for the main metabolic risk factors[32,34] or for markers of insulin resistance.[35] It was documented for both increasing TSH levels[36,37] and decreasing T4 levels.[38,39] In fact the association between low thyroid function and MASLD appears to be bidirectional as some studies have shown, in turn, that individuals with MASLD, whether children or adults, have a higher prevalence of SCH than controls.[40–42]

However, the question of causality is not settled. Longitudinal studies in Chinese individuals with SCH have shown an increased risk of incident MASLD, even after adjustment for indicators of the metabolic syndrome. The risk was commensurate to increasing baseline TSH levels.[43] In contrast, Mendelian randomisation studies provided a more nuanced picture: some studies demonstrated a causal effect of genetically determined TSH levels[44,45] on the occurrence of MASLD, although the risk became non-significant after adjustment for BMI.[45] Other studies did not find such a relationship but documented a potential inverse causality: that genetically determined MASLD significantly increased the risk of higher TSH levels.[37] While waiting for a firm demonstration of directional causality, the reasonable assumption is that in practice, thyroid function should be evaluated in patients with MASLD, while the risk of MASLD should be monitored in patients with hypothyroidism and SCH, especially in overweight and obese patients.

In support of the epidemiological association, there is evidence that increased TSH levels are associated with the severity of MASLD. Some studies have documented a higher proportion of steatohepatitis (rather than steatosis) in patients with higher TSH levels.[42] In a large study of patients with

biopsy-proven MASLD, high-normal TSH levels increased the risk of steatohepatitis (specifically that of hepatocyte ballooning) by 61% while SCH increased the same risk by 2.3 fold.[46] Remarkably, the association was also true for increased fibrosis, whether documented by biopsy,[46,47] serum fibrosis markers[35,48,49] or liver stiffness:[50] a two-fold increase in the prevalence of elastography-defined significant fibrosis was documented in patients with TSH >2.5 mIU/ml (*i.e.* above low-normal values).[36] Although most associations with steatosis are found between TSH and free T4, as robust markers of the hypothalamus-pituitary-thyroid axis, triiodothyronine (T3) was also shown to be significantly related to liver fibrosis in a large population-based study. [50] Due to its lower abundance in serum and more difficult measurement, T3 is less commonly used in clinical practice; however, low serum levels of T3 represent low intracellular levels of T3, which are related to fibrosis.[59]

In a large US population from NHANES III with a median follow-up of 23 years,[51] SCH was associated with higher overall and cardiovascular mortality compared to normal thyroid function in patients with MASLD. This association was significant even after adjustment for insulin resistance. Interestingly, the association was not found in individuals without MASLD.[51] Further analyses of the same cohort confirmed that individuals with MASLD, whether with low normal or SCH, have increased all-cause and cardiovascular mortality.[39] There are several mechanisms by which low thyroid function may have an adverse impact on cardiovascular mortality:[49] change in adipocytokine levels,[52] endothelial cell and arterial dysfunction,[53] decrease in cardiac contractility,[54] and decreased left ventricular diastolic dysfunction during rest and exercise.[55] Precisely how these mechanisms are synergised by the association with MASLD remains to be determined.

Thyroid hormone physiology

Regulation of intracellular T3 availability and deiodinase activity in health and disease

The thyroid mainly produces the pro-hormone T4 which is converted intracellularly into the bioactive hormone triiodothyronine (T3) to activate THRs within the cell (Fig. 1). This production of thyroid hormone is negatively regulated by pituitary TSH. Therefore, TSH and free T4 are used as markers of the hypothalamic-pituitary-thyroid-axis to indicate thyroid hormone dysfunction. There is a THR inside virtually every somatic cell, with thyroid hormone signalling regulating many basic processes involved in development, growth and metabolism. The THR is part of the nuclear receptor family and is activated mainly by T3.[56] Signalling through THR involves not only ligand receptor binding but also transport across the cell membrane of the ligand and its intracellular bioavailability, which is tightly regulated by specific enzymes called deiodinases. In humans, most T3 (~80%) is produced peripherally from T4. Type 1 deiodinase (Dio1) and type 2 deiodinase (Dio2) convert T4 to active T3 intracellularly in a regulated manner. Deiodinase type 3 (Dio3) converts T4 to an inactive metabolite, reverse T3 (rT3). This intracellular metabolism of thyroid hormones is believed to determine thyroid hormone availability and to protect the cell from high intracellular bioactive thyroid hormone levels, such as those occurring during hyperthyroidism. Furthermore, deiodinases regulate local T3 production according to cellular demands. As an example, during neonatal development, a surge in hepatic Dio2 expression helps regulate the expression of genes involved in fatty acid, triglyceride, and cholesterol synthesis, and increases the expression of genes involved in bile acid synthesis. Also, in rodents, prevention of the Dio2 surge alters the expression of >100 genes in the adult liver (with absent Dio2 activity) leading to an increased susceptibility to a

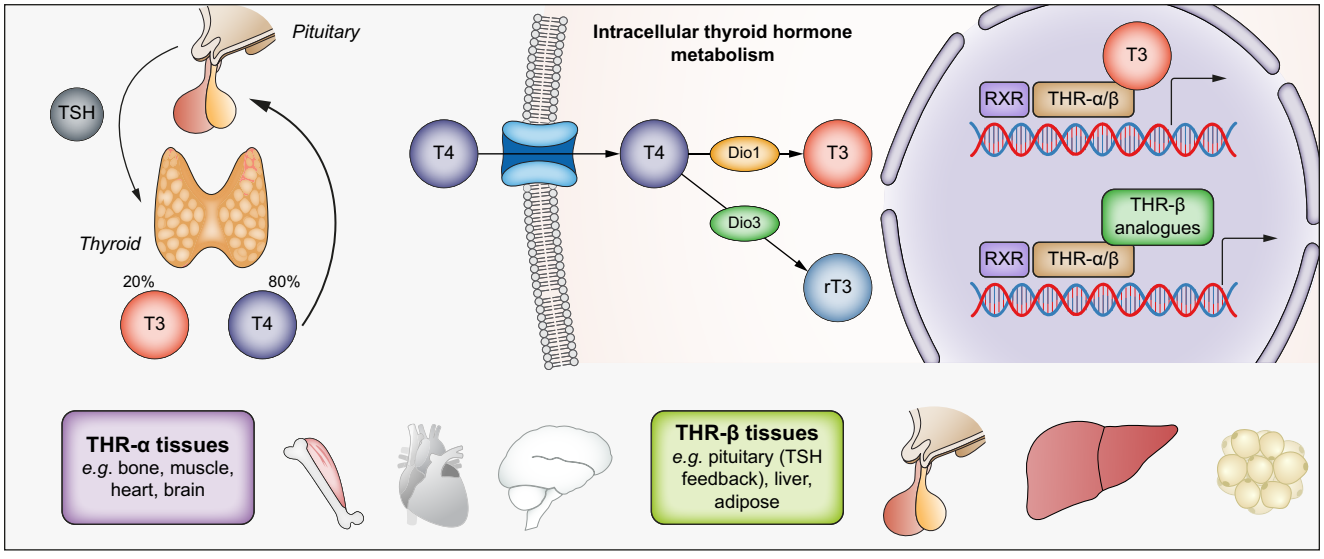

Fig. 1. Thyroid hormone metabolism. Thyroid hormone (T3 and T4) is produced by the thyroid after stimulation by TSH from the pituitary via a negative feedback loop. T3 and T4 are transported across the cell membrane in target organs by thyroid hormone transporters. Intracellularly T4 is converted to the active hormone T3 by deiodinases. Within the hepatocyte Dio1 is the major enzyme converting T4 to T3 and Dio3 converts T4 to rT3, an inactive form. Within the nucleus T3 binds to the THR to stimulate T3-mediated gene transcription (canonical signalling). THR-β analogues bind selectively to THR-β in target organs. Bone, skeletal muscle, heart and brain are organs expressing mainly THR-α whereas the pituitary and liver mainly express THR-β. Dio, deiodinase; rT3, reverse T3; T3, triiodothyronine; T4, thyroxine; THR, thyroid hormone receptor; TSH, thyroid-stimulating hormone.

high-fat diet. This shows that regulated T3 expression during development causes subsequent epigenetic modifications. [57] In adult life as well, T3 production is regulated by deiodinases: for instance, cold exposure in adults induces Dio2 expression in brown adipose tissue to increase local T3 production and trigger thermogenesis.

The main deiodinase converting T4 towards active T3 in the liver is Dio1 (Fig. 1). Single-cell RNA sequencing has established Dio1 expression mainly in hepatocytes and hepatic stellate cells with only minor contributions from macrophages and liver endothelial cells.[58,59] Dio1 activity is increased after administration of T3, decreased by inflammatory signals and modulated in MASLD. A high-fat diet induces hepatic Dio1 activity in mice on a western diet supplemented with fructose and in a hepatoma cell line cultured with a mixture of oleic acid and palmitic acid. Prevention of this Dio1 induction increases susceptibility to hepatic steatosis in mice fed a western diet supplemented with fructose, suggesting an early compensatory role of Dio1 against steatosis formation.[60] Concordant with these findings is the documentation of reduced Dio1 expression in advanced, fibrotic stages of human MASLD or in animal models of steatohepatitis.[59,61] The exact mechanism of reduced Dio1 expression is unknown but could involve both reduced intracellular thyroid hormone levels (which are positive regulators of Dio1 expression) and reduced Dio1 expression by inflammatory signals. In a human hepatoma cell line (HepG2 cells), treatment with the pro-inflammatory cytokine IL1-β reduces Dio1 activity.[62] Therefore, Dio1 plays a role both in nutrient excess and during inflammation; however, it needs to be established whether these changes are always compensatory or whether they can also be detrimental.

In hepatic stellate cells, Dio3 is highly expressed during liver injury.[59] Dio3 is involved in liver regeneration and inactivates T4 to generate the inactive metabolite rT3. The upregulation of hepatic Dio3 has been observed after partial hepatectomy in rodents and might represent a foetal-like reprogramming to induce regeneration.[63] Similar induction of Dio3 expression – mediated via hypoxia-inducible factor-dependent pathways – has been observed after myocardial infarction. [64] In liver fibrosis, Bohinc *et al.* showed that sonic hedgehog signalling increases Dio3 expression and decreases Dio1 expression.[59] Both decreased T3 production by Dio1 and increased T4 inactivation by Dio3 result in reduced intracellular thyroid hormone action in the liver, as previously shown in MASLD.[65,66]

Collectively these data show that a major determinant of thyroid hormone action is their intracellular bioavailability at the tissue level. This is particularly important in MASLD and possibly other chronic inflammatory conditions of liver injury, since decreased production of active T3 due to Dio1 downregulation and increased T4 inactivation by Dio3 result in an intrahepatic deficit of thyroid hormone activity that may impact lipid handling and promote inflammatory and fibrotic disease progression.

THR binding and development analogues

Genomic or canonical thyroid hormone action results from binding of ligand (T3) to the THR and subsequent binding of THR to cognate response elements in the regulatory regions of target genes (Fig. 1). In the absence of ligand, the THR recruits co-repressors such a NCor and SMRT which prevent transcription. Upon ligand binding, activator proteins are recruited with subsequent transcriptional activation of thyroid hormone target genes. Apart from this classical THR activation, other types of thyroid hormone action have been established including liganded THR exerting its action in the cytoplasm without DNA binding or binding of thyroid hormones to proteins in the plasma membrane or cytoplasm, independent of THR.[67] As the latter non-genomic or non-canonical binding of thyroid hormone does not require gene transcription or protein synthesis, it may account for rapid – within minutes – effects occurring after thyroid hormone administration.[67] It has been suggested that liver triglycerides are regulated via these non-canonical thyroid hormone actions. [68]

There are two types of THR exerting thyroid hormone action: thyroid hormone receptor-α1 (THR-α) and thyroid hormone receptor-β1 (THR-β) (Fig. 1). The predominant THR subtype expressed in the liver and pituitary is THR-β whereas THR-α predominates in the heart, bone, and skeletal muscle.[56] It was also shown that in adipose tissue THR-β mediates the effects of T3 on intracellular metabolism of glucose and lipids.[69] Mutations in THR-β result in impaired thyroid hormone signalling known as Refetoff's syndrome or resistance to thyroid hormone (RTH). Interestingly, dysfunction of THR-β has been linked to fat accumulation in the liver. Mice with this mutated THR-β display excessive lipid accumulation.[70] In humans, two separate studies have shown that RTH is associated with increased liver fat content.[71] Conversely, it has recently been shown in liver bulk RNA sequencing that THR-β activity is a critical suppressor of human MASLD progression.[66] Data presented above demonstrate that reduced intracellular thyroid hormone action increases liver steatosis. Based on this observation there have been several attempts to treat MASLD by increasing thyroid hormone action in the liver. To this end, euthyroid patients have been treated with synthetic thyroxine (LT4) which is converted into T3 locally and can serve as a ligand for the THR.[61] LT4 was titrated to suppress TSH into the low-normal range via negative feedback mechanisms. Liver fat was indeed reduced in this 16-week study. Although no changes in heart rate were observed, long-term effects on THR-α-expressing organs such as the heart (arrythmias) and bone (osteoporosis) cannot be ruled out. Thus, an optimal approach would not only specifically target THR-β with high selectivity over THR-α, but also have hepatic restricted actions in order to avoid dysregulation of the hypothalamus-pituitary-thyroid axis. Thyromimetics are synthetic compounds with THR-β subtype specificity that can restore thyroid hormone activity in hepatocytes (liver cells).

Putative mechanisms of action of thyroid hormones and thyromimetics in MASLD

Metabolic effects of T3 and thyromimetics in pre-clinical models

Triglycerides – Pre-clinical studies have shown clear effects of T3 and thyromimetics on reducing hepatic steatosis.[72–74] This is thought to be mediated by increased beta-oxidation of free fatty acids derived from triglycerides in lipid droplets (Fig. 2). Thyroid hormone is involved in the breakdown of triglyceride-containing lipid droplets by stimulating lipolysis, thereby leading to the release of free fatty acids and allowing for entry of

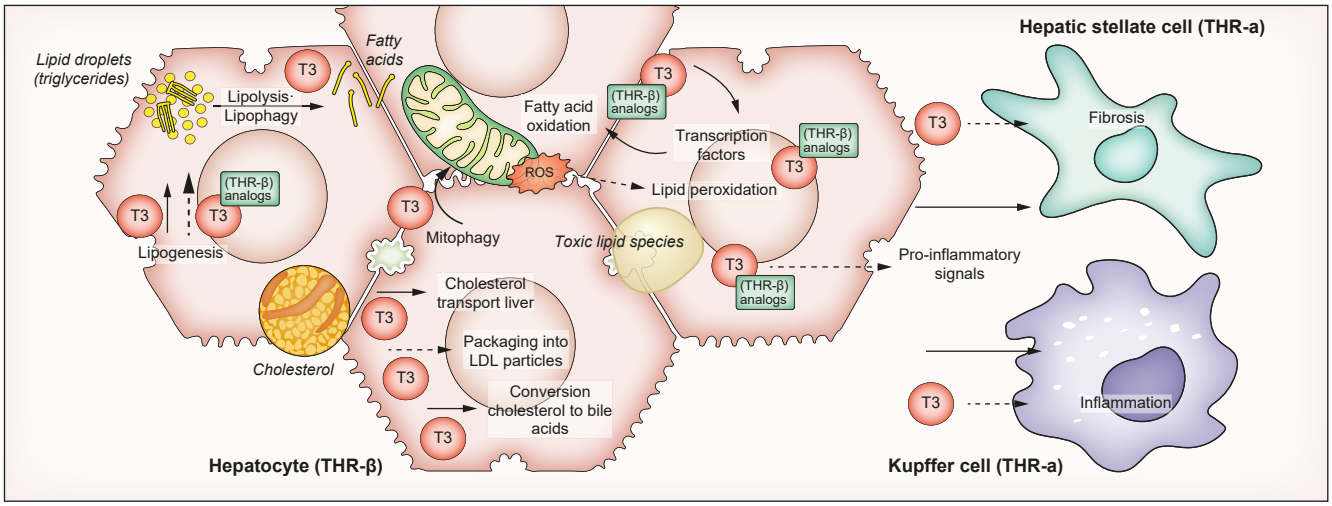

Fig. 2. Known mechanisms by which thyroid hormone (T3; orange circles) and THR-β agonists (green box) exert their beneficial effects on steatosis, hypercholesterolemia, inflammation and fibrosis. Lipid droplets consisting of triglycerides are hydrolysed by classical lipolysis and lipophagy known to be stimulated by T3. These fatty acids are shuttled towards mitochondria in which thyromimetics and T3 are known to stimulate beta-oxidation of fatty acids both directly and via transcription factors (*e.g.* PGC-1α). Furthermore, T3 is known to stimulate recycling of damaged mitochondria through mitophagy. T3 and thyromimetics are known to inhibit ROS-induced lipid peroxidation reducing the amount of toxic lipid species. T3 and thyromimetics inhibit pro-inflammatory signals (*e.g.* NLRP3 inflammasome, NF-KB, Jak-STAT3 signalling). Although T3 and thyromimetics are known to reduce inflammation and fibrosis it is unknown whether these effects depend on direct effects on macrophages and hepatic stellate cells, which both predominantly express THR-α. The effect of thyroid hormone on lipogenesis has been controversial, both showing stimulation of lipogenesis through CHREBP and inhibition of lipogenesis through SREBP. T3 has also shown beneficial effects on cholesterol metabolism stimulating cholesterol uptake by the liver and conversion towards bile acids and inhibiting packaging of LDL into LDL particles. Arrows: stimulatory effects, dashed arrows inhibiting effects of T3/THR agonists. ROS, reactive oxygen species; T3, triiodothyronine; THR, thyroid hormone receptor; TSH, thyroid-stimulating hormone.

fatty acids into mitochondria where they undergo beta-oxidation. It has been shown that the classical pathway of lipolysis is activated by increased expression of lipolytic enzymes (adipose tissue triglyceride lipase, hepatic lipase and zinc-α2-glycoprotein). In the context of thyroid hormone signalling, this classical pathway of lipolysis seems less important for the mobilisation of fatty acids compared to lipophagy, a process involving autophagy of lipids.[75] During lipophagy, intracellular lipid droplets are engulfed by the autophagosome and fuse with the lysosome, leading to the release of fatty acids.[76] This mechanism of lipophagy and increased beta-oxidation after T3 has also been shown to be important in a MASH animal model.[74] Thyroid hormone subsequently regulates the entry of fatty acids into mitochondria and beta-oxidation by upregulation of mitochondrial proteins (carnitine palmitoyltransferase 1A, mitochondrial trifunctional protein, medium-chain acyl-CoA dehydrogenase, pyruvate dehydrogenase kinase isoform 4, mitochondrial uncoupling protein 2). In mice treated with a high-fat methionine-and choline-deficient diet together with T3, the expression of peroxisomal acyl-CoA oxidase, the rate-limiting enzyme in peroxisomal beta-oxidation, was increased.[72] Increased beta-oxidation has also been observed after the administration of the thyromimetics VK2809 (also known as MB07811) *in vivo* and resmetirom *in vitro*.[73,77] Furthermore, thyroid hormone indirectly stimulates beta-oxidation by stimulation of liver transcription factors (*e.g.* peroxisome proliferator-activated receptor gamma coactivator 1-α [PGC-1α], ERRα [oestrogen-related receptor α], peroxisome proliferator-activated receptor α, and fibroblast growth factor 21).[78–80] Together with the stimulation of lipophagy and beta-oxidation, thyroid hormone stimulates the autophagy of damaged mitchochondria (mitophagy) and reduces reactive oxygen species production through ERRα.[79] Apart from stimulation of beta-oxidation, T3 has also been shown to decrease the protein levels of L-FABP in NAFLD models, regulating the uptake and transport of fatty acids for glycerolipid synthesis.[72]

It remains unclear whether thyroid hormone concurrently stimulates beta-oxidation and lipogenesis. It was shown that thyroid hormone stimulates lipogenesis via the transcription factor ChREBP.[81] Recently it was shown that VK2809 and T3 reduced the other important lipogenic pathway via SREB1c, indicating lipogenesis as a possible additional contributor to reduced steatosis.[73,74]

Cholesterol – Thyroid hormone is well known to reduce levels of the atherogenic lipid LDL-cholesterol. This is thought to be regulated at many levels including increased cholesterol clearance, decreased cholesterol secretion and increased reverse cholesterol transport to convert cholesterol into bile acids (Fig. 2). Thyroid hormone increases the expression of cholesteryl ester transfer protein and expression of hepatic LDL receptor, thus regulating clearance from serum, and negatively regulating the expression of both sterol O-acyltransferase 2 and apolipoprotein (Apo)B100, thus inhibiting re-esterification and packaging of cholesterol into VLDL and LDL. Furthermore, thyroid hormone has been shown to regulate PCSK9, promoting the recycling of the LDL receptor and contributing to lower serum levels of cholesterol.[75]

Glucose and insulin – The impact of thyroid hormone actions and thyromimetics on insulin resistance is incompletely understood. Insulin resistance and subsequent hyperinsulinemia cause excess substrate mobilisation of free fatty acids, which has been shown to be the major contributor to

increased hepatic *de novo* lipogenesis.[82] Associations between insulin resistance and hepatic steatosis have been clearly shown, though direct causality has been debated.[83] Thyroid hormone and thyromimetics reduce liver steatosis without affecting insulin resistance in humans.[84] In pre-clinical models, the thyromimetic GC1 (also known as sobetirome) significantly reduces steatosis while increasing fasting glucose and insulin.[85] Additionally, hyperinsulinemic euglycemic clamp explorations documented reduced suppression of endogenous glucose production, an indication of increased insulin resistance.[85] It is well known that thyroid hormone induces gluconeogenesis, and VK2809 increases PGC-1α but this does not appear to explain the increased insulin resistance.[73,85] Alternative explanations such as suppressed Akt phosphorylation after GC1 treatment[85] have been proposed. Although clinical trials do not support worsened glycaemic control it remains intriguing that a reduction of steatosis is not accompanied by an improvement of insulin resistance.

Anti-inflammatory effects of T3 and thyromimetics in pre-clinical models

Apart from the aforementioned effects of T3 and thyromimetics on metabolic parameters, thyroid hormone (analogues) may also have independent effects on inflammation. In a mouse model, T3 not only prevents steatohepatitis prophylactically, but it can also revert inflammation and fibrosis once established.[74] Increased lipid storage in hepatocytes induces the production of reactive oxygen species, leading to lipid peroxidation. Both T3 and the thyromimetic GC-1 can reduce lipid peroxidation products in a murine MASH model.[72] Hepatic oxidative stress, which is increased in mice with steatohepatitis, is reduced by administration of T3. [74] The inflammatory mediators JNK (Jun N-terminal kinase) and STAT3 (signal transducer activator of transcription-3) are phosphorylated in models of MASH. T3 almost completely inhibits the phosphorylation of these proteins.[72,74] Moreover, T3 reduces the mRNA and protein expression of MASH-induced NLRP3 inflammasome components NLRP3, ASC1, caspase 1, and Toll-like receptor 4. This may be mediated by increased AMPK phosphorylation.[74] Resmetirom inactivates the NF-κB and Jak-STAT3 signalling pathways in an *in vivo* MASH model.[86] This effect may be mediated via RGS5, a member of the regulators of G protein signalling family, which is involved in the regulation of inflammation.[86] T3 and GC-1 have been shown to stimulate TREM2 expression which produces a phenotypic switch in macrophages to an anti-inflammatory, restorative state.[87]

In summary, thyroid hormones and thyroid hormones analogues exhibit anti-inflammatory activities in addition to their metabolic effects. Since metabolic improvement of metabolic dysfunction can also result in less inflammation, disentangling these two effects is challenging.

Antifibrotic effects of T3 and thyromimetics in pre-clinical models

In murine MASH models, T3 administration results in a significant reduction of fibrosis as measured by fibrotic markers such as *Tgfb1* and *Col1a1* mRNA expression and liver hydroxyproline content.[74] Interestingly similar effects have been reported in non-metabolic liver fibrosis models, thus raising the possibility of a direct antifibrotic effect of T3, possibly mediated by hepatic stellate cells. In a carbon tetrachloride-induced model of fibrosis, thyroid hormone inhibited the pro-fibrotic TGF-β/SMAD pathway and reduced liver fibrosis.[88] In a bleomycin induced model of lung fibrosis, T3 improved mitochondrial function and suppressed mitochondria-regulated death pathways. These effects were dependent on intact PGC-1A and PINK1 pathways.[89] Improved mitochondrial function after T3 administration also potentially contributes to the antifibrotic effects in the liver.[74] While the metabolic actions of T3 do not appear to mediate the effects on fibrosis, the anti-inflammatory effects described above could certainly contribute to the reduction in liver fibrosis.

Recently it has been reported that THR-α modulates the activation of hepatic stellate cells and their response to pro-fibrogenic cytokines such as TGF-β.[90] This may suggest that the antifibrotic effects of THR-β-selective thyromimetics may not be exerted directly through stellate cells, but rather through effects on hepatocytes which preferentially express the β isoform of the THR.[90]

Pharmacology of thyromimetics

The use of thyromimetics as therapies for diseases other than thyroid hormone deficiency has been contemplated for decades. In the 1960s, a large clinical study dubbed the Coronary Drug Project assessed whether the unnatural D-enantiomer of T4 could extend life in euthyroid patients with coronary artery disease.[91] Rather than extending life, the treatment arm showed increased mortality, making it clear that the beneficial effects of T4 and T3 treatment in euthyroid patients are inseparable from the adverse effects associated with thyrotoxicosis. This prompted research aimed at discovering T3 analogues that retained the beneficial actions of excess T3, such as lipid lowering and antifibrotic activity, while sparing the adverse effects of excess T3 on the heart, bone, and skeletal muscle. Eprotirome was the first significant clinical stage thyromimetic to surface from these efforts and, in a 2010 phase II study, it was shown to significantly lower LDL-cholesterol levels in hypercholesterolemic patients with an acceptable safety profile.[92,93] Around this time, a different thyromimetic called sobetirome similarly showed potent LDL-cholesterol-lowering activity in patients without the anticipated thyrotoxicosis-related adverse effects.[94,95] Although these agents demonstrated potent liver-specific T3 action evidenced by LDL-cholesterol lowering, which occurs via liver-mediated reverse cholesterol transport, along with acceptable safety profiles in humans, neither were pursued to clinical approval. Eprotirome development was discontinued in phase III due to unanticipated cartilage abnormalities in non-clinical chronic toxicology studies,[96] and sobetirome was discontinued by the sponsor after phase I for strategic business reasons.

During the time eprotirome and sobetirome were being studied clinically as cholesterol-lowering agents, it was not widely appreciated that fatty liver disease and MASH represented major unmet clinical needs. Following the discovery of eprotirome and sobetirome, two other thyromimetics were discovered, resmetirom (MGL-3196) and VK2809, which progressed to late clinical stage development, and approval in the case of resmetirom, as MASH therapeutics. Like eprotirome and sobetirome, both of these thyromimetics bind and activate

the THR-β subtype in preference to THR-α, which is thought to be important for avoiding adverse T3-like effects in the heart, bone, and skeletal muscle.

A pharmacological property that is shared between resmetirom and VK2809, and clearly differentiated from eprotirome and sobetirome is reduced binding affinity and potency at THRs (Table 1). Eprotirome and sobetirome have THR affinity and potency that is similar to T3 in contrast to the affinity and potency at THR for resmetirom and VK2809 which is 2-3 orders of magnitude lower than that of T3 (Table 1). This point of differentiation is reflected in the dose ranges studied in human clinical trials. Human LDL-cholesterol lowering occurred in the 0.025-0.1 mg range with eprotirome and sobetirome whereas doses of 5-100 mg were needed to produce LDL-cholesterol lowering in humans with resmetirom and VK2809.[97,98]

Table 1. Selectivity and potency of thyromimetics.

Thyromimetic drug	Clinical dose (mg/day)	THR-β selectivity (fold *vs.* THR-α)	THR potency *vs.* T3
Sobetirome	0.025-0.1	10	T3-like
Eprotirome	0.1	Modest	T3-like
Resmetirom	80-100	30	<<<T3
VK2809	5	16	<<T3

THR, thyroid hormone receptor; T3, triiodothyronine.

Resmetirom has a similar molecular shape to T3 but differs substantially with the triazine-carbonitrile heterocycle replacing the carboxylic acid-containing alanine side chain of T3 (Fig. 3). Eprotirome and sobetirome which have similar THR affinity and potency to T3 also have carboxylate-containing substituents at this position. The resmetirom structure also contains an unusual dihydropyridazinyloxy heterocycle designed to mimic the phenolic outer ring of T3. These structural changes reduce resmetirom binding affinity for both THR-α and THR-β compared to T3 in a cell-free coactivator recruitment ligand binding assay.[99] Resmetirom binds with 14- and 374-fold lower affinity to THR-β and THR-α, respectively compared to T3. Resmetirom affinity for THR-β is about 30-fold higher than its affinity for THR-α,[97,99] which is desirable as previously mentioned for thyromimetics that target the liver. A second less than ideal physicochemical property of resmetirom in addition to on-target potency is solubility. The reported solubility of 0.0004 mg/ml at pH 7 is very low for an orally administered drug and suggests that resmetirom will have low permeability.[99] Because the liver is the first organ to receive absorbed orally administered drugs, this may be a benefit in that low

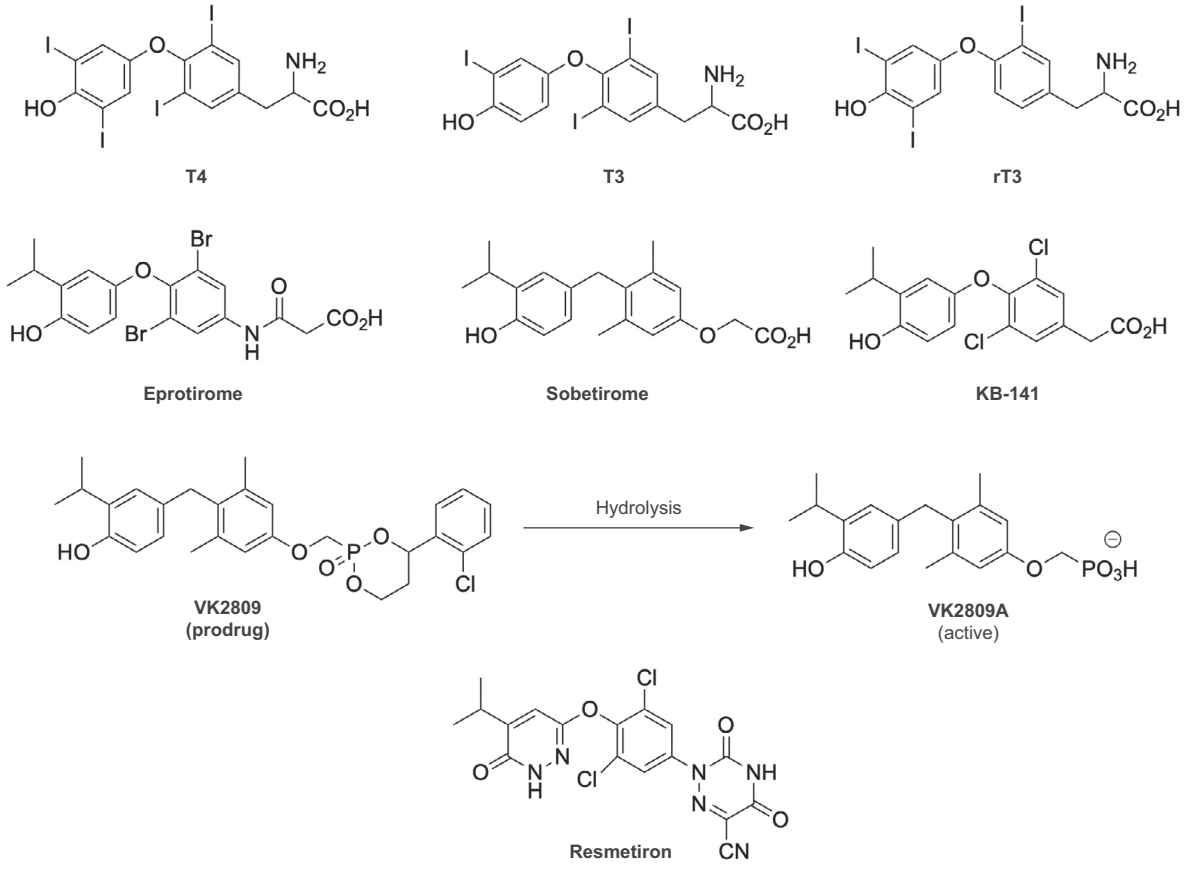

Fig. 3. Thyroid hormone and thyromimetics. Chemical structures of thyroid hormones and some synthetic T3 analogues, or thyromimetics. T4 is the predominant form of thyroid hormone in circulation and is converted to the active form T3 mostly in target tissues by activating Dio2. T4 can also be converted to the inactive metabolite rT3 by inactivating Dio3. Eprotirome, sobetirome, and KB-141 are first-generation thyromimetics discovered in the 1990s. Eprotirome and sobetirome were pursued in clinical development but discontinued after phase II for eprotirome and phase I for sobetirome. VK2809 is a prodrug that releases the active T3 agonist VK2809A upon selective enzymatic hydrolysis in the liver. VK2809 is currently being studied in phase II for NASH. Resmetirom is the first synthetic, non-natural T4 thyromimetic to be approved for clinical use. Dio, deiodinase; rT3, reverse T3; T3, triiodothyronine; T4, thyroxine.

permeability could limit resmetirom exposure in organs other than the liver where T3-driven adverse effects could arise. In a phase I multiple ascending dose study, doses of resmetirom from 5-200 mg were administered to healthy individuals, resulting in an up to 30% decrease in LDL-cholesterol after 14 days of once-daily oral dosing.[97] All doses were well-tolerated with a small ~20% decrease in free T4 at the highest dose. The sustained 20% decrease in free T4 as well as a decrease in T4 metabolites was also present with no change to TSH. T4 depletion with normal TSH is a hallmark of drugs that induce central hypothyroidism by initially suppressing TSH at the pituitary, leading to decreased production of T4 from the thyroid gland.[100]

The interplay between the decrease in endogenous thyroid hormones and the increase in blood levels of T3 agonistic drugs leads to inappropriate pituitary TSH secretion classic to drug-induced central hypothyroidism. While this small 20% depletion of T4 at a dose of 80-100 mg of resimetirom does not appear to coincide with safety signals related to hypothyroidism, this indicates that resmetirom is exiting the liver into the blood and producing T3 agonistic action at the pituitary.

VK2809 is a prodrug of an active THR-binding ligand (VK2809A) and the conversion of VK2809 to VK2809A occurs via an enzyme called cytochrome P3A4 (CYP3A4) abundantly expressed in the liver and gastrointestinal tract (Fig. 3). The chemical structure of the active thyromimetic VK2809A is identical to sobetirome with the one exception of a phosphonic acid in place of the carboxylic acid of the oxyacetic acid side chain. This chemical change results in a decrease in THR-binding affinity and THR potency for VK2809A compared to sobetirome and similar carboxylic acid-containing thyromimetics.[101] This is reflected in an approximate 100-fold lower potency for VK2809 to reduce cholesterol in diet-induced obese mice compared to KB-141, a carboxylate-containing thyromimetic with similar THR affinity and potency to sobetirome.[102] The liver selective conversion of VK2809 to VK2809A is designed to provide therapeutic exposure of the active thyromimetic in the liver, while reducing thyromimetic exposure in non-hepatic tissue. This is borne out in rats which show fewer heart and skeletal muscle T3-like effects when dosed with VK2809 *vs.* T3 or KB-141.[101] In addition, VK2809 reduced liver fat in mouse and rat fatty liver models without increasing heart rate or heart weight.[73] However, substantial dose-dependent systemic T4 and T3 depletion by VK2809 is observed in mice, indicating that T3-like thyromimetic action is occurring at the pituitary, resulting in TSH suppression leading to central hypothyroidism at high doses of VK2809.[101] This could arise from either the active thyromimetic VK2809A escaping the liver or conversion of the prodrug VK2809 in the pituitary by an enzyme other than CYP3A4 capable of cleaving phosphonate esters. At a liver steatosis-reducing dose, VK2809 showed no effect on pituitary *TSH* mRNA, a reproducible marker of hypothalamus-pituitary-thyroid axis regulation.[73]

In a phase I multiple ascending dose study, VK2809 was administered orally to healthy individuals with elevated cholesterol in the dose range of 0.25-40 mg/day for 14 days.[98] Significant reductions in LDL-cholesterol, triglycerides, ApoB, and lipoprotein (a) were observed at doses >5 mg, again demonstrating similar findings to other clinical stage thyromimetics albeit with decreased potency in humans for VK2809 compared to eprotirome and sobetirome.

Clinical data for thyromimetics in MASH

Resmetirom, the first-in-class thyromimetic for MASH, has been tested in patients with histologically confirmed MASH in a phase IIb trial[103] (125 patients randomised) including an open-label part[104] (31 patients included) and two phase III placebo-controlled randomised trials, one in patients with phenotypically defined MAFLD[105] (MAESTRO-NAFLD trial, 972 patients randomised) and one in an histologically confirmed MASH registrational trial[84] (MAESTRO-NASH, 966 patients randomised). A randomised, phase III trial in patients with MASH cirrhosis is ongoing (NCT05500222). These trials provided a wealth of information on the clinical effects of the drug and how it could be used in clinical practice.

The therapeutic effect of resmetirom is clearly dose dependent, with 60 mg daily being less effective than 80 mg daily, which is less effective than 100 mg daily. The optimal dose was determined by studying both plasma drug exposure – evaluated by plasma drug AUC – and liver exposure – evaluated by plasma sex hormone-binding globulin (SHBG) levels. SHBG is a target gene of THR-β signalling; thus, an increase, now set at >120% over baseline levels, is considered a pharmacodynamic marker of sufficient hepatic exposure. The dose dependency, well documented by the open-label extension study,[104] applies to liver fat content reduction but also to aminotransferase and lipid improvements, as well as to histological effects on MASH. However, it should be noted that these increases in SHBG result in increased concentrations of oestrogen (males) and total testosterone (males and females).[84] Weight is an important determinant of drug exposure and the prescribing information advises a dose of 100 mg daily for patients weighing more than 100 kg and 80 mg daily for those below that weight.[106]

Histological efficacy was demonstrated in two separate trials. The phase IIb 36-week trial tested a single 80 mg dose *vs.* placebo (n = 125 patients total), providing a clear indication of improvement of disease activity defined as resolution of steatohepatitis: 27% in the active arm *vs.* 6% in the placebo arm. This was confirmed as a primary endpoint in an intention-to-treat analysis in a much larger 1 year, phase III trial[84] with two pathologists: 29.9% for the 100 mg arm *vs.* 9.7% in the placebo arm. However, the definition of NASH (previous term for MASH) resolution used in these two trials includes a >2 point reduction in NAFLD activity score (NAS). Without this requirement, *i.e.* using the same FDA definition of NASH resolution as other trials,[107–110] the rate of response for the 100 mg arm was 36% *vs.* 13% in the placebo arm for one of the pathologists and 24% *vs.* 9% for the other[106] (Fig. 4). Fibrosis improvement (without worsening of total NAS) was not shown in the phase IIb trial but was suggested by AI-assisted digital pathology, especially after correction for liver volume and steatosis[111] (both significantly reduced by the antisteatogenic actions of resmetirom). The larger phase III trial confirmed a reduction in fibrosis in the 100 mg arm (25.9% *vs.* 14.2% in the placebo arm).[84] Again, using the standard FDA definition (which

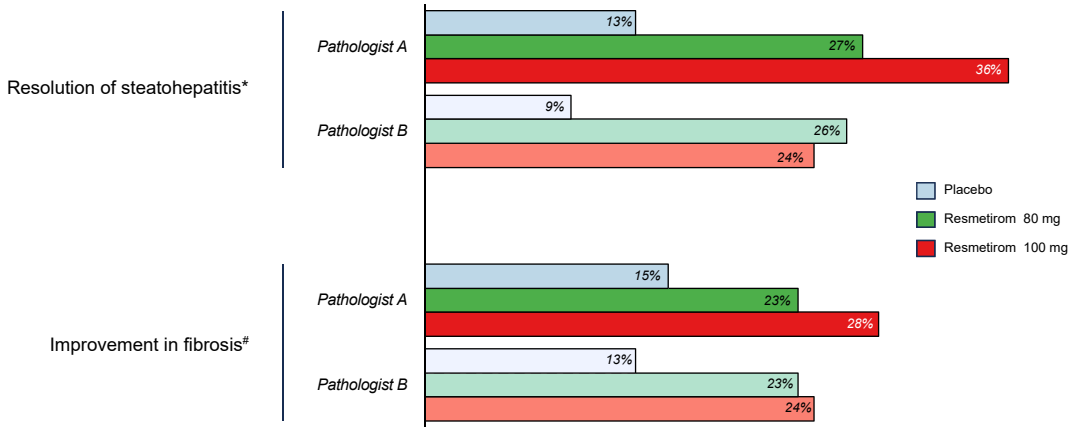

Fig. 4. Proportion of histological responders to resmetirom in the phase III MAESTRO-NASH registrational trial (adapted from[104]). Patients with missing liver biopsy at month 12 are considered non-responders. Resolution of steatohepatitis was defined as a score of 0–1 for inflammation, 0 for ballooning, and any value for steatosis according to the NASH-CRN classification. Improvement in fibrosis was defined as a reduction in fibrosis stage by 1 stage or more according to the NASH CRN classification. Both doses of resmetirom were significantly superior to placebo for both endpoints. *And no worsening of liver fibrosis; #and no worsening of steatohepatitis defined as no increase in score for ballooning, inflammation, or steatosis. Solid bars: pathologist A; hatched bars: Pathologist B. Both pathologists independently read the liver biopsies for each patient.

requires no worsening of NAS components rather than of the total score) the antifibrotic effect was confirmed: 28% *vs.* 15% for placebo for one of the pathologists and 24% *vs.* 13% placebo for the other when applied exclusively to the fibrosis stage 2 and stage 3 population[106] (Fig. 4). Improvement in fibrosis was also corroborated by non-invasive tests including magnetic resonance elastography, vibration-controlled transient elastography, ELF (enhance liver fibrosis) score and ProC3.[84] Importantly, at the 100 mg dose, improvement in fibrosis occurred independently of diabetes status, stage of fibrosis (stage 2 or 3) or weight loss changes during the trial. However, data is still needed to determine if the effect is as strong in patients on GLP1 receptor agonists as it is in those not receiving these medications.

A salient finding from the resmetirom clinical programme was that histological resolution of steatohepatitis predicted fibrosis improvement. Even in the phase IIb trial, which was overall negative for fibrosis improvement, patients who had resolution of steatohepatitis also had reduced fibrosis: 61% of those with MASH resolution also had 1 stage or more fibrosis improvement and 56% no longer had fibrosis (stage 0).[103] In fact, MASH resolution predicted fibrosis improvement better than the proton density fat fraction (PDFF) response, since only a third of those with a PDFF reduction >30% had fibrosis reversal.[103] One explanation put forth was that PDFF measures hepatic triglycerides and not specifically bioactive lipotoxic species and therefore PDFF reduction may not be an accurate surrogate of lipotoxic fat reduction. Nonetheless, for agents that act by reducing liver fat, PDFF reduction is still a good indicator of histological improvement: in the phase III trial, patients who did not achieve at least a 30% reduction in PDFF did not perform better than those receiving placebo for both primary histological endpoints. Conversely when PDFF declined by >30%, not only steatohepatitis resolution but also fibrosis improvement occurred more often than in the placebo arm. Moreover, earlier data have shown that the more profound the hepatic fat content reduction (*e.g.* 50% instead of 30%) the higher the histological response.[103,104]

Since both phase II and III trials incorporated serial measurements of PDFF and alanine aminotransferase (ALT) some insight into the kinetics of improvement on resmetirom is available. Most of the reduction in liver fat occurs in the first 16 weeks with only a marginal additional gain after 1 year.[105] Instead, the reduction in ALT follows a more delayed and protracted course. This should help refine prediction of response based on PDFF and ALT changes.[112]

As expected from earlier studies,[75] resmetirom improved multiple atherogenic lipids and lipoproteins (19% reduction for LDL-cholesterol, 21% reduction for ApoB, 27.5% reduction for triglycerides) including ApoC3 and lipoprotein (a),[105] small dense LDL, large VLDL and chylomicrons.[103] This reduction occurred after 6 months and was maintained at 1 year. No changes in body weight, nor improvements on glycaemic control (HbA1c) and insulin resistance (HOMA-IR) were observed after treatment. While glycaemic parameters did not change, there was a 33% increase in adiponectin tentatively explained by a decrease in hepatic turnover[103] or by a bona fide action of resmetirom on the adipose tissue.[113]

The effects of resmetirom on the pituitary/thyroid axis have been studied in detail. In patients with MASH, rT3, a marker of hepatic inflammation, is increased and the ratio of free T3/rT3 is reduced compared to control individuals, while the ratio of free T3/rT3 declines with fibrosis stage.[104] Treatment with resmetirom significantly reduced rT3 and increased the ratio of free T3/rT3,[104] which may reflect a correction of endogenous hepatic thyroid hormone activity. Resmetirom decreased free T4[104] with a minor change in TSH in the 80 mg group (-0.18 mIU/L) and no significant change in the 100 mg group.[84] The mechanisms accounting for these changes are under investigation. One possibility is that the thyromimetic increases Dio1 expression which converts T4 to T3. This is also shown by decreased rT3, the preferred substrate of Dio1, after resmetirom treatment. Another possibility is that the thyromimetic is acting on THR at the pituitary to suppress thyroid gland production of T4 by suppressing TSH secretion. While the initial studies of resmetirom excluded

patients with an L-thyroxine intake >75 μg per day, this was amended in the phase III trials and there is currently no restriction on resmetirom administration based on L-thyroxine supplementation.[106]

The excellent safety and tolerability profile of resmetirom is now confirmed. Overall, trial discontinuation due to adverse events occurred in 6.8-7.7% of patients in the 100 mg arm, 1.9-2.8% in the 80 mg arm and 2.2-3.4% in the placebo arm.[84,105] Diarrhoea and nausea are the two most common side effects. They appear to be self-limited and did not result in study withdrawal. Diarrhoea, which lasts for a median of 15-20 days, occurs in the early weeks of treatment.[105] The β selectivity of resmetirom appears to be sufficiently stringent to avoid adverse events related to α receptor activity, in particular cardiovascular symptoms or bone mineral density reduction, although in addition to β selectivity, restricted distribution of resmetirom from the blood to the heart and bone likely also plays a role. Elevations in oestradiol and total testosterone have been documented but their long-term relevance is unknown, as is the increase in SHBG, a glycoprotein which regulates tissue bioavailability of sex hormones.

VK2809, another THR-β selective, liver-enriched thyromimetic is currently being tested in VOYAGE, a phase IIb trial in patients with biopsy-proven steatohepatitis (NCT04173065). The study is being conducted in 248 participants and is testing four doses of drug *vs.* placebo. So far only top-line results, including of the primary endpoint (PDFF reduction at week 12) have been released: the mean relative change in liver fat ranges from -16.6% (1 mg QD) to -51.7% (1 mg QOD) *vs.* -3.7% for placebo. The proportion of patients achieving a 30% relative reduction ranged between 53% to 85% (*vs.* 13.6% for the placebo arm). Reductions in plasma lipids (LDL-cholesterol, ApoB, lipoprotein (a), Apo-CIII, and triglycerides) have been documented. Diarrhoea was reported in only 5% of the combined VK2809 arms *vs.* 3.1% in the placebo arm. Final results are expected for mid-2024.

Perspectives for clinical development and clinical use

Thyromimetics hold great promise for the treatment of MASLD due to their pleiotropic effects and, to date, acceptable safety profiles. The most recent EASL-EASO-EASD clinical practice guidelines on MASLD recommend that adults with non-cirrhotic MASH with significant liver fibrosis (stage >2) should be considered for treatment with resmetirom as a MASH-targeted therapy (whenever approved locally).[114] SHBG as a biomarker for thyroid hormone action within the liver could be used to monitor compliance, guide dosing and predict histological outcome. Long-term studies should indicate whether any detrimental long-term effects on the heart and bone occur. This could be assessed with clinical markers, such as heart rate and bone turnover, however, long-term markers such as risk of arrhythmia, fracture risk and symptoms related to bone cartilage damage are also important outcomes. Also, depletion of T4, which appears to be a common property of thyromimetics, could limit their doses if clinical signs of systemic or tissue-specific hypothyroidism become an issue, which should be carefully monitored in the development of novel, second-generation compounds. How long these compounds need to be administered for and whether discontinuation leads to reversal of the beneficial effect remain to be determined. Whether these drugs could also decrease hepatocellular carcinoma risk should be further investigated.[115,116] Since resemetirom is now an approved drug for MASH, future drug development programmes will need to identify which drugs will perform best in combination with resmetirom, while future real-world studies will need to determine the added benefit of *ad hoc* combinations of resmetirom in patients receiving incretin-based therapies for obesity or diabetes.[117] Hopefully, over the next years, these questions will be answered so that patients with MASH can benefit from long-term, safe and highly effective therapeutic regimens.

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Abbreviations

CHD, coronary heart disease; Dio, deiodinase; LNTF, low-normal thyroid function; MASLD, metabolic dysfunction-associated steatotic liver disease; MASH, metabolic dysfunction-associated steatohepatitis; NAS, NAFLD activity score; PDFF, proton density fat fraction; rT3, reverse T3; RTH, resistance to thyroid hormone; SCH, subclinical hypothyroidism; SHBG, sex hormone-binding globulin; T3, triiodothyronine; T4, thyroxine; THR, thyroid hormone receptor; TSH, thyroid-stimulating hormone.

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Conflict of interest

VR: Consulting for Boehringer-Ingelheim, Madrigal, Novo-Nordisk, 89Bio, Akero, LG Chem Sciences, Sagimet, Astra Zeneca. Grant to Institution: MSD; TSS. is a founder and senior advisor to Autobahn Therapeutics., EB: Received consultancy and speaker fees from Madrigal, on thyroid function monitoring board Aligos.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

VR, TS, EB all wrote the manuscript.

Supplementary data

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References

Author names in bold designate shared co-first authorship:

[1] Taylor PN, Albrecht D, Scholz A, et al. Global epidemiology of hyperthyroidism and hypothyroidism. Nat Rev Endocrinol 2018;14:301–316.
[2] Chaker L, Bianco AC, Jonklaas J, et al. Hypothyroidism. Lancet 2017;390:1550–1562.
[3] Klein I, Ojamaa K. Thyroid hormone and the cardiovascular system. N Engl J Med 2001;344:501–509.
[4] Surks MI, Ortiz E, Daniels GH, et al. Subclinical thyroid disease: scientific review and guidelines for diagnosis and management. Jama 2004;291:228–238.

[5] Biondi B, Cooper DS. The clinical significance of subclinical thyroid dysfunction. Endocr Rev 2008;29:76–131.
[6] Aoki Y, Belin RM, Clickner R, et al. Serum TSH and total T4 in the United States population and their association with participant characteristics: national Health and Nutrition Examination Survey (NHANES 1999-2002). Thyroid 2007;17:1211–1223.
[7] Inoue K, Ritz B, Brent GA, et al. Association of subclinical hypothyroidism and cardiovascular disease with mortality. JAMA Netw Open 2020;3:e1920745.
[8] Ochs N, Auer R, Bauer DC, et al. Meta-analysis: subclinical thyroid dysfunction and the risk for coronary heart disease and mortality. Ann Intern Med 2008;148:832–845.
[9] Rodondi N, den Elzen WP, Bauer DC, et al. Subclinical hypothyroidism and the risk of coronary heart disease and mortality. Jama 2010;304:1365–1374.
[10] Stott DJ, Rodondi N, Kearney PM, et al. Thyroid hormone therapy for older adults with subclinical hypothyroidism. N Engl J Med 2017;376:2534–2544.
[11] Gencer B, Collet TH, Virgini V, et al. Subclinical thyroid dysfunction and the risk of heart failure events: an individual participant data analysis from 6 prospective cohorts. Circulation 2012;126:1040–1049.
[12] Chang CH, Yeh YC, Caffrey JL, et al. Metabolic syndrome is associated with an increased incidence of subclinical hypothyroidism - a Cohort Study. Sci Rep 2017;7:6754.
[13] Roos A, Bakker SJ, Links TP, et al. Thyroid function is associated with components of the metabolic syndrome in euthyroid subjects. J Clin Endocrinol Metab 2007;92:491–496.
[14] Mehran L, Amouzegar A, Tohidi M, et al. Serum free thyroxine concentration is associated with metabolic syndrome in euthyroid subjects. Thyroid 2014;24:1566–1574.
[15] Ruhla S, Weickert MO, Arafat AM, et al. A high normal TSH is associated with the metabolic syndrome. Clin Endocrinol (Oxf) 2010;72:696–701.
[16] Muscogiuri G, Sorice GP, Mezza T, et al. High-normal TSH values in obesity: is it insulin resistance or adipose tissue's guilt? Obesity (Silver Spring) 2013;21:101–106.
[17] Han C, He X, Xia X, et al. Subclinical hypothyroidism and type 2 diabetes: a systematic review and meta-analysis. PLoS One 2015;10:e0135233.
[18] Liu D, Jiang F, Shan Z, et al. A cross-sectional survey of relationship between serum TSH level and blood pressure. J Hum Hypertens 2010;24:134–138.
[19] Cai Y, Ren Y, Shi J. Blood pressure levels in patients with subclinical thyroid dysfunction: a meta-analysis of cross-sectional data. Hypertens Res 2011;34:1098–1105.
[20] Kanaya AM, Harris F, Volpato S, et al. Association between thyroid dysfunction and total cholesterol level in an older biracial population: the health, aging and body composition study. Arch Intern Med 2002;162:773–779.
[21] Knudsen N, Laurberg P, Rasmussen LB, et al. Small differences in thyroid function may be important for body mass index and the occurrence of obesity in the population. J Clin Endocrinol Metab 2005;90:4019–4024.
[22] Fox CS, Pencina MJ, D'Agostino RB, et al. Relations of thyroid function to body weight: cross-sectional and longitudinal observations in a community-based sample. Arch Intern Med 2008;168:587–592.
[23] Svare A, Nilsen TI, Bjøro T, et al. Serum TSH related to measures of body mass: longitudinal data from the HUNT Study, Norway. Clin Endocrinol (Oxf) 2011;74:769–775.
[24] Wang X, Gao X, Han Y, et al. Causal association between serum thyrotropin and obesity: a bidirectional, mendelian randomization study. J Clin Endocrinol Metab 2021;106:e4251–e4259.
[25] Lautenbach A, Wernecke M, Mann O, et al. Thyroid-stimulating hormone levels in euthyroid patients 8 years following bariatric surgery. Int J Obes (Lond) 2022;46:825–830.
[26] Taylor PN, Razvi S, Pearce SH, et al. Clinical review: a review of the clinical consequences of variation in thyroid function within the reference range. J Clin Endocrinol Metab 2013;98:3562–3571.
[27] Asvold BO, Bjøro T, Nilsen TI, et al. Thyrotropin levels and risk of fatal coronary heart disease: the HUNT study. Arch Intern Med 2008;168:855–860.
[28] Asvold BO, Bjøro T, Vatten LJ. Association of thyroid function with estimated glomerular filtration rate in a population-based study: the HUNT study. Eur J Endocrinol 2011;164:101–105.
[29] Rhee CM, Kalantar-Zadeh K, Streja E, et al. The relationship between thyroid function and estimated glomerular filtration rate in patients with chronic kidney disease. Nephrol Dial Transpl 2015;30:282–287.
[30] Liangpunsakul S, Chalasani N. Is hypothyroidism a risk factor for non-alcoholic steatohepatitis? J Clin Gastroenterol 2003;37:340–343.
[31] Mantovani A, Nascimbeni F, Lonardo A, et al. Association between primary hypothyroidism and nonalcoholic fatty liver disease: a systematic review and meta-analysis. Thyroid 2018;28:1270–1284.
[32] Tao Y, Gu H, Wu J, et al. Thyroid function is associated with non-alcoholic fatty liver disease in euthyroid subjects. Endocr Res 2015;40:74–78.
[33] Bril F, Kadiyala S, Portillo Sanchez P, et al. Plasma thyroid hormone concentration is associated with hepatic triglyceride content in patients with type 2 diabetes. J Investig Med 2016;64:63–68.
[34] Chung GE, Kim D, Kim W, et al. Non-alcoholic fatty liver disease across the spectrum of hypothyroidism. J Hepatol 2012;57:150–156.
[35] Kim D, Yoo ER, Li AA, et al. Low-normal thyroid function is associated with advanced fibrosis among adults in the United States. Clin Gastroenterol Hepatol 2019;17:2379–2381.
[36] Martínez-Escudé A, Pera G, Costa-Garrido A, et al. TSH levels as an independent risk factor for NAFLD and liver fibrosis in the general population. J Clin Med 2021;10:2907.
[37] Fan H, Liu Z, Zhang X, et al. Thyroid stimulating hormone levels are associated with genetically predicted nonalcoholic fatty liver disease. J Clin Endocrinol Metab 2022;107:2522–2529.
[38] Xu C, Xu L, Yu C, et al. Association between thyroid function and nonalcoholic fatty liver disease in euthyroid elderly Chinese. Clin Endocrinol (Oxf) 2011;75:240–246.
[39] Chen YL, Tian S, Wu J, et al. Impact of thyroid function on the prevalence and mortality of metabolic dysfunction-associated fatty liver disease. J Clin Endocrinol Metab 2023;108:e434–e443.
[40] Sheikhi V, Heidari Z. Association of subclinical hypothyroidism with nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus: a cross-sectional study. Adv Biomed Res 2022;11:124.
[41] Kaltenbach TE, Graeter T, Oeztuerk S, et al. Thyroid dysfunction and hepatic steatosis in overweight children and adolescents. Pediatr Obes 2017;12:67–74.
[42] Guo Z, Li M, Han B, et al. Association of non-alcoholic fatty liver disease with thyroid function: a systematic review and meta-analysis. Dig Liver Dis 2018;50:1153–1162.
[43] Xu L, Ma H, Miao M, et al. Impact of subclinical hypothyroidism on the development of non-alcoholic fatty liver disease: a prospective case-control study. J Hepatol 2012;57:1153–1154.
[44] Qiu S, Cao P, Guo Y, et al. Exploring the causality between hypothyroidism and non-alcoholic fatty liver: a mendelian randomization study. Front Cel Dev Biol 2021;9:643582.
[45] Xie J, Huang H, Liu Z, et al. The associations between modifiable risk factors and nonalcoholic fatty liver disease: a comprehensive Mendelian randomization study. Hepatology 2023;77:949–964.
[46] Kim D, Kim W, Joo SK, et al. Subclinical hypothyroidism and low-normal thyroid function are associated with nonalcoholic steatohepatitis and fibrosis. Clin Gastroenterol Hepatol 2018;16:123–131 e121.
[47] Li R, Zhou L, Chen C, et al. Sensitivity to thyroid hormones is associated with advanced fibrosis in euthyroid patients with non-alcoholic fatty liver disease: a cross-sectional study. Dig Liver Dis 2023;55:254–261.
[48] Fan H, Li L, Liu Z, et al. Low thyroid function is associated with an increased risk of advanced fibrosis in patients with metabolic dysfunction-associated fatty liver disease. BMC Gastroenterol 2023;23:3.
[49] Klein I, Danzi S. Thyroid disease and the heart. Curr Probl Cardiol 2016;41:65–92.
[50] Bano A, Chaker L, Plompen EP, et al. Thyroid function and the risk of nonalcoholic fatty liver disease: the rotterdam study. J Clin Endocrinol Metab 2016;101:3204–3211.
[51] Kim D, Vazquez-Montesino LM, Escober JA, et al. Low thyroid function in nonalcoholic fatty liver disease is an independent predictor of all-cause and cardiovascular mortality. Am J Gastroenterol 2020;115:1496–1504.
[52] Yu H, Yang Y, Zhang M, et al. Thyroid status influence on adiponectin, acylation stimulating protein (ASP) and complement C3 in hyperthyroid and hypothyroid subjects. Nutr Metab (Lond) 2006;3:13.
[53] Kim SK, Kim SH, Park KS, et al. Regression of the increased common carotid artery-intima media thickness in subclinical hypothyroidism after thyroid hormone replacement. Endocr J 2009;56:753–758.
[54] Owen PJ, Sabit R, Lazarus JH. Thyroid disease and vascular function. Thyroid 2007;17:519–524.
[55] Biondi B. Cardiovascular effects of mild hypothyroidism. Thyroid 2007;17:625–630.
[56] van der Spek AH, Fliers E, Boelen A. The classic pathways of thyroid hormone metabolism. Mol Cel Endocrinol 2017;458:29–38.

[57] Fonseca TL, Fernandes GW, McAninch EA, et al. Perinatal deiodinase 2 expression in hepatocytes defines epigenetic susceptibility to liver steatosis and obesity. Proc Natl Acad Sci U S A 2015;112:14018–14023.
[58] MacParland SA, Liu JC, Ma XZ, et al. Single cell RNA sequencing of human liver reveals distinct intrahepatic macrophage populations. Nat Commun 2018;9:4383.
[59] Bohinc BN, Michelotti G, Xie G, et al. Repair-related activation of hedgehog signaling in stromal cells promotes intrahepatic hypothyroidism. Endocrinology 2014;155:4591–4601.
[60] Bruinstroop E, Zhou J, Tripathi M, et al. Early induction of hepatic deiodinase type 1 inhibits hepatosteatosis during NAFLD progression. Mol Metab 2021:101266.
[61] Bruinstroop E, Dalan R, Cao Y, et al. Low-dose levothyroxine reduces intrahepatic lipid content in patients with type 2 diabetes mellitus and NAFLD. J Clin Endocrinol Metab 2018;103:2698–2706.
[62] Kwakkel J, Wiersinga WM, Boelen A. Differential involvement of nuclear factor-kappaB and activator protein-1 pathways in the interleukin-1beta-mediated decrease of deiodinase type 1 and thyroid hormone receptor beta1 mRNA. J Endocrinol 2006;189:37–44.
[63] Kester MH, Toussaint MJ, Punt CA, et al. Large induction of type III deiodinase expression after partial hepatectomy in the regenerating mouse and rat liver. Endocrinology 2009;150:540–545.
[64] Simonides WS, Mulcahey MA, Redout EM, et al. Hypoxia-inducible factor induces local thyroid hormone inactivation during hypoxic-ischemic disease in rats. J Clin Invest 2008;118:975–983.
[65] Pihlajamäki J, Boes T, Kim EY, et al. Thyroid hormone-related regulation of gene expression in human fatty liver. J Clin Endocrinol Metab 2009;94:3521–3529.
[66] Kendall TJ, Jimenez-Ramos M, Turner F, et al. An integrated gene-to-outcome multimodal database for metabolic dysfunction-associated steatotic liver disease. Nat Med 2023;29:2939–2953.
[67] Flamant F, Cheng SY, Hollenberg AN, et al. Thyroid hormone signaling pathways: time for a more precise nomenclature. Endocrinology 2017;158:2052–2057.
[68] Hones GS, Rakov H, Logan J, et al. Noncanonical thyroid hormone signaling mediates cardiometabolic effects in vivo. Proc Natl Acad Sci U S A 2017;114:E11323–E11332.
[69] Ma Y, Shen S, Yan Y, et al. Adipocyte thyroid hormone beta receptor-mediated hormone action fine-tunes intracellular glucose and lipid metabolism and systemic homeostasis. Diabetes 2023;72:562–574.
[70] Araki O, Ying H, Zhu XG, et al. Distinct dysregulation of lipid metabolism by unliganded thyroid hormone receptor isoforms. Mol Endocrinol 2009;23:308–315.
[71] Moran C, McEniery CM, Schoenmakers N, et al. Dyslipidemia, insulin resistance, ectopic lipid accumulation, and vascular function in resistance to thyroid hormone beta. J Clin Endocrinol Metab 2021;106:e2005–e2014.
[72] Perra A, Simbula G, Simbula M, et al. Thyroid hormone (T3) and TRbeta agonist GC-1 inhibit/reverse nonalcoholic fatty liver in rats. Faseb j 2008;22:2981–2989.
[73] Cable EE, Finn PD, Stebbins JW, et al. Reduction of hepatic steatosis in rats and mice after treatment with a liver-targeted thyroid hormone receptor agonist. Hepatology 2009;49:407–417.
[74] Zhou J, Tripathi M, Ho JP, et al. Thyroid hormone decreases hepatic steatosis, inflammation, and fibrosis in a dietary mouse model of nonalcoholic steatohepatitis. Thyroid 2022;32:725–738.
[75] Sinha RA, Bruinstroop E, Singh BK, et al. Nonalcoholic fatty liver disease and hypercholesterolemia: roles of thyroid hormones, metabolites, and agonists. Thyroid 2019;29:1173–1191.
[76] Sinha RA, You S-H, Zhou J, et al. Thyroid hormone stimulates hepatic lipid catabolism via activation of autophagy. J Clin Invest 2012;122:2428–2438.
[77] Hones GS, Sivakumar RG, Hoppe C, et al. Cell-specific transport and thyroid hormone receptor isoform selectivity account for hepatocyte-targeted thyromimetic action of MGL-3196. Int J Mol Sci 2022;23.
[78] Adams AC, Astapova I, Fisher FM, et al. Thyroid hormone regulates hepatic expression of fibroblast growth factor 21 in a PPARalpha-dependent manner. J Biol Chem 2010;285:14078–14082.
[79] Singh BK, Sinha RA, Tripathi M, et al. Thyroid hormone receptor and ERRalpha coordinately regulate mitochondrial fission, mitophagy, biogenesis, and function. Sci Signal 2018;11.
[80] Zhang Y, Ma K, Song S, et al. Peroxisomal proliferator-activated receptor-gamma coactivator-1 alpha (PGC-1 alpha) enhances the thyroid hormone induction of carnitine palmitoyltransferase I (CPT-I alpha). J Biol Chem 2004;279:53963–53971.
[81] Mendoza A, Tang C, Choi J, et al. Thyroid hormone signaling promotes hepatic lipogenesis through the transcription factor ChREBP. Sci Signal 2021;14:eabh3839.
[82] Ter Horst KW, Vatner DF, Zhang D, et al. Hepatic insulin resistance is not pathway selective in humans with nonalcoholic fatty liver disease. Diabetes Care 2021;44:489–498.
[83] Birkenfeld AL, Shulman GI. Nonalcoholic fatty liver disease, hepatic insulin resistance, and type 2 diabetes. Hepatology 2014;59:713–723.
[84] Harrison SA, Bedossa P, Guy CD, et al. A phase 3, randomized, controlled trial of resmetirom in NASH with liver fibrosis. N Engl J Med 2024;390:497–509.
[85] Vatner DF, Weismann D, Beddow SA, et al. Thyroid hormone receptor-beta agonists prevent hepatic steatosis in fat-fed rats but impair insulin sensitivity via discrete pathways. Am J Physiol Endocrinol Metab 2013;305:E89–E100.
[86] Wang X, Wang L, Geng L, et al. Resmetirom ameliorates NASH-model mice by suppressing STAT3 and NF-kappaB signaling pathways in an RGS5-dependent manner. Int J Mol Sci 2023;24.
[87] Ferrara SJ, Chaudhary P, DeBell MJ, et al. TREM2 is thyroid hormone regulated making the TREM2 pathway druggable with ligands for thyroid hormone receptor. Cell Chem Biol 2022;29:239–248 e234.
[88] Alonso-Merino E, Martín Orozco R, Ruíz-Llorente L, et al. Thyroid hormones inhibit TGF-β signaling and attenuate fibrotic responses. Proc Natl Acad Sci 2016;113:E3451–E3460.
[89] Yu G, Tzouvelekis A, Wang R, et al. Thyroid hormone inhibits lung fibrosis in mice by improving epithelial mitochondrial function. Nat Med 2018;24:39–49.
[90] Manka P, Coombes JD, Sydor S, et al. Thyroid hormone receptor alpha modulates fibrogenesis in hepatic stellate cells. Liver Int 2024;44:125–138.
[91] The coronary drug project. Findings leading to further modifications of its protocol with respect to dextrothyroxine. The coronary drug project research group. JAMA 1972;220:996–1008.
[92] Berkenstam A, Kristensen J, Mellstrom K, et al. The thyroid hormone mimetic compound KB2115 lowers plasma LDL cholesterol and stimulates bile acid synthesis without cardiac effects in humans. Proc Natl Acad Sci U S A 2008;105:663–667.
[93] Ladenson PW, Kristensen JD, Ridgway EC, et al. Use of the thyroid hormone analogue eprotirome in statin-treated dyslipidemia. N Engl J Med 2010;362:906–916.
[94] Scanlan TS. Sobetirome: a case history of bench-to-clinic drug discovery and development. Heart Fail Rev 2010;15:177–182.
[95] Lin VW, Klepp HM, Hanley RM. Sobetirome is a TRβ- and liver-selective thyromimetic that can affect substantial LDL-C lowering without significant changes in heart rate or the thyroid axis in euthyroid men [abstract]. In: San francisco: the endocrine society annual meeting ENDO 08; 2008. OR36-33.
[96] Angelin B, Kristensen JD, Eriksson M, et al. Reductions in serum levels of LDL cholesterol, apolipoprotein B, triglycerides and lipoprotein(a) in hypercholesterolaemic patients treated with the liver-selective thyroid hormone receptor agonist eprotirome. J Intern Med 2015;277:331–342.
[97] Taub R, Chiang E, Chabot-Blanchet M, et al. Lipid lowering in healthy volunteers treated with multiple doses of MGL-3196, a liver-targeted thyroid hormone receptor-beta agonist. Atherosclerosis 2013;230:373–380.
[98] Lian B, Hanley RM, Schoenfeld S. A phase 1 randomized, double-blind, placebo-controlled, multiple ascending dose study to evaluate safety, tolerability and pharmacokinetics of the liver-selective TR-beta agonist VK2809 (MB07811) in hypercholesterolemic subjects. J Am Coll Cardiol 2016;67(13):1932.
[99] Kelly MJ, Pietranico-Cole S, Larigan JD, et al. Discovery of 2-[3,5-dichloro-4-(5-isopropyl-6-oxo-1,6-dihydropyridazin-3-yloxy)phenyl]-3,5-dio xo-2,3,4,5-tetrahydro[1,2,4]triazine-6-carbonitrile (MGL-3196), a Highly Selective Thyroid Hormone Receptor beta agonist in clinical trials for the treatment of dyslipidemia. J Med Chem 2014;57:3912–3923.
[100] Ferrara SJ, Bourdette D, Scanlan TS. Hypothalamic-pituitary-thyroid Axis perturbations in male mice by CNS-penetrating thyromimetics. Endocrinology 2018;159:2733–2740.
[101] Erion MD, Cable EE, Ito BR, et al. Targeting thyroid hormone receptor-beta agonists to the liver reduces cholesterol and triglycerides and improves the therapeutic index. Proc Natl Acad Sci U S A 2007;104:15490–15495.
[102] Grover GJ, Mellstrom K, Ye L, et al. Selective thyroid hormone receptor-beta activation: a strategy for reduction of weight, cholesterol, and lipoprotein (a) with reduced cardiovascular liability. Proc Natl Acad Sci U S A 2003;100:10067–10072.
[103] Harrison SA, Bashir MR, Guy CD, et al. Resmetirom (MGL-3196) for the treatment of non-alcoholic steatohepatitis: a multicentre, randomised,

double-blind, placebo-controlled, phase 2 trial. Lancet 2019;394:2012–2024.
[104] Harrison SA, Bashir M, Moussa SE, et al. Effects of resmetirom on noninvasive endpoints in a 36-week phase 2 active treatment extension study in patients with NASH. Hepatol Commun 2021;5:573–588.
[105] Harrison SA, Taub R, Neff GW, et al. Resmetirom for nonalcoholic fatty liver disease: a randomized, double-blind, placebo-controlled phase 3 trial. Nat Med 2023;29:2919–2928.
[106] Food and Drug Administration. Highlits of prescribing information Rezdiffra. [cited Accessed April 17th 2024. April 17th 2024]; Available from:, 2024. https://www.accessdata.fda.gov/drugsatfda_docs/label/2024/217785s000lbl.pdf; 2024.
[107] **Younossi ZM**, **Ratziu V**, Loomba R, et al. Obeticholic acid for the treatment of non-alcoholic steatohepatitis: interim analysis from a multicentre, randomised, placebo-controlled phase 3 trial. Lancet 2019;394:2184–2196.
[108] Sanyal AJ, Ratziu V, Loomba R, et al. Results from a new efficacy and safety analysis of the REGENERATE trial of obeticholic acid for treatment of pre-cirrhotic fibrosis due to non-alcoholic steatohepatitis. J Hepatol 2023;79:1110–1120.
[109] Newsome PN, Buchholtz K, Cusi K, et al. A placebo-controlled trial of subcutaneous semaglutide in nonalcoholic steatohepatitis. N Engl J Med 2021;384:1113–1124.
[110] Francque SM, Bedossa P, Ratziu V, et al. A randomized, controlled trial of the pan-PPAR agonist lanifibranor in NASH. N Engl J Med 2021;385:1547–1558.
[111] Tai D, Bashir M, Taub R, et al. Impact of resmetirom-mediated reductions in liver volume and steatosis compared with placebo on the quantification of fibrosis using second harmonic generation in a serial liver biopsy study. J Hepatol 2022;77:S32.
[112] Loomba R, Sanyal AJ, Kowdley KV, et al. Factors associated with histologic response in adult patients with nonalcoholic steatohepatitis. Gastroenterology 2019;156:88–95 e85.
[113] Viguerie N, Millet L, Avizou S, et al. Regulation of human adipocyte gene expression by thyroid hormone. J Clin Endocrinol Metab 2002;87:630–634.
[114] European Association for the Study of the Liver. European association for the study of diabetes, European association for the study of obesity. EASL-EASD-EASO clinical practice guidelines on the management of metabolic dysfunction-associated steatotic liver disease (MASLD). J Hepatol 2024.
[115] Frau C, Loi R, Petrelli A, et al. Local hypothyroidism favors the progression of preneoplastic lesions to hepatocellular carcinoma in rats. Hepatology 2015;61:249–259.
[116] Kowalik MA, Puliga E, Cabras L, et al. Thyroid hormone inhibits hepatocellular carcinoma progression via induction of differentiation and metabolic reprogramming. J Hepatol 2020;72:1159–1169.
[117] Ratziu V, Charlton M. Rational combination therapy for NASH: insights from clinical trials and error. J Hepatol 2023;78:1073–1079.

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