Impact of peripheral thyroid hormone balance on liver fat: insights from the NutriAct trial

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Abstract

Objective

Hypothyroidism has been proposed as a potential contributor to steatotic liver disease (SLD), but existing data shows conflicting results in euthyroid subjects. Therefore, we investigated the association between thyroid function and intrahepatic lipids (IHLs) during a 36-months randomized controlled trial evaluating a diet known to reduce liver fat.

Design

502 eligible subjects (aged 50 to 80 y, ≥ 1 risk factor for unhealthy aging) were randomly assigned to either follow a diet rich in unsaturated fatty acids, plant protein and fiber (intervention group, IG), or dietary recommendations of the German Nutrition Society (control group (CG)).

Methods

Serum levels of thyroid hormones (THs) as well as IHLs, defined via magnetic resonance spectroscopy, were measured within an euthyroid subgroup without significant alcohol consumption at baseline (n = 332) and after 12 months (n = 243). Ratio of T3/T4 was used to assess whole body deiodinase activity. Estimates of glucose and lipid metabolism were analyzed.

Results

Only fT3 and T3/T4 ratio showed a significant positive correlation with IHL at baseline. We observed a significant decline in fT3, T3, fT3/fT4 ratio and T3/T4 ratio in CG and IG after 12 months without significant differences between groups. TSH, fT4 and T4 remained stable. A larger improvement of IHL during dietary intervention was seen in those subjects with a lower decline in T3 concentrations.

Conclusions

Altered TH balance indicates a possible compensatory upregulation of whole body TH activity in subjects with increased liver fat. This might be also relevant during improvement of hepatic steatosis.

Significance Statement

Our study examines the relationship between liver fat content, assessed by magnetic resonance spectroscopy, and thyroid hormone (TH) balance using data from a randomized controlled dietary intervention trial. Baseline analysis reveals a significant association between increased intrahepatic lipid content and higher peripheral T3 concentrations and T3/T4 ratio. Throughout the intervention, improvements in liver fat and declines in T3 are observed in both dietary groups. Subjects maintaining higher T3 levels during the dietary intervention loose more intrahepatic lipids over 12 months. Notably, this link is driven by peripheral TH balance rather than central axis regulation, as evidenced by stable TSH levels. This suggests a compensatory upregulation of T3/T4 ratio to counterbalance liver fat accumulation in steatotic liver disease.

Introduction

Non-alcoholic fatty liver disease (NAFLD), according to the new nomenclature metabolic dysfunction-associated steatotic liver disease (MASLD) [1], is characterized by accumulation of fatty acids and lipid metabolites in hepatocytes. It comprises a spectrum of progressive liver disease ranging from simple steatosis to steatohepatitis, fibrosis and cirrhosis which can progress to hepatocellular carcinoma [2–5]. Recent research consistently demonstrates an overlap of 96% or more between subjects with NAFLD and those who meet the criteria for MASLD [6,7]. Therefore, we decided to use the term MASLD also for previous studies. MASLD is closely intertwined with other metabolic diseases like dyslipidemia, obesity, insulin resistance and type 2 diabetes [8,9]. The increasing global prevalence of metabolic syndrome made MASLD one of the most important liver diseases worldwide [10].

Thyroid hormones (THs) play a pivotal role in governing local energy metabolism by modifying the metabolism of carbohydrates and fatty acids via effects on gluconeogenesis, glycolysis, lipogenesis, lipolysis and beta-oxidation [11–13]. While the influence of TH on liver energy metabolism has been well known for decades, recently more evidence accumulated showing that systemic

hypothyroidism could be an independent risk factor for the development of MASLD and metabolic dysfunction-associated steatohepatitis (MASH) [14–17]. Additionally, also subclinical hypothyroidism (i.e., TSH > 4.5 mIU/L, fT3 and fT4 within reference range) potentially serves as a predictor of steatosis, advanced NASH and liver fibrosis [18,19]. However, conflicting results were reported by others [20,21], which might be attributed to methodological variations in existing studies. Many studies assessed thyroid status solely based on serum levels of TSH and fT4, with most relying on ultrasound for MASLD diagnosis. Thus, the link between thyroid function and steatotic liver disease (SLD) is still not fully clarified, despite documented roles of TH as key regulators of hepatic fat and glucose metabolism at the local level[11,22]. Especially local mechanisms involved in TH availability and action might play an important role. The prohormone thyroxine (T4) is produced in the thyroid gland, while its activation to the receptor-active hormone 3,3'-5-triiodothyronine (T3) through 5'-deiodination occurs predominantly in peripheral tissues like the liver by deiodinases (DIO) with DIO type 1 (DIO1) being the predominant isoform in the liver. Current therapeutic strategies especially in the early stages of MASLD focus on lifestyle and dietary recommendations, given the absence of any approved pharmacological treatments in Europe to date [13,23]. The recent approval of the thyroid hormone receptor (TR) beta agonist Resmetirom in the US for the treatment of MASH emphasizes the role of TH metabolism in hepatic steatosis. Dietary patterns rich in unsaturated fatty acids (UFA) proved to be successful in reducing liver fat [24]. This had been impressively confirmed recently in the long-term randomized dietary intervention NutriAct trial [25], which demonstrated a stronger improvement of liver steatosis (based on assessment of intrahepatic lipid (IHL) via proton magnetic resonance spectroscopy (1H-MRS)) in 258 elderly subjects with rather mildy elevated IHL content after 12 months of a specific diet focusing on high intake in fiber, protein and UFA. If the underlying mechanisms also include changes in TH state and metabolism is unclear.

We aimed to analyze, whether the observed improvement in liver fat in this cohort with mildly elevated IHL content during this dietary intervention is related to changes in TH status.

Materials and Methods

Study design and Participants

The 36-month NutriAct randomized controlled multi-center parallel group trial compared long-term effects of two different dietary patterns in a German aging population. In short, subjects between 50 and 80 years of age were included if they met at least one condition for unhealthy aging (i.e., arterial hypertension, cardiovascular disease, heart failure or cognitive impairment) (see supplemental material for more details). Among other criteria, participants with severe hepatic disease, severe substance abuse or an active cancer disease, type 1 diabetes or individuals with insulin therapy in type 2 diabetes were excluded. In summary, a total of 502 subjects was randomly assigned to either intervention (IG) or control group (CG). Due to medical contraindications, decline of the procedure or insufficient data quality IHL data is available only for a part of the participants. Here, we report data of the first 12 months of the trial in individuals with available 1H-MRS data as well as evaluation of thyroid hormone status (Fig. 1). Detailed information on data collection is provided in the supplemental material and in previous reports [26]. This study was conducted in accordance with the Declaration of Helsinki.

The study protocol was approved by the Institutional Review Board of Charité-Universitätsmedizin Berlin (approval EA1/315/15). The trial was registered at German Clinical Trials Register (drks.de) as DRKS00010049.

Procedures

Dietary intervention

In the intervention group, a specific dietary pattern consisted of 35% to 40% of total energy (%E) from fat, with an emphasis on UFA; 15%E to 25%E from protein, with a focus on plant-based proteins; and 35%E to 45%E from carbohydrates and a minimum of 30 grams of fiber daily was implemented, while the CG received standard care following local dietary recommendations from

the GNE (German Nutrition Society). Details were already reported previously [26] and described in the supplements.

Phenotyping

At baseline and after 12 months metabolic phenotyping procedures were performed including collection of anthropometric data and blood sampling. All participants were assessed at 8:00 AM after a 12-hour fasting period. Using a digital column scale equipped with an integrated stadiometer (Charité: Seca, Hamburg, Germany; DIfE: Soehnle, Nassau, Germany), body weight (rounded to the nearest 0.1 kg) and height were measured. Further on, participants underwent a fasting venous blood sample and 75-g oral glucose tolerance test. Blood samples were centrifuged and frozen immediately at -80 °C. Type 2 diabetes (T2D) was coded in participants if at baseline either a T2D was known or in subjects with antidiabetic medication, HbA1c > 6,5%, fasting plasma glucose concentrations exceeded ≥ 7 mmol/L, plasma glucose exceeded ≥ 11.1 mmol/L 120 min after oral glucose load. A Body-Mass-Index (BMI, weight [kg]/ (height [m])^2) value at baseline ≥ 30 kg/m^2 was used for the diagnosis of obesity. A subset of patients underwent magnetic resonance spectroscopy (1H-MRS) on a 1.5T whole-body scanner (Magnetom Avanto, Siemens Healthcare, Erlangen, Germany) for quantification of intrahepatic lipid (IHL) via single voxel stimulated echo acquisition mode (STEAM). IHL measurement via 1H-MRS is a highly accurate, reproducible noninvasive technique for hepatic fat quantification and showed a higher sensitivity to reflect change in IHL as compared to combined liver fat scores[27–29]. IHL was quantified as ratio of fat divided by the sum of water using a voxel with a volume of 30 x 30 x 20 mm in the posterior part of liver segment 7. IHL > 5.56% was used as cut-off criterion for diagnosis of significantly increased IHL content indicating SLD [30].

Exclusion criteria

In addition to the general exclusion criteria cited above, for our substudy we applied additional exclusion criteria. Subjects with significant consumption of alcohol (> 20g alcohol/day for women and >30g alcohol/day for men), intake of T3 or thyreostatic medication (thiamazole), missing data for IHL (due to MRI contraindication or declined procedure or data quality not sufficient for analysis) or missing thyroid parameters were additionally excluded in this study evaluation. Even though TSH was screened before enrollment and only subjects with TSH values within the reference range were enrolled, 10 participants displayed manifest hyperthyroid (TSH < 0.27 mIU/l and fT4 > 17 ng/l) or hypothyroid (TSH > 4.2 and fT4 < 9.3 ng/l) values in the baseline laboratory exam and were excluded from further analysis. Subjects under levothyroxine replacement therapy and euthyroid state at evaluation were not excluded from further analysis in order to preserve the statistical power.

Laboratory analyses

Details are described in the supplements.

Outcomes

The primary endpoint of the study was a composite measure that encompassed various age-related conditions. This included cardiovascular issues, cognitive function decline associated with aging as well as aspects related to muscle health, such as lean body mass and muscular function. Further details have been reported elsewhere [26]. In this substudy we focused on the relationship between changes in liver fat and parameters of TH action following a 12-month intervention period within the 36-month randomized controlled trial.

Statistical analysis

Statistical analyses are described in detail in the supplements. In summary, T3/T4 ratio was calculated by dividing T3 by T4 and multiplication by factor 100. Change in IHL was calculated as absolute change by subtracting the baseline IHL value from the value at month 12. Independent samples t-test or Wilcoxon rank-sum test were used for baseline comparison as appropriate while correlation analyses were performed using Spearman's rank coefficient. Change in parameters within the groups from baseline to 12 month was evaluated using Wilcoxon signed-rank test or related samples t-test. An analysis of covariance model adjusted for baseline age, sex and BMI was used to assess the effect of the intervention between groups.

Results

Our subcohort consisted of 332 subjects (219 females, 113 males) with available IHL and TH data, which were included in the baseline analysis (Table 1). Median IHL at baseline was 3.62%. Increased IHL content (> 5.56%) was present in 117 subjects, 138 subjects had a BMI > 30kg/m^2 (41.6%) and type 2 diabetes was prevalent in less than 20%. TH substitution was performed in 42 participants (levothyroxine). Participants with intake of T3 or thyreostatic medication were excluded. All subjects were characterized by TSH, fT4 and fT3 concentrations within the reference range at baseline. There were no significant differences in TH concentrations, IHL or anthropometric parameters between IG and CG at baseline (Table 1).

Higher IHL content at baseline was associated with higher fT3 levels and T3/T4 ratio (Table 2, Fig. 2), while correlation with T3 level marginally failed to reach significance (p = 0.060). When excluding the 42 subjects with intake of levothyroxine from the analysis, the effect remains stable: p = 0.04 for correlation between IHL and fT3 (rho = 0.120, N = 290) and p = 0.005 for correlation between IHL and T3/T4 ratio (rho = 0.163, N = 290). In contrast, BMI at baseline was not significantly associated with TH parameters except for total T3 levels (p = 0.027, Rho = 0.12).

Most strikingly, the correlation between T3/T4 ratio and baseline IHL remained significant also upon correction for baseline BMI ($p = 0.024$), while the association between IHL and fT3 remains positive but failed to be significant ($p = 0.095$).

Effect of dietary intervention

After 12 months of dietary intervention a small but similar reduction in BMI was observed in IG (-0.4 kg/m^2, $p < 0.001$) and CG (-0.6 kg/m^2, $p < 0.001$ (p for between group difference = 0.36)) of our subcohort, which was comparable to the results within the entire cohort with 1H-MRS [25]. 1H-MRS and TH data was available for 243 subjects at month 12. Median IHLs declined from 3.50% to 2.78% (CG, $p < 0.001$) and 3.62% to 2.10% (IG, $p < 0.001$) from baseline to month 12.

A similar reduction in fT3, T3, fT3/fT4 ratio and T3/T4 ratio could be observed over 12 months in both groups (Table 3), while no change in T4, fT4 and TSH was observable in CG and IG. These results remained stable also after exclusion of subjects with intake of T4 supplementation. Further on we repeated this analysis for subgroups categorized by the presence of increased IHL content (IHL > 5.56%), type 2 diabetes or obesity. Due to the limited sample size minor discrepancies could not be evaluated. All subgroups showed the previously observed reduction in fT3 and T3, as well as fT3/fT4 ratio and T3/T4 ratio, even though this not always remained significant within the subgroup analyses (Tables S1-3).

A change in TH parameters between month 0 and 12 was not associated with changes in BMI (Table S4). However, a smaller decrease in T3 from baseline to month 12 was associated with a stronger concomitant decline of IHL (Rho = -0.133, $p = 0.039$). Thus, subjects that rather maintained their baseline T3 concentrations experienced greater IHL reduction at month 12 (Fig. 3). The result remained stable after adjustment for change in BMI ($p= 0.014$). Exclusion of subjects with intake of TH-active medication, which resulted in a substantial reduction of the sample size, attenuated this relationship (Rho = -0.123, $p = 0.07$). In contrast, changes in TSH, fT3, fT4 or T4 as well as fT3/fT4 ratio and T3/T4 ratio were not related to IHL improvement (Table S4).

Discussion

In this study, we investigated the relationship between TH homeostasis and liver fat during a randomized dietary intervention trial in a cohort characterized by early stages of liver steatosis. Higher peripheral T3 availability, indicated by higher concentrations of fT3, T3 and T3/T4 ratio, were associated with increased liver fat content and were associated with stronger reduction of liver fat during a dietary intervention focusing on liver fat reduction. Both findings might suggest, that higher T3 activation reflects an adaptive mechanism to counterbalance increased liver fat. Accordingly, a decline in IHL through low-dose levothyroxine supplementation was reported in patients with MASLD without the need for supplementation [31] and a lower prevalence of MASLD and LDL-cholesterol could be demonstrated upon substitution of low-dose levothyroxine in subclinical hypothyroid patients [32]. Moreover, recently published promising results of the TR beta agonist Resmetirom on liver steatosis are highly supportive [33,34] and TR beta agonistic actions seem to effectively reduce disease consequences such as steatohepatitis and fibrosis as well [33,35]. These data support the importance of novel liver-targeted TH activity modifying therapeutic agents to alleviate the global burden of MASLD [13].

In contrast, the missing relationship with TSH and T4 as well as unchanged TSH levels during our dietary intervention contradict an interaction with the hypothalamic-pituitary-thyroid axis.

The role of THs in hepatic lipid metabolism leading to changes in hepatic lipid accumulation, lipolysis and beta-oxidation, is well known [11,12,36]. Hepatic TH availability and action depends on their uptake via transmembrane transporters as well as their activation and inactivation via deiodinases (mainly DIO1 in the liver) and local expression of TRs [13,37]. Therefore, increased local TH presence and subsequent stimulation of hepatic TRs likely support hepatic lipid clearance. In line with our results, higher fT3 has been already described in euthyroid patients with elevated biochemical estimates of MASLD [38]. As data regarding measurement of liver fat via 1H-MRS and

association with TH were not available so far, our data expand current findings. Due to the lack of liver biopsy samples, as in most published studies on this topic, we could not measure liver-specific parameters of TH availability. Nevertheless a higher T3/T4 ratio seems to reflect an increased conversion of T4 into T3 within peripheral tissues including e.g. muscle, adipose tissue, central nervous system and liver due to peripheral conversion by deiodinases [39,40]. Thus, our data regarding circulating levels of fT3, T3 and T3/T4 ratio are congruent with a TH activation by DIO [41], which might not be limited to the liver.

In line with such an assumption, recently increased hepatic DIO1 expression and activity in early stages of a MASLD mouse model was reported, linked to an increased T3/T4 ratio [42,43] while another study found a regulatory element in the DIO1 promoter region to be a potential enhancer of SLD [44]. Similarly, other studies observed a higher fT3/fT4 ratio in subjects with ultrasound-diagnosed SLD as compared to controls without MASLD [45,46]. Although, some authors have already posited our hypothesis that this might be a compensatory mechanism as reaction to hepatic lipid accumulation, our long term results during the dietary intervention further supports this assumption. Most interestingly, this mechanism seems to exist only in steatosis and disappears in later stages with increasing inflammation as higher fT3 levels were found in subjects with mild MASLD compared to advanced MASLD [47]. Additionally a higher fT3/fT4 ratio has been reported in subjects with steatosis as compared to subjects with biopsy-proven NASH [48]. This would further underline a possible influence of DIO1, since DIO1 is downregulated by inflammation signals which are increasing upon NASH development.

Within the randomized dietary intervention trial, we compared the effect of two dietary regimes. Although both interventions reduced liver fat, this effect was more pronounced in the group which followed the dietary pattern focusing on high intake of UFA, protein and fiber [25]. Both dietary approaches resulted in a comparable decline of fT3 and T3 without any effect on TSH, T4 or fT4. Thus, differences in the dietary pattern might not have specific effects on THs. In general, reduced T3 and/or fT3 concentration through diet has been shown before in high-fat or low-carbohydrate

dietary patterns [49–51]. Yet these studies can not directly be compared with our data, as they used small sample sizes (n =11 to 42), short intervention time (3 to 12 weeks), did not provide a control group and more drastic changes in nutrition composition between groups regarding fat or carbohydrate content than in our study[49–51]. Most importantly, all studies led to a substantial weight reduction between 2 – 4.6 kg despite no caloric restriction was intended. This is of high relevance, as weight loss is known to directly affect THs [52]. In line with this finding, the association between a Mediterranean diet – favoring fruits, vegetables, vegetable protein – and lower fT3 levels, found in a cross-sectional study by Zupo et al., was no longer significant upon correction for BMI [53]. The fact that peripheral TH balance was altered by diet in our as well as previous studies indicates that the used dietary approaches might induce changes of peripheral conversion of T4 to T3. Nevertheless, we could not exclude an effect of the observed small weight loss , as even a moderate weight loss has been shown to affect the peripheral thyroid status [54,55].

The current study presents some limitations. Firstly, our study participants do not reflect the general population as only subjects over 50 years of age were included. We decided not to exclude patients with T4 replacement from the study to preserve the statistical power of the study. However, we believe this is a rather small limitation, as subgroup analyses excluding those participants showed no substantial changes in the results. No subject was taking amiodarone. However, no data regarding exposure to iodinated contrast agents prior to the study visits are available. On the other hand, the large sample size allowed us to carry out subgroup analyses and to analyze potentially influential metabolic parameters like obesity or diabetes state, which is not the case in most publications on this topic. Since we used MRI, a highly validated method to assess liver fat content, only a reduced number of participants could undertake these evaluations mainly due to medical exclusion criteria for MRI. The number of cases did not allow sufficiently valid conclusions to be drawn for further specific questions. For example, the association between changes in IHL and T3 from baseline to follow up could still be observed as a trend but did not reach significance, after repeating the analyses in those subjects without T4-substitution. This

might potentially be due to the limited sample size as no substantial reduction in effect size could be found. Given the only mildly elevated median IHL at baseline, the study cohort may not necessarily be suited to address advanced stages of MASLD, which could explain divergent results compared to large meta-analyses. On the other hand, this study population with low IHL holds information on possible dietary interventions in early stages of MASLD, since our results provide an indication that compensatory mechanisms play a decisive role especially at the beginning of the development of steatosis.

Although we saw significant changes for both fT3 and T3 in our longitudinal analysis, improvements in IHL were associated only with T3 but not with fT3 or fT3/fT4 ratio. However, the laboratory measurement of peripheral TH is fraught with some difficulties for some parameters. fT3 assays are less validated and robust than those for fT4, leading to a preference for total T3 measurement, a recommendation also given by the American Thyroid Association (ATA) [56–59]. Variations in thyroid hormone-binding proteins affect fT4 and fT3 measurements, potentially causing misleading results in certain physiological and disease states [60]. The consistent fT4/T4 ratio (table 1 and 3) during the intervention and between groups contradicts changes in TBG levels as potential cause of our findings. Therefore, we deem the usage of total T3 appropriate in our study.

Besides the mentioned limitations, notable strengths of the study include its randomized controlled trial design, providing valuable longitudinal insights into TH concentration changes during the intervention. This is of high importance, as mostly cross-sectional data were published so far. Moreover, several studies investigating the relationship between TH activity and SLD are limited to the use of serum parameters and approximative liver fat scores like HSI and FLI to assess steatosis. Within the NutriAct cohort we carried out a comprehensive phenotyping using 1H-MRS based IHL measurement, which has been proven to be a valid method for evaluating IHL and has been used in many clinical studies [29,30].

Conclusion

Overall, our findings suggest that a higher peripheral concentration of active THs might reflect a compensatory mechanism in subjects with mildly increased IHL content and early stages of MASLD. This speculation is especially supported by the finding that a stronger improvement of liver fat was seen in subjects who did not demonstrate a diet-induced decline in T3. Given the impact of DIO1 in peripheral activation of T4 the role of DIO1 in this context needs further investigation.

Conflict of Interest:

MSB, THN, CW, JM, TS, DP, EKW, JS and KM declare no conflict of interest.

The lab of FT has received funding from Gilead, MSD and AstraZeneca. FT has served as a consultant for Novo Nordisk, AstraZeneca, Gilead, Abbvie, Alnylam, BMS, Intercept, Falk, Inventiva, MSD, Pfizer, GSK, Novartis and Sanofi. LPB was employed by Charité Universitätsmedizin Berlin during the conduct of the study, but as of 1st of August 2023 is employed by Novo Nordisk.

Authors Contributions:

KM, EKW and JS designed research, KM, CW, LPB, THN, JM, FT, TS, DP and MSB conducted research, CW, JM, MSB and KM analyzed data. MSB, EKW and KM wrote the manuscript and have primary responsibility for final content. All authors contributed to interpretation of the results. All authors critically read and approved the final version of the manuscript.

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Data Availability Statement:

The datasets generated during and/or analyzed during this study and the study protocol are available from the corresponding author upon reasonable request. The study design and study protocol are already publicly accessible.

Figure legends:

Figure 1: Trial profile. 502 eligible men and women were randomly assigned. The dietary pattern of the intervention group (NutriAct dietary pattern) focused on a high proportion of unsaturated fat and plant proteins. The control group received usual care including dietary recommendations of the German Nutrition Society. For this substudy participants with significant consumption of alcohol (> 20g alcohol/day for women and >30g alcohol/day for men) or thyroid specific exclusion criteria (intake of T3 supplementation or thyreostatic medication, non-euthyroid state at baseline, missing TH parameters) or missing 1H-MRS data were additionally excluded. TH: thyroid hormone, 1H-MRS: proton magnetic resonance spectroscopy.

Figure 2: Association between intrahepatic lipid (IHL) and fT3 (A) and T3/T4 ratio (B) at baseline. N = 332. Higher IHL content at baseline is associated with higher fT3 and T3/T4 ratio. Correlation was analyzed using Spearman's rank correlation coefficient, grey area indicates 95% confidence interval.

Figure 3: Association between change in intrahepatic lipid (IHL) from baseline to month 12 and corresponding changes in T3 level. N = 243. Smaller decrease in T3 is associated with a steeper decline in IHL over 12 months. Correlation was investigated using Spearman's rank correlation coefficient, grey area indicates 95% confidence interval.

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Table 1: Descriptive characteristics of the study cohort with available 1H-MRS data at baseline.

	Control	**Intervention**	**Total**	**P value**
	n = 166	**n = 166**	**n = 332**	
Baseline parameters	**n (%) or mean (SD) or median [IQR]**	**n (%) or mean (SD) or median [IQR]**	**n (%) or mean (SD) or median [IQR]**	
Age (years)	64.99 (6.75)	65.87 (7.00)	65.43 (6.88)	0.25
Female sex (n (%))	110 (66,3)	109 (65,7)	219 (66,0)	0.59
BMI (kg/m^2)	29.38 (4.97)	29.25 (5.02)	29.31 (4.99)	0.81
IHL (%)	3.50 [1.51, 7.82]	3.62 [1.36, 7,80]	3.62 [1.39, 7.93]	0.89
Type 2 diabetes (n (%))	27 (16.3)	30 (18.1)	57 (17.2)	0.45
Obesity (n (%))	68 (41.0)	70 (42.2)	138 (41.6)	0.58
IHL > 5.56% (n (%))	58 (34.9)	59 (35.5)	117 (35.2)	0.78
TH medication (n (%))	25 (15.1)	17 (10.2)	42 (12.7)	0.37
TSH (mIU/l)	0.73 [0.41, 1.17]	0.75 [0.39, 1.18]	0.74 [0.40, 1.18]	0.35
free T3 (pmol/l)	4.58 [4.09, 5.15]	4.52 [4.04, 5.02]	4.53 [4.06, 5.13]	0.96
free T4 (pmol/l)	11.80 [10.74, 13.13]	12.23 [11.14, 13.24]	12.03 [10.78, 13.17]	0.30
total T3 (nmol/l)	1.07 [0.97, 1.25]	1.09 [0.96, 1.25]	1.08 [0.96, 1.25]	0.81
total T4 (nmol/l)	105.29 (21.44)	106.83 (20.07)	106.06 (20.75)	0.50
fT3/fT4 ratio	0.39 (0.09)	0.38 (0.10)	0.39 (0.09)	0.34

T3/T4 ratio	1.66 [1.43, 1.87]	1.63 [1.39, 1.91]	1.64 [1.41, 1.89]	0.65
fT4/T4 ratio	0.11 [0.10, 0.13]	0.12 [0.10, 0.13]	0.12 [0.10, 0.13]	0.39
TG (mmol/l)	1.15 [0.92, 1.46]	1.21 [0.90, 1.58]	1.18 [0.91, 1.53]	0.32
HbA1c (%)	5.70 [5.50, 6.00]	5.70 [5.50, 6.00]	5.70 [5.50, 6.00]	0.61
Total cholesterol (mmol/l)	5.36 (1.04)	5.40 (1.04)	5.38 (1.04)	0.70
LDL-C (mmol/l)	3.34 (0.91)	3.37 (0.89)	3.35 (0.90)	0.78
HDL-C (mmol/l)	1.43 (0.32)	1.44 (0.33)	1.44 (0.32)	0.92

1H-MRS: proton magnetic resonance spectroscopy; TG: triglycerides; IHL: intrahepatic lipids; HDL-C: HDL cholesterol; LDL-C: LDL cholesterol; BMI: body-mass index; TH: thyroid hormone; TH medication: intake of levothyroxine or thiamazole. IHL > 5.56% is used as criterion for steatotic liver disease. Data were reported as mean (SD) for normally distributed data, median [IQR] for skewed data or as n (%) for categorical parameters.

Table 2: Correlations of estimates of thyroid hormone (TH) parameters with intrahepatic lipid (IHL) at baseline. Analyses were performed using Spearman's rank correlation coefficient.

Correlation of baseline TH parameters with IHL content (n = 332)		
	Rho	**P value**
TSH (mIU/l)	0.010	0.85
free T3 (ng/l)	0.118	0.03
free T4 (ng/l)	0.001	0.90
total T3 (nmol/l)	0.103	0.06
total T4 (nmol/l)	-0.068	0.22
fT3/fT4 ratio	0.085	0.12
T3/T4 ratio	0.149	0.01

Table 3: Change of thyroid hormone parameters from baseline to 12 months in control and intervention group with available proton magnetic resonance spectroscopy (1H-MRS) data at baseline and month 12.

	Control (n = 123)			Intervention (n = 120)			Control vs. Intervention
	Baseline	12 mo		Baseline	12 mo		
Parameters	Mean (SD) or Median [IQR]	Mean (SD) or Median [IQR]	P[1]	Mean (SD) or Median [IQR]	Mean (SD) or Median [IQR]	P[1]	P[2]
TSH (mIU/l)	0.72 [0.41, 1.10]	0.79 [0.43, 1.24]	0.27	0.76 [0.48, 1.19]	0.73 [0.46, 1.17]	0.95	0.76
free T3 (ng/l)	2.99 [2.65, 3.35]	2.86 [2.54, 3.16]	0.001	3.01 [2.66, 3.35]	2.83 [2.54, 3.13]	<0.001	0.95
free T4 (ng/l)	9.17 [8.32, 10.22]	9.18 [8.08, 10.27]	0.90	9.43 [8.58, 10.23]	9.39 [8.50, 10.57]	0.77	0.39
total T3 (nmol/l)	1.08 [0.99, 1.26]	1.02 [0.89, 1.15]	<0.001	1.09 [0.97, 1.25]	1.03 [0.92, 1.18]	0.002	0.93
total T4 (nmol/l)	107.52 [92.47, 118.17]	106.22 [91.31, 119.67]	0.80	103.88 [89.35, 120.52]	105.87 [92.90, 116.52]	0.81	0.76
fT3/fT4 ratio	0.39 (0.09)	0.37 (0.08)	0.001	0.39 (0.09)	0.36 (0.07)	<0.001	0.59
T3/T4 ratio	1.65 [1.43, 1.85]	1.52 [1.29, 1.84]	0.002	1.65 [1.42, 1.91]	1.55 [1.34, 1.84]	0.004	0.86
fT4/T4 ratio	0.11 [0.10, 0.13]	0.11 [0.10, 0.13]	0.71	0.12 [0.11, 0.13]	0.12 [0.11, 0.13]	0.28	0.33

1 Related-samples t tests or Wilcoxon signed-rank tests were used for within-group comparisons.
2 Baseline-adjusted analysis of covariance models were used for between-group comparisons of change

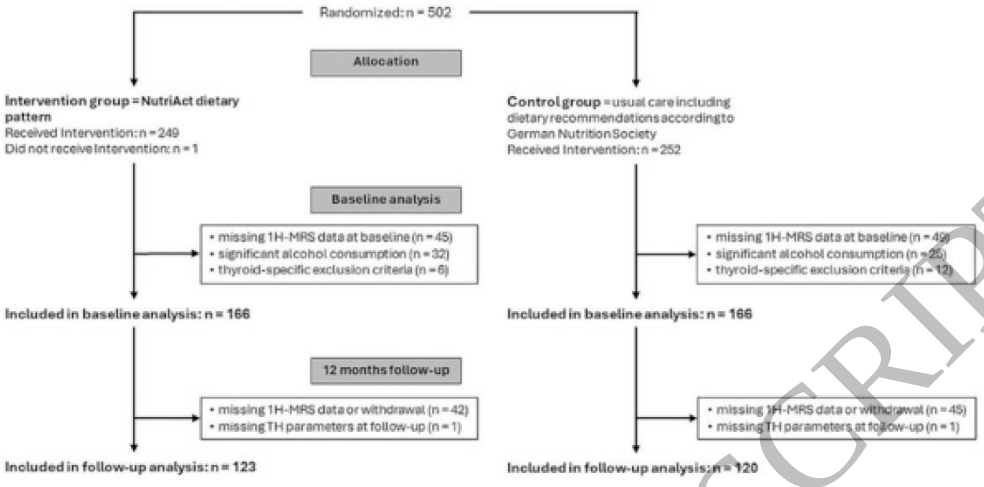

Figure 1
51x26 mm (DPI)

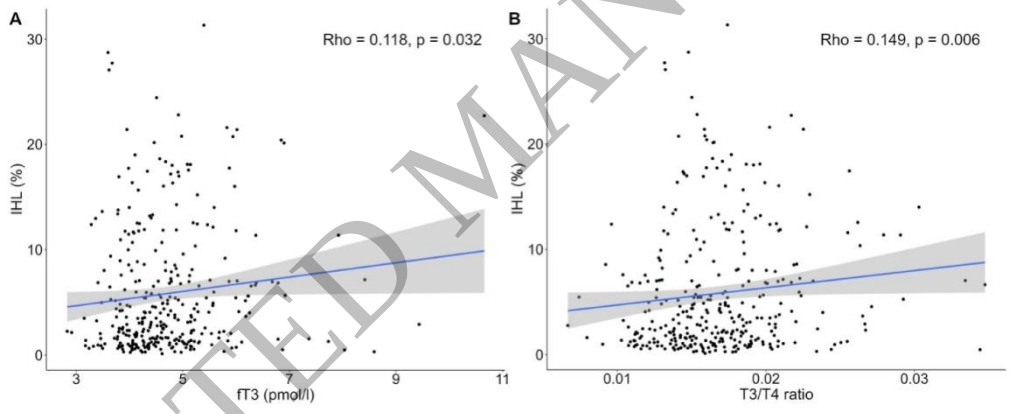

Figure 2
127x51 mm (DPI)

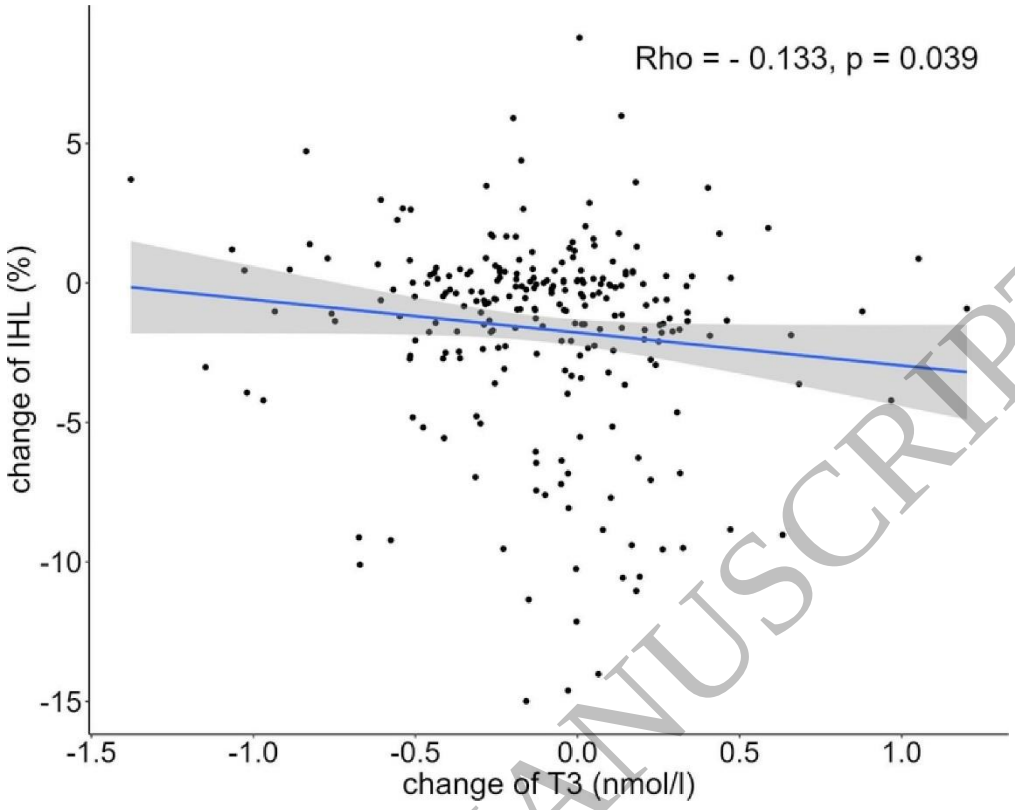

Figure 3
63x50 mm (DPI)