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Reversal of insulin resistance in people with obesity by lifestyle-induced weight loss does not impact the proportion of circulating 12α-hydroxylated bile acids

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Abstract

Aim: Bile acids (BAs) are implicated in the pathogenesis of several metabolic syndrome-related diseases, including insulin resistance (IR) and type 2 diabetes (T2D). It has been reported that IR and T2D are associated with an increased ratio of 12α/non-12α-hydroxylated BAs in the circulating BA pool. It is, however, unknown whether the improvement of insulin sensitivity inversely affects BA composition in humans. Therefore, we assessed whether lifestyle-induced weight loss induces changes in BA metabolism in people with obesity, with or without T2D, and if these changes are associated with metabolic parameters.

Materials and Methods: Individual BAs and C4 were quantified by ultra-high-performance liquid chromatography-tandem mass spectrometry in plasma samples collected from two cohorts of people with obesity (OB) and with T2D and obesity (T2D), before and after a lifestyle intervention.

Results: Lifestyle-induced weight loss improved glycaemic control in both cohorts, with plasma BA concentrations not affected by the lifestyle interventions. The ratio of 12α/non-12α-hydroxylated BAs remained unchanged in OB ($p = .178$) and even slightly increased upon intervention in T2D ($p = .0147$). Plasma C4 levels were unaffected in OB participants ($p = .20$) but significantly reduced in T2D after intervention ($p = .0003$). There were no significant correlations between the ratio of 12α/non-12α-hydroxylated

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BAs and glucose, insulin, or homeostatic model assessment-IR, nor in plasma triglycerides, low-density lipoprotein cholesterol, lipoprotein (a) in the T2D cohort.

Conclusions: Lifestyle-induced weight loss did improve glycaemic control but did not affect BA concentrations. Improvements in insulin sensitivity were not associated with changes in BA parameters in people with obesity, with or without T2D.

KEYWORDS
dietary intervention, cohort study, glycaemic control, liver, type 2 diabetes

1 | INTRODUCTION

The World Health Organization estimates that over 1 billion people live with obesity, with 167 million expected to be overweight or obese by 2025. Obesity negatively impacts health[1] and raises the risk of several comorbidities, including insulin resistance (IR), type 2 diabetes (T2D), cardiovascular diseases and metabolic dysfunction-associated fatty liver disease. Altered bile acid (BA) metabolism in obesity may contribute to these pathological features.[2]

BAs, amphipathic molecules produced in the liver, facilitate fat-soluble nutrient absorption and regulate lipid, glucose and energy metabolism by signalling via nuclear and membrane-bound receptors.[3,4] The human BA pool includes different species of BA with distinct detergent and physicochemical properties that impact their function.[3] BAs exert hormone-like functions via the activation of various receptors, such as the farnesoid X receptor and the Takeda G protein-coupled receptor 5.[4] By doing this, BAs are involved in the control of lipid, glucose, energy metabolism and insulin sensitivity.[3,5–10] These observations suggest that BAs may play a role in the development and progression of obesity and associated diseases.[11,12] In fact, changes in BA composition have been indicated to prevent diet-induced obesity and the development of IR in preclinical studies.[13,14] Several studies have reported links between plasma BA concentrations, markers of BA synthesis, and IR in humans.[15,16] It has been shown that plasma secondary BAs correlate with diabetes and liver fat content, and that plasma C4 is associated with characteristics of diabetic dyslipidaemia in obesity.[17] Plasma C4 levels are used as markers for the rate of hepatic BA synthesis.[18] Furthermore, plasma BAs are indicated to be elevated in individuals with T2D and IR,[15] as well as in animal models of type 1 diabetes (T1D) and T2D.[19–24] Interestingly, it has been shown that the expression and activity of the enzyme CYP8B1, responsible for C12 hydroxylation of BAs, are controlled by insulin[25,26] and intracellular glucose, the latter via ChREBP.[27] Consistently, several studies have shown that ratios of 12α-hydroxylated BAs (cholic acid, deoxycholic acid, and their conjugated forms) to non-12α-hydroxylated BAs [chenodeoxycholic acid, ursodeoxycholic acid, lithocholic acid (LCA)] are elevated in the plasma of individuals with T2D and IR.[15,28] Importantly, this ratio has recently been identified as a potential determinant of cholesterol absorption in mouse experiments[29] and in people with T1D.[30] However, it remains to be elucidated whether BA composition is causally involved in the aetiology of T2D and IR, as well as how it affects metabolic parameters that are often dysregulated in patients with T2D and IR. In addition, it is unknown whether improvement of insulin sensitivity by, for instance, lifestyle-induced weight loss affects BA metabolism in people with obesity, with or without T2D.

The primary aim of the current study is therefore to determine the effects of lifestyle-induced weight loss on plasma BA concentrations and composition as well as on hepatic BA synthesis as reflected by plasma C4 levels in people with obesity and T2D (cohort T2D), and to relate any possible change to alterations in metabolic parameters [glucose, insulin, homeostatic model assessment (HOMA)-IR], including lipid parameters [triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), lipoprotein a (LPa), plant sterols].

In addition, it has been established that sulphated BAs, particularly sulphated LCAs, are present in human serum and urine in variable amounts.[31] Sulphation is considered to serve as a mechanism to eliminate potentially hepatotoxic BAs.[32,33] However, the effects of obesity and T2D on the absolute and relative concentrations of sulphated BAs are unknown. Therefore, the presence of sulphated BAs in patients with obesity, with and without T2D, has also been evaluated in this study.

2 | MATERIALS AND METHODS

2.1 | Participants and interventions

Two distinct cohorts were used to examine the effects of diet-induced weight loss. The primary cohort (n = 45, OB) included individuals with obesity, who were recruited at the Obesity Center CGG, Erasmus University Medical Center, Rotterdam, the Netherlands. A minority of those participants (n = 5) met the criteria for T2D without the use of insulin. Details of the lifestyle intervention are described extensively elsewhere.[34] In short, participants underwent a structured combined lifestyle intervention consisting of advice on healthy eating, exercise and behavioural therapy. Participants received a total of 18 sessions with a dietitian and psychologist, including 1.5 h of exercise under the supervision of a physical therapist, during 75 weeks. Inclusion criteria for OB: body mass index (BMI) ≥30 kg/m^2, age ≥18 years and presence of at least one obesity-related comorbidity (e.g. hypertension, diabetes mellitus, dyslipidaemia, non-alcoholic fatty liver disease, obstructive sleep apnoea syndrome, polycystic ovarian syndrome, or osteoarthritis). Exclusion criteria for OB: inability to speak Dutch (because of the nature of the treatment in group sessions), wish to become pregnant in the near future, intellectual disability and (severe) behavioural problems that would impede functioning in a group setting (e.g. motoric disability or factors that necessitate

individual therapy such as major depressive disorder or binge-eating disorder). The second cohort (n = 162, T2D) consisted of people with obesity and T2D who participated in the run-in phase of the Prevention of Weight Regain (POWER) trial (trial registration no. NTR2264). The participants with T2D underwent a very low-calorie diet (3000 kJ/day = 750 kcal/day) for 8 weeks, followed by a 12-week low-energy diet (5500 kJ/day = 1300 kcal/day). Basal parameters and some outcome measurements (BMI, waist circumference, systolic blood pressure, use and dosage of antidiabetic agents have previously been reported).[35] Inclusion criteria for T2D: Overweight and obese (BMI ≥27 kg/m^2) adults with type 2 diabetes aged 18-75 years. Exclusion criteria for T2D: pregnancy; lactation; inadequate understanding of the Dutch language; severe psychiatric problems; significant cardiac arrhythmias; unstable angina; decompensated congestive heart failure; carcinomas; major organ system failure; untreated hypothyroidism; end-stage renal disease; myocardial infarction, cerebrovascular accident or major surgery during the previous 3 months. A condensed overview of the subject characteristics of OB and T2D is provided in Table S1.

2.2 | Sampling

After an overnight fast, blood samples were collected and stored at −20°C and −80°C until analysis. In addition to body weight, height and waist circumference were recorded. Using standard laboratory techniques, metabolic [fasting glucose, fasting insulin, glycated haemoglobin (HbA1c)] and lipid (LDL-cholesterol, high-density lipoprotein cholesterol and triacylglycerol) parameters were determined.[36,37]

2.3 | Plasma bile acids and plasma lipid measurements

Plasma BA species and C4 levels were quantified by liquid chromatography-tandem mass spectrometry (LC/MS/MS) procedures using a Nexera X2 ultra-high-performance liquid chromatography system (SHIMADZU), connected to a SCIEX QTRAP 4500 MD triple quadrupole MS (SCIEX) (UHPLC-MS/MS) as previously described.[38] Plasma plant sterols were determined by gas chromatography-MS, as described,[39] and normalized to plasma total cholesterol levels. Plasma levels of total cholesterol were determined using commercially available diagnostic system solutions (DiaSys Diagnostic Systems).

2.4 | Statistical analysis

Data are presented as Tukey box and whisker plots, produced using GraphPad Prism 8 (GraphPad Software). For statistical significance between the variables, a two-sided paired-sample t-test was used. Values for $p < .05$ were considered significant. The level of significance is indicated as $^*p \le .05$, $^{**}p \le .01$ and $^{***}p \le .0001$. Spearman rank correlation was used to measure the degree of association between variables. The Wilcoxon ranking test was used to evaluate pre- and post-treatment measurements. A Corrplot graphic was used to visualize a correlation matrix among variables.

3 | RESULTS

3.1 | Lifestyle-induced weight loss does not affect total plasma bile acids, ratios of 12α-hydroxylated/non-12α-hydroxylated bile acids or bile acid synthesis in people in the obesity cohort

The average weight loss in patients with obesity (OB cohort) was 6.5% of their initial body weight, leading to significant reductions in BMI and waist circumference ($p < .001$).[35,36] Despite the fact that the majority of these individuals did not have T2D, their HbA1c and fasting glucose levels improved with a significant reduction in HOMA-IR (Figure 1). To assess whether this lifestyle-induced weight loss resulted in changes in BA homeostasis, we quantified concentrations of individual BA species as well as C4 levels at different time points after the start of the intervention. To our surprise, we found no correlation between HOMA-IR and the ratio of 12α-hydroxylated to non-12α-hydroxylated BAs at baseline ($r = 0.191$, $p = .215$). Plasma total BA levels remained unchanged in all participants upon the intervention (Figure 2A). In contrast with our expectations, the ratio of 12α-hydroxylated to non-12α-hydroxylated BAs also remained unchanged ($p = .178$) (Figure 2B). In addition, there were no changes in the ratio of secondary to primary BAs ($p = .052$) (Figure 2C) or in the ratio of conjugated versus unconjugated BAs ($p = .223$) (Figure 2D) upon intervention. The absolute and relative concentrations of sulphated LCAs were also not affected by weight loss (Figure 2E). Plasma C4 levels, used as markers for the rate of BA synthesis, did not change after the lifestyle intervention ($p = .20$) (Figure 2F), indicating that the hepatic BA synthesis rate was not impacted by weight loss in these individuals.

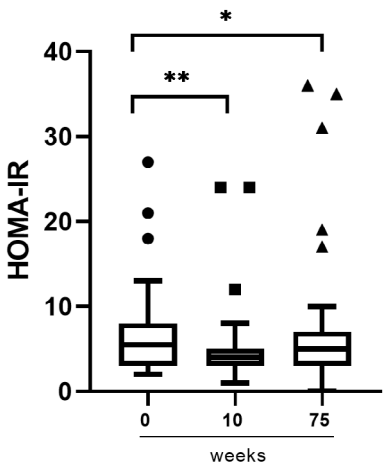

FIGURE 1 Lifestyle-induced weight loss improves HOMA-IR in people with obesity (OB cohort). HOMA-IR in people with obesity; OB cohort N = 45. Data are presented as Tukey's box. Data are presented as Tukey's box and p values represent $^*p < .05$ and $^{**}p < .01$ by a two-sided paired-sample t test. HOMA-IR, homeostatic model assessment of insulin resistance.

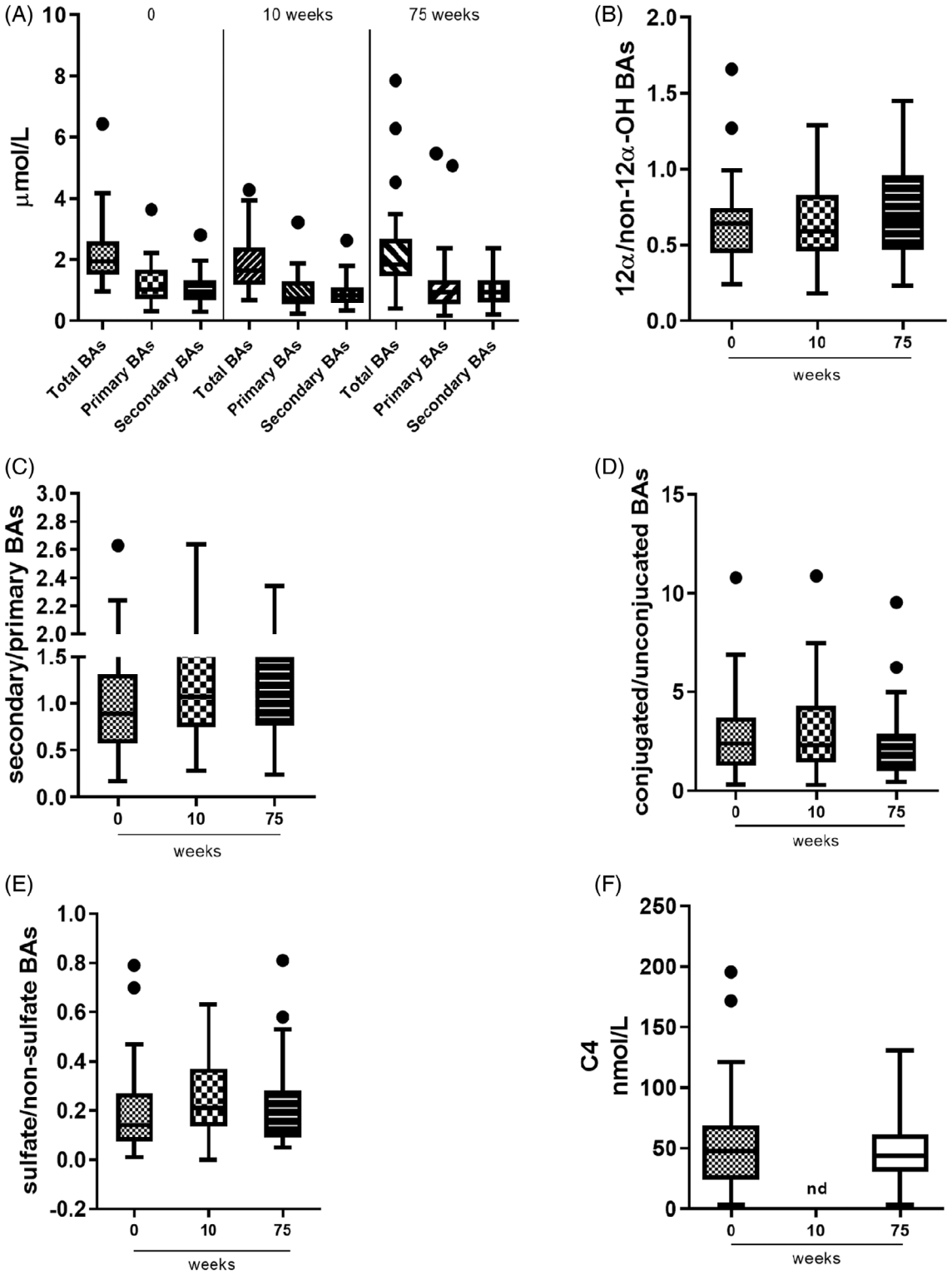

FIGURE 2 Lifestyle-induced weight loss does not affect total plasma BAs, ratios of 12α-hydroxylated/non-12α-hydroxylated (12α-/non-12α-OH) BAs or C4 in people with obesity (0B cohort). (A) Plasma total, primary and secondary BAs before, after 10 and 75 weeks of lifestyle interventions in people with obesity. (B) Ratios of 12α/non-12α-OH BAs in people with obesity. (C) Ratios of secondary to primary BAs in people with obesity. (D) Ratios of conjugated versus un-conjugated BAs in people with obesity. (E) Ratios of sulphated to non-sulphated BAs in people with obesity. (F) C4 levels before and after 75 weeks of lifestyle intervention in people with obesity. Data at 10 weeks are not determined. Obesity cohort N = 45. Data are presented as Tukey's box. BAs, bile acids.

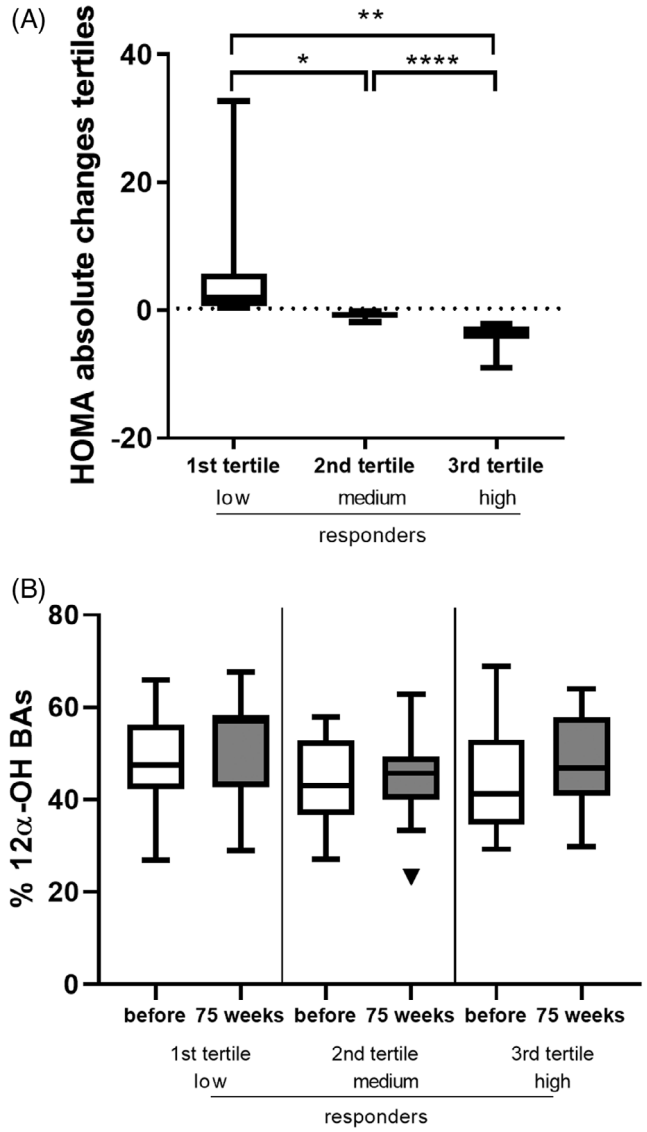

FIGURE 3 No relation between percentage of 12α-OH BAs in plasma and change in HOMA-insulin resistance in people with obesity upon weight loss. (A) HOMA-insulin resistance absolute change tertiles (low, medium and high responders) in people with obesity. (B) Percentage of 12α-OH BAs before and after 75 weeks of lifestyle intervention in the three tertiles. Obesity cohort N = 45. Data are presented as Tukey's box and correlation line. 12α-OH, 12α-hydroxylated; BAs, bile acids; HOMA, homeostatic model assessment.

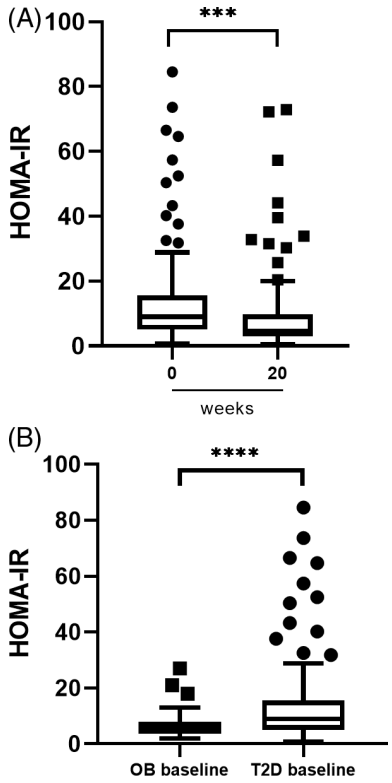

FIGURE 4 Lifestyle-induced weight loss improves HOMA-IR in people with obesity and type 2 diabetes (T2D cohort). (A) HOMA-IR in people with obesity and T2D at baseline and after 20 weeks of lifestyle intervention. (B) Comparison between HOMA-IR in the two cohorts. People with obesity (OB cohort). N = 45; T2D cohort N = 162. Data are presented as Tukey's box and *p* values represent $^{**}p < .01$ and $^{****}p < .0001$ by a two-sided paired-sample t test. HOMA-IR: homeostatic model assessment of insulin resistance; T2D: type 2 diabetes. HOMA-IR, homeostatic model assessment of insulin resistance.

3.2 | No relation between percentage of 12α-hydroxylated bile acids in plasma and change in homeostatic model assessment-insulin resistance in people with obesity upon weight loss (obesity cohort)

IR per se has been reported to be associated with increased ratios of 12α-hydroxylated/non-12α-hydroxylated BAs in humans. Therefore, we divided the participants into tertiles based on their change in HOMA-IR during the lifestyle intervention (75 weeks vs. start) (Figure 3A). Yet, the percentage of 12α-hydroxylated BAs within these three tertiles was similar and remained unaltered during the lifestyle intervention ($p = .35$, $p = .79$, $p = .56$) (Figure 3B).

3.3 | Lifestyle-induced weight loss does not affect total plasma bile acids, increases the ratio of 12α-hydroxylated/non-12α-hydroxylated bile acids and reduces hepatic bile acid synthesis in people in the obesity and type 2 diabetes cohort

The lifestyle intervention in patients with obesity and T2D (T2D cohort) resulted in a significant reduction (9.9%) of initial body weight. The intervention improved glycaemic control,[35,36] resulting in a significantly improved HOMA-IR ($p < .01$) (Figure 4A). Comparing the HOMA-IR scores of the OB and T2D cohorts revealed that the participants in the OB cohort began treatment with a significantly lower HOMA-IR than the participants with diabetes in the T2D cohort ($p < .0001$) (Figure 4B).

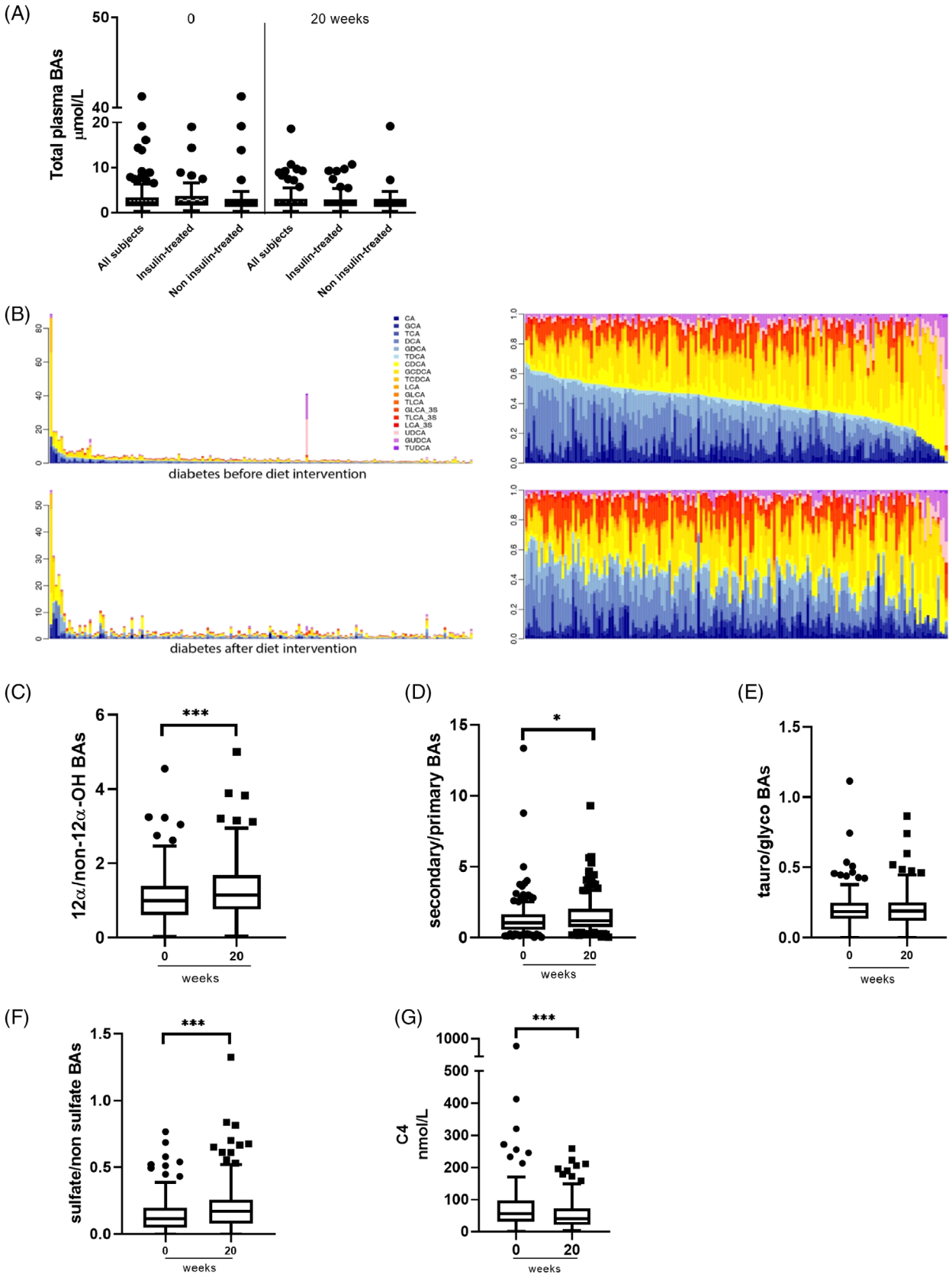

FIGURE 5 Legend on next page.

Again, no correlation between HOMA-IR and the ratio of 12α-hydroxylated to non-12α-hydroxylated BAs was found at baseline ($r = 0.003$, $p = .97$). Plasma total BA levels were unchanged upon the lifestyle intervention in T2D (mean = 0.248, SD = 3674, $p = .39$) (Figure 5A). Plasma BA composition showed a large intra-individual variation, with some participants showing a preponderance of 12α-hydroxylated BAs, while non-12α-hydroxylated BAs were the most abundant in others. No concomitant effect of weight loss was evident (Figure 5B). As 50.6% of the 162 participants were treated with insulin, which is involved in the control of the expression of CYP8B1, we next compared plasma BA levels in insulin-treated participants and non-insulin-treated participants before and after intervention. However, there were no differences in plasma BA levels between these subgroups (Figure 5A). Furthermore, the sum of 12α-hydroxylated BA species and the sum of non-12α-hydroxylated BA species were unaltered after the intervention in all participants ($p = .216$; $p = .149$) as well as in insulin- and non-insulin-treated participants (data not shown). The ratio of 12α-hydroxylated/non-12α-hydroxylated BAs was slightly increased ($p = .014$), opposite to what was expected, after intervention in all participants (Figure 5C), but there were no significant differences between insulin- and non-insulin-treated participants (data not shown). The ratio of secondary to primary BAs, however, was significantly increased upon intervention across all participants (mean = −0.256, SD = 1.285, $p = .012$) (Figure 5D). Meanwhile, there were no significant differences in the ratio of tauro- versus glyco-conjugated BAs ($p = .941$) (Figure 5E). Of interest is the finding that the concentrations of LCA increased upon dietary intervention: the median range of sulphated BAs/non-sulphated LCA increased from 0.22 to 0.26 ($p = .009$) (Figure 5F). Plasma C4 levels were significantly lower after the intervention ($p = .0003$) (Figure 5G), indicating reduced hepatic BA synthesis (median C4 before diet: 55.7 nmol/L; median C4 after diet: 40.4 nmol/L; Wilcoxon ranking test before – after, $p = .001$).

3.4 | Lifestyle-induced weight loss and reversal of insulin resistance are not associated with changes in the ratio of 12α-hydroxylated/non-12α-hydroxylated bile acids in people with obesity or overweight and type 2 diabetes cohorts

Based on the changes in HOMA-IR in response to the lifestyle intervention, the T2D population was categorized into three tertiles (Figure S1). The percentage of 12α-hydroxylated BAs within the three tertiles remained unaltered upon intervention ($p = .67$, $p = .148$, $p = .563$) (Figure S1) and there were no differences in correlations between ratios of 12α-hydroxylated/non-12α-hydroxylated BAs before and after treatment between the tertiles (Figure S1).

3.5 | Lifestyle-induced weight loss and changes in glucose and lipid parameters are not associated with bile acid parameters in people in the obesity and type 2 diabetes cohort

In the T2D cohort, there were no correlations between the ratios of secondary to primary BAs and glucose, insulin, HOMA, TG, LDL-C, or LP(a), and there were no correlations between sulphated BAs and TG, LDL, or total cholesterol. The only significant correlation found ($r = 0.188$, $p = .017$) was between delta LP(a) and the delta ratio of primary/secondary BAs.

3.6 | Lifestyle-induced weight loss and changes in lipid parameters do not significantly impact bile acid parameters in people with obesity and type 2 diabetes

As expected,[17,28] C4 and TG were positively correlated at baseline ($r = 0.410$, $p = .001$), underscoring the important role of hepatic BA synthesis in the control of plasma TG concentrations. At baseline, C4 and sulphated BAs were not correlated ($r = -0.099$, $p = .209$), but delta C4 and delta sulphated BAs were negatively correlated ($r = -0.272$, $p < .001$). In addition, delta LP(a) was unrelated to delta-sulphated BAs, while there was no significant correlation between C4 and LDL at baseline ($r = 0.127$, $p = .116$) or between delta C4 and delta LP(a) ($r = -0.041$, $p = .610$).

3.7 | Ratios of 12α-hydroxylated/non-12α-hydroxylated bile acids are negatively correlated with total plant sterols in people in the obesity and type 2 diabetes cohort

Plasma concentrations of non-cholesterol sterols (brassicasterol, campesterol, sitosterol and total plant sterols), as biomarkers of cholesterol absorption, were measured in all individuals of the T2D cohort before the start of the intervention to evaluate the relationship between plant sterols (total concentrations) and the ratio of 12α-

FIGURE 5 Lifestyle-induced weight loss does not affect total plasma BAs, increases ratios of 12α-hydroxylated/non-12α-hydroxylated (12α/non-12α-OHs) BAs and reduces BA synthesis in people with obesity and T2D (T2D cohort). (A) Plasma total, primary and secondary BAs before and after 20 weeks of lifestyle interventions in theT2D cohort. (B) BA species in waterfall plot for each individual in the T2D cohort before and after 20 weeks of lifestyle intervention. (C) Ratios of 12α-OH/non-12α-OH BAs in people with obesity and T2D. (D) Ratios of secondary to primary BAs in people in theT2D cohort. (E) Ratios of tauro-conjugated versus glycol-conjugated BAs in people in theT2D cohort. (F) Ratios of sulphated to non-sulphated BAs in people with obesity and T2D. (G) C4 levels people in theT2D cohort. T2D cohort N = 162. Data are presented as Tukey's box and as waterfall chart in which each bar represents a single individual and colours represent different BAs. Values represent $^{*}p < .05$ and $^{***}p < .001$ by a two-sided paired-sample t-test. BAs, bile acids.

hydroxylated/non-12α-hydroxylated BAs (Figure S2). In contrast to what was expected, the ratio of 12α-hydroxylated/non-12α-hydroxylated BAs negatively correlated, although only moderately, with total plant sterols ($r = -0.2137$, $p = .0063$) (Figure S2). Plasma concentrations of plant sterols were normalized to cholesterol, but no effects were detected (Figure S2).

4 | DISCUSSION

In the current study, we aimed to determine whether lifestyle-induced weight loss can reverse IR-related changes in BA metabolism in people in the OB cohort and in people in the T2D cohort, and to evaluate whether changes in BA composition are associated with changes in metabolic parameters such as plasma lipids. Plasma BA concentrations have been linked to IR in humans.[15] In particular, individuals with T2D and IR were reported to display elevated plasma total BAs, plasma secondary BAs and high ratios of 12α-hydroxylated/non-12α-hydroxylated BAs.[15] Thus, it has been suggested that 12α-hydroxylated BAs might play a role in the aetiology of T2D. Based on these findings, we hypothesized that total plasma BA concentrations, ratios of 12α-hydroxylated/non-12α-hydroxylated BAs, ratios of secondary/primary BAs and C4 concentrations would be elevated in people with obesity, and with obesity and T2D and decrease upon weight loss-associated improvements in insulin sensitivity. In addition, as the effects of T2D on the concentrations of sulphated BAs in people with obesity are unknown, we also evaluated their values and how their concentrations change in response to lifestyle intervention in individuals with obesity who did or did not have T2D. Several studies have reported on the BA sulphation process in humans, showing the presence of sulphated BAs in blood, bile and urine.[32,33,40] BA sulphation is generally considered a mechanism for eliminating potentially toxic secondary BAs, but, according to our knowledge, their signalling properties have not been evaluated.[32,33]

Contrary to our expectations, our data show that lifestyle-induced improvement of insulin sensitivity in individuals with obesity and with T2D does not have a distinct impact on BA parameters that were studied. In particular, total plasma BAs, secondary BAs, BA synthesis markers and sulphated BAs were not affected by the lifestyle intervention in the two independent cohorts. In line with our earlier observation in obese subjects (17), we did not observe correlations between HOMA-IR and the ratio of 12α-hydroxylated/non-12α-hydroxylated BAs in OB and in T2D. In addition, this ratio remained unchanged in the OB cohort and even slightly increased upon intervention in the T2D cohort. To further delineate the potential correlation between 12α-hydroxylated/non-12α-hydroxylated BAs and insulin sensitivity, participants of both cohorts were divided into three tertiles based on their HOMA-IR response to the lifestyle intervention: low responders, medium responders and high responders. We observed no changes in the percentage of 12α-hydroxylated BAs after the diet in each tertile, suggesting that there is no relationship between the percentage of 12α-hydroxylated BAs and HOMA-IR changes upon lifestyle intervention in these cohorts. This was unexpected as we did observe that lifestyle-induced weight loss improved HbA1c and fasting glucose levels, resulting in a significantly improved HOMA-IR score in both cohorts. Interestingly, in the T2D cohort, the lifestyle-induced weight loss did not affect total plasma BAs but reduced BA synthesis, and, surprisingly, it increased the ratios of 12α-hydroxylated/non-12α-hydroxylated BAs. This indicates that the improvement in IR caused by diet-induced weight loss is not accompanied by a reduction in the ratio of 12α-hydroxylated/non-12α-hydroxylated BAs. This conclusion might also be supported by the findings obtained from a study we conducted on a mouse model with a human-like BA composition (*Cyp2c70*$^{-/-}$ mice) in which the manipulation of 12α-hydroxylated/non-12α-hydroxylated BA ratio by colesevelam, a BA sequestrant, had no effect on insulin sensitivity.[41] The expression and activity of the enzyme CYP8B1, responsible for 12α-hydroxylated BA production, are controlled by insulin, intracellular glucose and glucose-6-phosphate. In particular, insulin suppresses CYP8B1 via FOXO1 and glucose-6-phosphate induces its expression via ChREBP.[27,42] It has recently been shown that CYP8B1 haploinsufficiency in humans, leading to reduced CYP8B1 activity, decreases ratios of 12α-hydroxylated/non-12α-hydroxylated BAs and is associated with increased insulin sensitivity.[5] The latter was partially explained by the potential selectivity of the non-12α-hydroxylated chenodeoxycholic acid in modulating skeletal muscle insulin sensitivity by increasing muscle FOXO1 activity.[5] Therefore, it has been proposed that changes in BA composition caused by decreased CYP8B1 activity may play a role in the regulation of peripheral insulin sensitivity in humans. However, in the current study, we show that the converse, i.e. improvement of insulin sensitivity as induced by lifestyle changes, does not impact ratios of 12α-hydroxylated/non-12α-hydroxylated BAs in individuals with obesity. Taken together, these data suggest that increased CYP8B1 activity may contribute to IR but that the reversal of IR in obesity does not impact the proportion of circulating 12α-hydroxylated BAs. An important factor to consider is the large interindividual variation in BA composition that exists in individuals with obesity[17] and healthy weight,[43] underscoring that genetic, microbial factors and diet composition all contribute to this variation. In fact, in 300 individuals with obesity, the ratio of 12α-hydroxylated/non-12α-hydroxylated BAs ranged from 0.1 to 6.8 (mean value 1.5)[17] and in 1200 healthy subjects, from 0.1 to 7.3 (mean value 1.3).[44] The ranges (0.4-2.2; 0.02-2.1) and mean values (0.9; 0.8) in the respective OB and T2D cohorts are considerably smaller than previously reported in the literature. However, it could be debated whether differences in the proportion of 12α-hydroxylated/non-12α-hydroxylated BAs could be identified in relatively small cohorts. In a recent study, Chen et al. showed that diet components and the gut microbiome are more important than genetic factors in determining the inter-individual variability in human plasma metabolomes, including BAs.[43] Alterations in lifestyle interventions can strongly influence the abundance and composition of gut microbiome and, hence, BAs.[45] Therefore, all data considered, the regulation of human CYP8B1 and other factors that contribute to the ratio of 12α-hydroxylated/non-12α-hydroxylated BAs appear to be highly complex and not governed by insulin (sensitivity) alone.

It has been established that sulphated BAs, particularly sulphated LCAs, are present in human serum and urine in variable amounts,[32] but whether obesity and T2D might have effects on the absolute and relative concentrations of sulphated BAs remains unknown. We now report these values in people with OB and OB + T2D. Indeed, the contribution of sulphated BAs to total plasma BAs showed large intra-individual variation, but appeared unrelated to the metabolic state. Intriguingly, in the T2D cohort, the concentrations of sulphated BAs increased upon dietary interventions, indicating that the diet may play a role in the sulphation process or in the conservation of sulphated BAs in the enterohepatic circulation. Changes in sulphated BAs did not correlate to any of the glucose or lipid parameters quantified in the current study. It has been reported that cholesterol absorption markers (plant sterols, campesterol and β-sitosterol) are increased in T1D[46,47] and positively correlated with HbA1c and fasting glucose, while cholesterol synthesis (lathosterol) is decreased,[30] suggesting that T1D is associated with higher cholesterol absorption and lower cholesterol synthesis. We have shown that impaired intestinal cholesterol absorption in Western-type diet-fed mice with a human-like BA composition correlates positively with the ratio of 12α-hydroxylated/non-12α-hydroxylated BAs indicating that a low presence of 12α-hydroxylated BAs in the BA pool reduces the efficiency of intestinal cholesterol absorption.[29] Therefore, plasma plant sterols were measured in the T2D cohort before the intervention. We expected the ratio of 12α-hydroxylated/non-12α-hydroxylated BAs to be positively correlated with plant sterols. Yet, to our surprise, we found that this ratio actually negatively correlated with plant sterol levels in people with obesity and T2D. To exclude the possibility that these unexpected results were attributable to treatment with medications that could interfere with cholesterol absorption,[48,49] we corrected plant sterol levels for metformin use. Although plant sterol levels are widely used as markers of cholesterol absorption, their validity has been controverted. It has been shown that in a large population of subjects with mild hypercholesterolaemia, plasma plant sterol concentrations do not reflect cholesterol absorption.[37] Likewise, Stellaard et al. also observed that sitosterol and cholestanol represent poor markers of cholesterol absorption.[50] In summary, our unexpected findings should be interpreted with caution, as plant sterols may be inaccurate markers of cholesterol absorption. Therefore, a real quantification of cholesterol absorption using stable isotopes is needed in future studies.

In conclusion, our data indicate that there is no correlation between weight loss-induced improvement in insulin sensitivity and changes in BA parameters (concentrations, ratios of 12α/non-12α-hydroxylated BAs and BA synthesis) in individuals with obesity and with obesity and T2D. Specifically, ratios of 12α/non-12α-hydroxylated BAs are unrelated to indices of improved insulin sensitivity.

AUTHOR CONTRIBUTIONS

AP, MK, MVH, ATP, MF and JFB performed experiments, analysis and wrote the manuscript. KB, ESV and EFCR performed data collection, analysis and wrote the manuscript. MT and FK designed the study, supervised analysis and wrote the manuscript. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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