Harreiter Jürgen (Orcid ID: 0000-0003-2508-9403)
Kautzky-Willer Alexandra (Orcid ID: 0000-0002-3520-4105)

Combined EXENatide and DApagliflozin has no additive effects on reduction of hepatocellular lipids despite better glycemic control in patients with type 2 diabetes mellitus treated with metformin –EXENDA, a 24 week, prospective, randomized, placebo-controlled pilot trial.

Jürgen Harreiter, PhD 1, Ivica Just, PhD 2,3, Michael Leutner, PhD 1, Magdalena Bastian, BSc 1, Helmut Brath, MD 4, Christian Schelkshorn, MD 5, Radka Klepochova, PhD 2,3, Martin Krššák, PhD 2,3, Alexandra Kautzky-Willer, MD 1

1 Gender Medicine Unit, Division of Endocrinology & Metabolism, Department of Medicine III, Medical University Vienna, Vienna, Austria.

2 Department of Biomedical Imaging and Image-Guided Therapy, High-Field MR Center, Medical University of Vienna, Vienna, Austria.

3 Division of Endocrinology & Metabolism, Department of Medicine III, Medical University Vienna, Vienna, Austria.

4 Diabetes Outpatient Clinic, Health Centre Favoriten, 1100, Vienna, Austria

5 Department of Medicine, State Hospital Stockerau, Stockerau, Austria

Corresponding author: Jürgen Harreiter, MD, PhD, MSc, Gender Medicine Unit, Division of Endocrinology & Metabolism, Department of Medicine III, Medical University Vienna, Waehringerguertel 18-20, 1090 Vienna. telephone contact: +43 1 40400 43110, Fax +43 1 40400 43090, email: juergen.harreiter@meduniwien.ac.at

Short running title: GLP1-RA&SGLT2-I in hepatic lipid reduction

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/dom.14319

Abbreviations:

ALT	alanine transaminase
ANCOVA	analysis of covariance
AST	aspartate aminotransferase
BMI	body mass index
CV	coefficient of variation
CVD	cardiovascular disease
DAPA	dapagliflozin
EXE	exenatide
EXENDA	name of the trial
GCP	good clinical practice
GGT	gamma-glutamyl transpeptidase
GLP1-RA	Glucagon-like Peptid 1 receptor agonists
HbA1c	glycated haemoglobin
HCL	hepatocellular lipids
HDL-C	high-density lipoprotein cholesterol
HOMA	homeostasis model assessment
ISO	International Organization for Standardization
LDL-C	low-density lipoprotein cholesterol
NAFLD	non-alcoholic fatty liver disease
MRS	magnetic resonance spectroscopy
PLAC	placebo
RCT	randomized controlled trial
SAT	subcutaneous abdominal fat
SGLT2-I	sodium-glucose linked transporter 2 inhibitor
T2DM	type 2 diabetes mellitus
VAT	visceral abdominal fat

Abstract:

Aims:

To investigate potential synergistic effects of combined exenatide and dapagliflozin (EXE+DAPA) versus placebo and dapagliflozin (PLAC+DAPA) on hepatocellular lipid (HCL) reduction after 24 weeks of treatment.

Materials and Methods

Thirty patients with T2DM were randomized to weekly EXE and daily DAPA(n=16) or weekly PLAC and daily DAPA(n=14). Inclusion criteria were HbA1c of 6.5-11% (48-97mmol/mol), age 18-75y, BMI>=25kg/m² and metformin>=1000mg. The primary endpoint HCL was measured at baseline and after 24 weeks of treatment using magnetic resonance spectroscopy. Between group effects were analysed using general linear models adjusted for baseline outcome variables, age, sex and BMI. Within group differences were assessed using paired t-test.

Results:

After 24 weeks HCL was reduced in both treatment groups (absolute change from baseline,95%CI, EXE+DAPA:-4.4%;-8.2,-0.7,p<0.05;PLAC+DAPA:-3.9%;-6.0,-1.7,p<0.01, relative change: EXE+DAPA:- 35.6%;PLAC+DAPA -32.3%) with no difference between groups. Similar findings were observed for subcutaneous (SAT) and visceral adipose tissue (VAT). HbA1c (EXE+DAPA:-1.6%(=-17.8mmol/mol), -2.3,-1.0;,p<0.001;PLAC+DAPA-0.6%(=-6.9mmol/mol);-1.0.,-0.3,p=0.001) and fasting glucose significantly decreased in both groups although EXE+DAPA achieved better glycemic control than PLAC+DAPA (adj.diff. HbA1c:-0.55%(=-6.0mmol/mol);-0.88,-0.20,p<0.01). Body weight was reduced in both treatment groups (EXE+DAPA: -7.3kg;-9.9,-4.8,p<0.001;PLAC+DAPA -4.6kg;-7.4,-

1.8,p<0.01) with comparable results between groups. Changes of HCL and weight, hip, waist circumference, VAT and SAT were positively associated.

Conclusion:

After 24 weeks HCL was significantly but comparably reduced in EXE+DAPA and PLAC+DAPA despite significantly better glycemic control in the combined group EXE+DAPA. Changes of HCL were associated with weight loss and reduction of visceral adiposity but not with glucose control. Further studies are necessary to evaluate possible additional long-term effects of a combined treatment.

keywords: type 2 diabetes mellitus, SGLT2 inhibitor, GLP1 receptor agonist, NAFLD, magnetic resonance imaging, prevention, metabolic syndrome, CVD, ectopic lipids, obesity, weight loss, glycaemic control.

Introduction:

Type 2 diabetes mellitus (T2DM) is associated with several co-morbidities such as cardiovascular disease (CVD), obesity, hyperlipidaemia or non-alcoholic fatty liver disease (NAFLD), the most prevalent chronic liver disease worldwide. NAFLD is common among patients with T2DM demonstrating a global prevalence of about 55% in patients with T2DM with highest prevalence rates of 70% reported in Europe (1). An elevated risk for progression to more severe liver diseases as NASH, fibrosis and cirrhosis as well as end-stage liver disease is evident (2, 3). Recently, a population-based cohort study found a significant increase of the indication for liver transplantation associated with NAFLD from 2.0% to 6.2% over the last three decades (4). Facing these complications of NAFLD, interventions need to be identified urgently, which are able to effectively and safely reduce excess hepatocellular lipids (HCL).

Recent national and international clinical practice guidelines recommend new drug classes such as sodium-glucose linked transporter 2 inhibitors (SGLT2-I) or Glucagon-like Peptide 1 receptor agonists (GLP1-RA) as the main treatment options in high risk CVD patients, based on their cardiovascular and renal benefits (5-7). Moreover, disease-modifying effects on NAFLD were reported for several glucose-lowering drugs, including some GLP1-RA and SGLT2-I. Indeed, more and more patients in clinical routine are using combined GLP1-RA and SGLT2-I therapy showing strong glucose lowering potential and weight reduction (8, 9). Whether treatment combinations also have synergistic cardiovascular or renal effects, are more beneficial in patients with heart failure or are able to reduce HCL more effectively is not well investigated and thus needs further examination in randomized controlled trials (RCT).

NAFLD is associated with increased CVD mortality, an even higher mortality was reported in patients with both, NAFLD and T2DM (2, 3, 10). Thus a dual strategy using glucose lowering

compounds to optimize glycemic control and to reduce HCL seems a promising approach in patients with T2DM. So far combinations of above mentioned drug classes to investigate synergistic effects of these compounds in reducing HCL are hardly examined. A RCT combining exenatide and pioglitazone observed significantly higher reductions in HCL compared with pioglitazone (11). Recently, a study demonstrated beneficial effects of a combination of the SGLT2-I dapagliflozin (DAPA) with the GLP1-RA exenatide (EXE) on reducing markers and scores of liver steatosis and fibrosis in patients with T2DM on metformin therapy (12). However, further studies to prove these findings are required.

In our EXENDA trial, a prospective, monocentric, double-blind, randomized, controlled trial, we aimed to investigate the potential synergistic effects of a treatment combination of once weekly exenatide and once daily dapagliflozin on HCL measured by magnetic resonance spectroscopy (MRS) compared with once weekly EXE matched placebo (PLAC) and once daily DAPA in patients with T2DM and metformin monotherapy after 24 weeks of treatment.

Methods

Study design and participants

EXENDA included female and male patients with T2DM (n=30) according to the following criteria: HbA1c (glycated hemoglobin) >=6.5% and <=11% (48-97mmol/mol) age 18-75 years, body mass index (BMI)>=25kg/m², metformin>=1000mg daily with at least 8 weeks stable dose. Exclusion criteria are shown in the supplementary material section (supplementary material S1). Patients were recruited from our diabetes outpatient clinic and affiliated hospitals. The study was performed at a single centre at the Medical University of Vienna and approved by the local research ethics committee (EKNR 1306/2016). EXENDA was registered in approved clinical trial registries (EudraCT 2016-000574-38). The study was conducted in accordance with the ethical and good clinical practice (GCP) standards of the

responsible ethics committee. Informed consent was obtained from all patients and recruitment was conducted between June 2017 to May 2019. In total 7 study visits were conducted during the study, which was a screening visit (week -4 to 0) followed by the randomization visit at baseline (week 0), study visits at 4,8,16,24 weeks and a follow-up visit at week 28.

Sample size (n=16 per arm) was determined to detect a 4% change in hepatocellular lipids between EXE+DAPA and PLAC+DAPA, with 80% power at a significance level of 5%, based on results of a previously published RCT examining the effects of exenatide on excess hepatic fat reduction after 26 weeks of treatment compared with a reference group (13).

Study objectives

The primary objective was to investigate the effects of a combination therapy with EXE+DAPA compared with PLAC+DAPA given for 24 weeks on the reduction of HCL in patients with T2DM and metformin therapy only. Secondary and exploratory objectives were the investigation on changes in subcutaneous and visceral fat, glycemic control (change in HbA1c, % of patients reaching target (HbA1c ≤6.5 %), fasting plasma glucose and fasting plasma insulin), weight loss, waist and hip circumference, liver function parameters (ALT, AST, GGT), lipid parameters, treatment safety and tolerability.

Randomization and blinding

Patients were randomized in a 1:1 ratio and stratified for BMI (<30/>30kg/m²) to either combined EXE+DAPA or PLAC+DAPA with a GCP compliant computer randomization program (www.randomizer.at) applying a block randomization in permuted blocks of six. The unique coding key was only known by the programmer, who was not working with the study staff or patients enrolled in this study. The study staff received electronic information of the actual randomization of the patient via email after logging into the electronic randomization

tool with their own access users. They received one unique number assigning the patient to either treatment group in a blinded fashion.

Interventions

DAPA was distributed in the officially approved original boxes available in Austria. Both groups received DAPA in a similar route, dosage and frequency and thus it was not randomized. The investigational drugs were provided by AstraZeneca. EXE and PLAC looked identical, and did not differ in appearance, colour, smell or handling. Participants were randomized to either receive EXE+DAPA (n=16) or PLAC+DAPA (n=14) for a total duration of 24 weeks. The initial and maintenance doses of EXE and PLAC were 2mg. EXE/PLAC was administered subcutaneously once weekly by the patients. The study participants received guided application training of EXE/PLAC with material provided by AstraZeneca. The initial and maintenance doses of DAPA were 10mg. DAPA was administered orally once daily. Treatment compliance was documented with drug accountability and by asking patients directly as well as measurement of urinary glucose to document regular SGLT2-I intake. Participants were asked to bring empty blisters and used vials to the study visits to check compliance of medication intake.

Assessments

Blood sampling, laboratory methods and calculations of insulin resistance and NAFLD/ fibrosis scores are described in detail in the supplementary material section (supplementary material S2).

Assessment of lipid distribution by magnetic resonance spectroscopy (MRS)

All measurements were performed on 3-Tesla whole body scanner (Magnetom PrismaFit, Siemens Healthcare, Erlangen, Germany). Participants were placed supine with head first and combination of spine array coil and flex coil placed over the upper abdomen. MR

measurements were performed at baseline and after 24 weeks. HCL was measured by localized short echo time 1H single voxel spectroscopy sequence similarly to previous studies without water signal suppression during single breath hold by placing the volume of interest within the right lateral liver lobe (14, 15). Signals of lipids from both (CH2)n (1.3 ppm) and CH3 (0.9 ppm) group resonances and water (4.7ppm) were corrected for T1 and T2 relaxation with relaxation times measured at 3T. HCL values were calculated from the processed signals as the fat fraction percentage ratio between lipid and the sum of water and lipid integrals. A hepatic triglyceride cut off of >=5.56% was used to assess hepatic steatosis based on previous work (16).

Visceral and subcutaneous abdominal fat (VAT, SAT) were measured with an axial T_1-weighted turbo spin echo technique using the whole body coil of the MR system. Within one breath hold, 10 slices of 10 mm thickness and 10 mm gap were recorded covering the area from sacroiliacum to L1. SAT and VAT was quantified in 3 slices (L5/L4, L4/L3 and L3/L2) by semiautomatic delineation of the compartments in ImageJ software (17).

Statistical analyses

Continuous variables were summarized by means and standard deviations and categorical variables by counts and percentages. Assumption of Gaussian distribution of parameters was decided using Kolmogorov-Smirnov test. Non-parametrically distributed parameters were log transformed. Baseline categorical and continuous variables parameters were analysed using Chi2 test or Student t-test as appropriate. Associations were tested using Pearson correlation. Differences between treatment groups after 24 weeks were tested with an analysis of covariance (ANCOVA) with treatment as fixed factor and adjustment for the baseline outcome as covariate. Adjustment for sex, age and BMI was conducted in a further statistical model. Within group effects from baseline to end of study were assessed with a paired t-test. The efficacy analysis was based on the intention to treat population. To test the robustness of

the results, per protocol sensitivity analyses was performed. Last observation carried forward was used to substitute missing results. Treatment efficacy and safety between treatment groups was assessed by logistic regression models. Statistical analysis was performed using SPSS 26.0 (SPSS Inc. Chicago, IL) and GraphPad Prism 7 (GraphPad Software, La Jolla, CA). A two-sided p-value <0.05 was considered statistically significant.

Results

Thirty participants were randomized to EXE+DAPA (n=16) or PLAC+DAPA (n=14). One participant of the PLAC+DAPA group was excluded due to withdrawal of the study by the participant after the baseline visit (figure 1). Baseline characteristics of both groups were comparable (table 1). The majority of the participants was of Caucasian ethnicity (97%) and33% of participants were female. All participant received metformin only before entering the study. The mean diabetes disease duration was 6.6 years.

Hepatocellular lipids, visceral and subcutaneous adipose lipids

After 24 weeks of treatment no significant differences were found in HCL between both treatment groups (table 2, figure 2). However, in both treatment groups HCL significantly decreased from baseline (table 3). Hepatic steatosis resolved (persons with less than 5.56% HCL) in 5 of 30 (16.7%) patients (EXE+DAPA: 3/16, 14.3%, PLAC+DAPA:2/14, 18.8%, p=0.74). VAT and SAT were reduced significantly in EXE+DAPA and PLAC+DAPA, but no differences in VAT and SAT were observed between groups (table 2+3, figure 2). Per protocol analysis showed comparable results (data not shown). Relative changes of HCL, VAT, SAT and metabolic parameters are presented by treatment group (supplementary material S3). Characteristics of subjects more responsive and less responsive to treatment (both treatment arms) discriminated by the 50th percentile of HCL reduction are shown in the supplementary material section (supplementary material S4). Baseline characteristics were

similar between groups. High responders had significant higher weight loss and higher reductions of waist circumference, VAT and SAT, whereas glycemic parameters were comparable.

Glycemic control and efficacy

Fasting glucose and HbA1c significantly decreased in EXE+DAPA and PLAC+DAPA and were significantly lower in EXE+DAPA compared with PLAC+DAPA at end of study (table 2+3, figure 2). HOMA-IR was significantly lower in PLAC+DAPA, however, no differences between treatment groups were found. At 24 weeks HbA1c<6.5% (48mmol/mol) was observed in 11/16 participants (68.8%) compared with 0/16 at baseline (p=0.001) in EXE+DAPA and 5/14 participants (35.7%) versus 2/14 (14.3%) (p=0.25) in PLAC+DAPA. Logistic regression analysis adjusted for baseline outcome levels identified significant differences in treatment efficacy (HbA1c<6.5%) and higher reductions in EXE+DAPA (RR 0.15, 95% CI 0.03;0.81, p=0.028).

Weight and anthropometrics

After 24 weeks of treatment BMI, weight, waist and hip circumference were significantly lower in EXE+DAPA and PLAC+DAPA with no significant difference found between the two treatment arms (table 2+3, figure 2).

Liver function parameters

At the end of study AST was significantly lower in both treatment groups, whereas ALT was significantly lower in EXE+DAPA and showed only a trend towards lower levels in PLAC+DAPA. GGT was significantly reduced in PLAC+DAPA only. No between group differences were found in all three parameters at the end of study (table 2+3, figure 2).

Lipids:

After 24 weeks of treatment LDL-, HDL-cholesterol and triglycerides did not differ between treatment groups. LDL- and HDL-cholesterol did not change significantly from baseline, whereas triglycerides significantly decreased in PLAC+DAPA and were close to significant in EXE+DAPA (table 2+3).

Calculated predictors of NAFLD and fibrosis

No differences between groups were found at the end of study in fatty liver index or FIB4 score (table 2+3). Fatty liver index was significantly lower in both treatment groups. FIB4 score was significantly lower in PLAC+DAPA.

Correlations

Correlation analysis demonstrated that the primary outcome parameter HCL was associated with changes of weight (r=0.54, p=0.002), hip circumference (r=0.40, p=0.03), waist circumference (r=0.40, p=0.03), VAT (r= 0.41, p=0.04) and SAT (r=0.62, p=0.001) but not with changes of glycemic parameters (HOMA IR, r=-0.10, fasting glucose, r=0.23, HbA1c, r=0.33, all non-significant).

Adverse events:

In total 24/30 participants reported adverse events (AEs) in our study with a total of 89 different AEs reported (supplementary material S5). No hypoglycemic event (glucose<70mg/dl, symptomatic or asymptomatic, lowest self-reported glucose value=77mg/dl) and no ketoacidosis occurred. Genital mycosis occurred in 4/16 participants in EXE+DAPA and 1/14 in PLAC+DAPA (25.0% vs. 7.1%, p=ns.). Three of these infections were in women. Urinary tract infections occurred in two participants, one in each treatment group. Gastrointestinal side effects were reported in 4/16 participants in EXE+DAPA and 1/14 in PLAC+DAPA (25.0 vs 7.7%, p=ns.). Local skin reactions at the injection site were

reported in 2/16 participants in EXE+DAPA and 1/14 in PLAC+DAPA (12.5% vs. 7.1%, p=ns).

Three serious adverse events occurred which resolved immediately after treatment and were not related to study medication (otitis media, hypertensive crisis, vertigo).

Conclusion

As one of the first studies assessing synergistic effects of a combined GLP1-RA and SGLT2-I therapy on HCL quantified by MRS, EXENDA revealed a significant reduction of HCL after 24 weeks of treatment in EXE+DAPA and PLAC+DAPA. However, we were not able to identify differences between these treatment groups, arguing against an additive effect of EXE combined with DAPA, despite better glycemic control in EXE+DAPA, at least during 6 months of treatment. Weight reduction and decreases of hip and waist measures as well as reduction of VAT and SAT were significant in both groups but did not differ between groups either. Furthermore, decreases in liver function parameters (AST) were observed in both groups, with again no differences found between groups after 24 weeks.

In alignment with our results several SGLT2-I and GLP1-RA have been associated with an improvement of NAFLD (18-27). Studies investigating the effects of dapagliflozin on NAFLD in patients with T2DM corroborate our findings and found significantly decreased hepatic fat assessed by MRS, markers of liver injury and VAT, decreased steatosis and fibrosis assessed by transient elastography, next to improved glycemic control after 8 to 24 weeks of treatment (22-24). Interestingly, a recently published real world observational study observed reductions in HCL only in patients receiving SGLT2-I but not in patients receiving GLP1-RA, which partly corroborates our findings in EXENDA of no additive effects found in combined EXE+DAPA and effects on HCL mainly driven by SGLT2-I treatment (28). However, other trials are not consistent with these findings by observing significant decreases of HCL, VAT and SAT measured by MRS in patients with T2DM receiving exenatide after 6

months of treatment (13, 29). Furthermore, lower liver function parameters, body weight, waist circumference and postprandial glucose were observed (29).

In accordance with our findings Gastaldelli et al (12) found in a secondary analysis of the Duration 8 trial a significant reduction of the fatty liver index – a parameter with high correlation with HCL - after 28 weeks of treatment in all of their three treatment arms, combined EXE+DAPA, EXE or DAPA monotherapy (12). Different to our findings the combined EXE+DAPA group showed strongest effects on markers of hepatic steatosis and fibrosis with superior improvements observed for biomarkers of fatty liver/steatosis compared with each monotherapy (12). However, the combined EXE+DAPA group in Duration 8 had significantly higher weight loss compared to the monotherapy groups after 28 weeks of treatment (12). This was corroborated in a recently published study testing a combination of liraglutide and canagliflozin compared with monotherapies of canagliflozin or liraglutide (9). In our EXENDA trial we were not able to detect significant differences in weight loss in EXE+DAPA compared with PLAC+DAPA, which might serve as an explanation for a similar decrease of HCL in both treatment arms. The weight reduction in PLAC+DAPA (-4.6kg) was relatively high compared with a systematic review assessing weight loss in patients with T2DM receiving dapagliflozin (-1.6kg), possibly leading to higher HCL reduction in the PLAC+DAPA group (30). Interestingly, an earlier study reported higher weight reduction in patients with longer diabetes duration (11y) compared with patients with short T2DM duration (1y) after treatment with dapagliflozin for 12 weeks (31). The reason for this difference was not clear, but changes in food intake or metabolic rate were discussed (31). In our study we did not find any associations between diabetes duration and weight or HCL reduction (data not shown).

We found significant associations of HCL with changes in weight, anthropometric parameters and especially visceral and abdominal fat in the total study population. In Duration 8

significant direct associations of weight loss on ALT:AST ratio as well as indirect treatment effects on ALT:AST ratio and Adipo-IR mediated by weight loss in EXE+DAPA compared with the other treatment groups were found (12). A recent systematic review applying different weight loss interventions (behavioural, pharmaceutical and surgical) reported evidence of the associations between different weight loss treatment options and improved NAFLD biomarkers (32). Significant associations were found between weight loss interventions and the amelioration of laboratory, radiologic and histologic markers of NAFLD. Thus weight loss interventions are essentially recommended in the treatment of NAFLD in guidelines (2).

Results of the Diabetes Remission Clinical Trial (DiRECT) support above data in a T2DM population and found significant reductions in HCL in their participants with T2DM, who had an average decrease of 15% of body weight with reversible beta-cell function found predominately in those patients with shorter T2DM duration (33). According to these results the remission of T2DM seems to be associated with a reduction of HCL. Ameliorations of fasting glucose levels and hepatic insulin resistance and hepatocellular insulin signalling were associated with decreases of HCL in prior studies (3, 34). Our findings of significantly improved glucose parameters along with significant reductions of HCL support these observations. However, no direct associations were found. We observed significantly lower HbA1c and fasting glucose in EXE+DAPA versus PLAC+DAPA, which is in accordance with findings of Duration 8 with superior effects on glucometabolic parameters in EXE+DAPA (8). However, opposing results were seen in a study combining liraglutide and canagliflozin with absence of the synergistic effect on gluco-metabolic parameters (9). Further research is necessary to investigate the mechanisms of additive effects of GLP1-RA and SGLT2-I on glucose metabolism.

A main limitation is that this study is a pilot trial with low participant numbers and thus outcomes have to be considered exploratory and with care. Thus, further trials will be needed to assess synergistic effects of GLP1-RAs and SGLT2-I on HCL. Furthermore, our method to assess HCL was MRS, which is not the gold standard for NAFLD assessment. Nonetheless, it is a highly reliable and accurate, non-invasive method with good correlation with histological methods, which can assess lipid distribution in liver and other organs in one session and relatively short duration (35). However, measurement facilities are limited and MRI costs are high. Another limitation was the fact that HCL was not defined as an inclusion criteria and thus patients with low hepatic lipid content participated in EXENDA.

In conclusion EXENDA - as one of the first studies - demonstrated improvements in HCL in a combined GLP1-RA and SGLT2-I intervention group and a SGLT2-I comparator group measured with MRS. While earlier studies show a potential beneficial additive effect on calculated steatosis/fibrosis scores in their combined treatment group, we were not able to confirm these findings, although significant better glycemic control was achieved. Weight reduction seems to have an essential role in the disease modifying effects on NAFLD. Further studies will have to assess potential additive effects of GLP1-RA and SGLT2-I on ectopic lipid accumulation and other potential comorbidities.

Competing interests:

The authors declare no competing interests.

Authors' contributions:

JH was the study coordinator and Co-PI, initiated and designed the study, did the statistical analyses, wrote the first draft of the paper, edited and finalized the manuscript. AKW was the principal investigator and sponsor of the study and designed and executed the EXENDA

study, provided input for the interpretation of the results, read and corrected draft versions. JH and AKW applied for and received funding by AZ. IJ, RT and MKS acquired and processed MRS data, read and corrected draft version and approved the final manuscript. ML, MB, HB, CS contributed to the conception of the trial, read and corrected draft versions of the manuscript and approved the final manuscript. JH and AKW are the guarantors of the study.

Funding: EXENDA was an investigator initiated study and was funded by AstraZeneca, project number ESR 15-10882

Data sharing statement:

The datasets analysed within this manuscript may be obtained in accordance with AstraZeneca's data sharing policy. For further information see https://astrazenecagrouptrials.pharmacm.com/ST/Submission/Disclosure

Acknowledgements:

The project was funded by AstraZeneca as an investigator initiated project. The funders had no role in any aspect of the study beyond funding and initial design. We thank the research team, and health professionals collaborating in the recruitment. Finally, we thank all participants for their good collaboration.

References:

1. Younossi ZM, Golabi P, de Avila L, et al. The global epidemiology of NAFLD and NASH in patients with type 2 diabetes: A systematic review and meta-analysis. J Hepatol. 2019;71(4):793-801.
2. 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 for the management of non-alcoholic fatty liver disease. Diabetologia. 2016;59(6):1121-40.
3. Dewidar B, Kahl S, Pafili K, Roden M. Metabolic liver disease in diabetes - From mechanisms to clinical trials. Metabolism. 2020:154299.

4. Holmer M, Melum E, Isoniemi H, et al. Nonalcoholic fatty liver disease is an increasing indication for liver transplantation in the Nordic countries. Liver Int. 2018;38(11):2082-90.
5. Davies MJ, D'Alessio DA, Fradkin J, et al. Management of Hyperglycemia in Type 2 Diabetes, 2018. A Consensus Report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). Diabetes Care. 2018;41(12):2669-701.
6. Clodi M, Abrahamian H, Brath H, et al. [Antihyperglycemic treatment guidelines for diabetes mellitus type 2 (Update 2019)]. Wien Klin Wochenschr. 2019;131(Suppl 1):27-38.
7. Grant PJ, Cosentino F. The 2019 ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD: New features and the 'Ten Commandments' of the 2019 Guidelines are discussed by Professor Peter J. Grant and Professor Francesco Cosentino, the Task Force chairmen. Eur Heart J. 2019;40(39):3215-7.
8. Frias JP, Guja C, Hardy E, et al. Exenatide once weekly plus dapagliflozin once daily versus exenatide or dapagliflozin alone in patients with type 2 diabetes inadequately controlled with metformin monotherapy (DURATION-8): a 28 week, multicentre, double-blind, phase 3, randomised controlled trial. Lancet Diabetes Endocrinol. 2016;4(12):1004-16.
9. Ali AM, Martinez R, Al-Jobori H, et al. Combination Therapy With Canagliflozin Plus Liraglutide Exerts Additive Effect on Weight Loss, but Not on HbA1c, in Patients With Type 2 Diabetes. Diabetes Care. 2020;43(6):1234-41.
10. Adams LA, Harmsen S, St Sauver JL, et al. Nonalcoholic fatty liver disease increases risk of death among patients with diabetes: a community-based cohort study. Am J Gastroenterol. 2010;105(7):1567-73.
11. Sathyanarayana P, Jogi M, Muthupillai R, et al. Effects of combined exenatide and pioglitazone therapy on hepatic fat content in type 2 diabetes. Obesity (Silver Spring). 2011;19(12):2310-5.
12. Gastaldelli A, Repetto E, Guja C, et al. Exenatide and dapagliflozin combination improves markers of liver steatosis and fibrosis in patients with type 2 diabetes. Diabetes Obes Metab. 2020;22(3):393-403.
13. Dutour A, Abdesselam I, Ancel P, et al. Exenatide decreases liver fat content and epicardial adipose tissue in patients with obesity and type 2 diabetes: a prospective randomized clinical trial using magnetic resonance imaging and spectroscopy. Diabetes Obes Metab. 2016;18(9):882-91.
14. Leutner M, Klimek P, Gobl C, et al. Glucagon-like peptide 1 (GLP-1) drives postprandial hyperinsulinemic hypoglycemia in pregnant women with a history of Roux-en-Y gastric bypass operation. Metabolism. 2019;91:10-7.
15. Winhofer Y, Wolf P, Krssak M, et al. No evidence of ectopic lipid accumulation in the pathophysiology of the acromegalic cardiomyopathy. J Clin Endocrinol Metab. 2014;99(11):4299-306.
16. Szczepaniak LS, Nurenberg P, Leonard D, et al. Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. Am J Physiol Endocrinol Metab. 2005;288(2):E462-8.
17. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. Nat Methods. 2012;9(7):671-5.
18. Gallo S, Calle RA, Terra SG, et al. Effects of Ertugliflozin on Liver Enzymes in Patients with Type 2 Diabetes: A Post-Hoc Pooled Analysis of Phase 3 Trials. Diabetes Ther. 2020;11(8):1849-60.
19. Kuchay MS, Krishan S, Mishra SK, et al. Effect of Empagliflozin on Liver Fat in Patients With Type 2 Diabetes and Nonalcoholic Fatty Liver Disease: A Randomized Controlled Trial (E-LIFT Trial). Diabetes Care. 2018;41(8):1801-8.
20. Kahl S, Gancheva S, Strassburger K, et al. Empagliflozin Effectively Lowers Liver Fat Content in Well-Controlled Type 2 Diabetes: A Randomized, Double-Blind, Phase 4, Placebo-Controlled Trial. Diabetes Care. 2020;43(2):298-305.
21. Akuta N, Watanabe C, Kawamura Y, et al. Effects of a sodium-glucose cotransporter 2 inhibitor in nonalcoholic fatty liver disease complicated by diabetes mellitus: Preliminary prospective study based on serial liver biopsies. Hepatol Commun. 2017;1(1):46-52.

22. Latva-Rasku A, Honka MJ, Kullberg J, et al. The SGLT2 Inhibitor Dapagliflozin Reduces Liver Fat but Does Not Affect Tissue Insulin Sensitivity: A Randomized, Double-Blind, Placebo-Controlled Study With 8-Week Treatment in Type 2 Diabetes Patients. Diabetes Care. 2019;42(5):931-7.
23. Shimizu M, Suzuki K, Kato K, et al. Evaluation of the effects of dapagliflozin, a sodium-glucose co-transporter-2 inhibitor, on hepatic steatosis and fibrosis using transient elastography in patients with type 2 diabetes and non-alcoholic fatty liver disease. Diabetes Obes Metab. 2019;21(2):285-92.
24. Eriksson JW, Lundkvist P, Jansson PA, et al. Effects of dapagliflozin and n-3 carboxylic acids on non-alcoholic fatty liver disease in people with type 2 diabetes: a double-blind randomised placebo-controlled study. Diabetologia. 2018;61(9):1923-34.
25. Armstrong MJ, Gaunt P, Aithal GP, et al. Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): a multicentre, double-blind, randomised, placebo-controlled phase 2 study. Lancet. 2016;387(10019):679-90.
26. Newsome P, Francque S, Harrison S, et al. Effect of semaglutide on liver enzymes and markers of inflammation in subjects with type 2 diabetes and/or obesity. Aliment Pharmacol Ther. 2019;50(2):193-203.
27. Seko Y, Sumida Y, Tanaka S, et al. Effect of 12-week dulaglutide therapy in Japanese patients with biopsy-proven non-alcoholic fatty liver disease and type 2 diabetes mellitus. Hepatol Res. 2017;47(11):1206-11.
28. Mittag-Roussou V, Wagenpfeil S, Lammert F, Stokes CS. Noninvasive monitoring of liver fat during treatment with GLP-1 analogues and SGLT-2 inhibitors in a real-world setting. Endocrinol Diabetes Metab. 2020;3(3):e00131.
29. Liu L, Yan H, Xia M, et al. Efficacy of exenatide and insulin glargine on nonalcoholic fatty liver disease in patients with type 2 diabetes. Diabetes Metab Res Rev. 2020;36(5):e3292.
30. Zhang M, Zhang L, Wu B, et al. Dapagliflozin treatment for type 2 diabetes: a systematic review and meta-analysis of randomized controlled trials. Diabetes Metab Res Rev. 2014;30(3):204-21.
31. Zhang L, Feng Y, List J, Kasichayanula S, Pfister M. Dapagliflozin treatment in patients with different stages of type 2 diabetes mellitus: effects on glycaemic control and body weight. Diabetes Obes Metab. 2010;12(6):510-6.
32. Koutoukidis DA, Astbury NM, Tudor KE, et al. Association of Weight Loss Interventions With Changes in Biomarkers of Nonalcoholic Fatty Liver Disease: A Systematic Review and Meta-analysis. JAMA Intern Med. 2019.
33. Taylor R, Al-Mrabeh A, Zhyzhneuskaya S, et al. Remission of Human Type 2 Diabetes Requires Decrease in Liver and Pancreas Fat Content but Is Dependent upon Capacity for beta Cell Recovery. Cell Metab. 2018;28(4):667.
34. Petersen KF, Dufour S, Befroy D, et al. Reversal of nonalcoholic hepatic steatosis, hepatic insulin resistance, and hyperglycemia by moderate weight reduction in patients with type 2 diabetes. Diabetes. 2005;54(3):603-8.
35. Idilman IS, Aniktar H, Idilman R, et al. Hepatic steatosis: quantification by proton density fat fraction with MR imaging versus liver biopsy. Radiology. 2013;267(3):767-75.

Table 1: Baseline characteristic of the study population

	EXE+DAPA			PLAC+EXE			all			
	n	**mean**	**SD**	**N**	**mean**	**SD**	**n**	**mean**	**SD**	**p**
Lipid distribution										
HCL %	16	12.85	9.26	14	13.17	8.91	30	13.00	8.94	0.92
VAT mm²*	14	8.33	3.12	12	8.49	3.28	26	8.41	3.13	0.90
SAT mm²*	14	18.36	5.20	12	16.55	4.95	26	17.52	5.07	0.38
Glycemic parameters										
fasting glucose mg/dl *	16	169.3	71.4	14	150.1	31.2	30	160.4	56.3	0.36
fasting insulin µU/mL *	16	14.8	11.3	14	14.7	9.3	30	14.7	10.2	0.98
HbA1c %	16	7.8	1.3	14	7.3	0.6	30	7.5	1.0	0.17
HbA1c mmol/mol	16	62.2	15.0	14	55.9	6.8	30	59.2	12.2	0.16
HOMA IR *	16	6.4	5.9	14	5.6	4.0	30	6.0	5.1	0.67
Diabetes duration y	16	7.3	5.2	14	5.8	4.7	30	6.6	5.0	0.43
Anthropometrics										
Age y	16	59.4	8.5	14	60.9	7.4	30	60.1	7.9	0.63
Height cm	16	175.6	0.1	14	174.3	0,1	30	175	0.1	0.72
Weight kg	16	99.1	20.6	14	93.5	14.2	30	96.5	17.9	0.40
BMI kg/m²	16	31.9	4.6	14	30.7	3.5	30	31.3	4.1	0.45
Neck cf. cm	16	41.9	4.4	14	41.6	3.4	30	41.7	3.9	0.83
Waist cf. cm	16	113.0	12.3	14	111.3	8.9	30	112.2	10.7	0.67
Hip cf. cm *	16	113.1	11.0	14	111.0	8.3	30	112.1	9.8	0.56
Blood pressure										
sys. BP mmHg	16	137	15	14	131	18	30	134	16	0.34
dia.BP mmHg	16	85	9	14	79	9	30	82	9	0.08
HR bpm	16	73	13	14	75	15	30	74	14	0.76
Liver function parameters										
AST U/L	16	30.2	13.0	14	34.4	17.1	30	32.1	14.9	0.46
ALT U/L	16	40.0	20.9	14	47.2	28.7	30	43.4	24.7	0.43
AST:ALT ratio	16	0,91	0.59	14	0.81	0.25	30	0.87	0.46	0.56
GGT U/L	16	45.4	34.9	14	45.0	34.3	30	45.2	34.0	0.98
Lipids										
LDL-C mg/dl	16	86.1	36.0	14	92.7	36.8	30	89.2	35.9	0.29
HDL-C mg/dl	16	47.8	11.3	14	47.0	9.9	30	47.4	10.5	0.62
Triglyceride mg/dl	16	133.3	56.9	14	160.9	83.4	30	146.1	70.7	0.88
NAFLD/ Fibrosis scores										
FLI	16	78.3	19.4	14	77.9	17.4	30	78.1	18.2	0.96
FIB-4	16	1.46	0.81	14	1.40	0.64	30	1.42	0.72	0.81
	N	**%**		**N**	**%**		**N**	**%**		
Female sex	6/16	37.5		4/14	28.6		10/30	33.3		0.71
Caucasian ethnicity	15/16	93.8		14/14	100		29/30	96.7		1.0
Alcohol	10/16	62.5		9/14	64.3		19/30	63.3		0.92
Smoking	4/16	25.0		1/14	7.1		5/30	16.7		0.19
Steatosis hepatis	11/16	68.8		11/14	78.6		22/30	73.3		0.54

EXE= exenatide, PLAC= placebo, DAPA= dapagliflozin, n= number, SD= standard deviation, BMI= body mass index, Cf.= circumference, BP= blood pressure, sys.= systolic, dia.= diastolic, HOMA IR= Homeostasis Model Assessment for Insulin Resistance, HCL= hepatocellular lipids, VAT= visceral adipose tissue, SAT= subcutaneous adipose tissue, AST= aspartate aminotransferase, ALT= alanine aminotransferase, GGT= gamma-glutamyl transpeptidase, LDL-C= low-density lipoprotein cholesterol, HDL-C= high-density lipoprotein cholesterol, FLI= Fatty Liver Index, FIB-4= fibrosis score

Table 2: Comparison EXE&DAPA vs PLAC&DAPA after 24 weeks of treatment adjusted for the baseline outcome variables and adjusted for sex, age, BMI, at baseline and the baseline outcome variable

		Adjustment for baseline outcome variable			Multiple adjustment		
	N	**Mean difference**	**95% CI**	**p**	**Mean difference**	**95% CI**	**p**
Lipid distribution							
HCL %	30	-0.65	-4.40 to 3,10	0.72	- 0.77	-4.84 to 3.30	0.70
VAT mm²	26	-0.10	-1.02 to 0.82	0.82	0.06	-1,01 to 1,13	0.91
SAT mm²	26	-0.66	-2.53 to 1.21	0.47	-0.43	-2.41 to 1.55	0.66
Glycemic parameters							
HbA1c %	30	-0.55	-0.88 to -0.20	<0.01	-0.52	-0.86 to -0.17	0.005
Fasting glucose mg/dl	30	-12.85	-25.48 to -1.22	0.03	-11.55	-23.17 to 0.06	0.051
Fasting insulin U/L	30	4.23	-4.19 to 12.65	0.31	3.69	-5.28 to 12.65	0.40
HOMA IR	30	0.89	-1.41 to 3.18	0.44	0.44	-2.10 to 2.98	0.73
Anthropometrics							
Weight kg	30	-2.32	-5.84 to 1.21	0.19	-2.45	-5.45 to 0.54	0.10
BMI kg/m²	30	-0.70	-1.78 to 0.38	0.19	-0.62	-1.70 to 0.47	0.25
Waist cf.cm	30	-0.07	-2.89 to 2.76	0.96	0.02	-3.00 to 3.04	0.99
Hip cf. cm	30	0.07	-3.51 to 3.66	0.97	0.36	-3.45 to 4.16	0.85
Liver function parameters							
AST U/L	30	-0.20	-5.18 to 4.79	0.94	0.28	-5.06 to 5.62	0.92
ALT U/L	30	-1.72	-11.76 to 8.33	0.73	-0.85	-11.65 to 9.94	0.87
AST:ALT ratio	30	0.05	-010 to 0.20	0.50	0.05	-0.09 to 0.20	0.48
GGT U/L	30	2.17	-8.68 to 13.03	0.69	3.18	-8.10 to 14.47	0.57
Lipids							
LDL-C mg/dl	30	-13.08	-5.91 to 32.06	0.17	-12.25	-31.93 to 7.44	0.21
HDL-C mg/dl	30	-1.85	-5.94 to 2.24	0.36	-1.56	-5.72 to 2.61	0.45
Triglyceride mg/dl	30	6.92	-17.74 to 31.59	0.570	7.96	-17.93 to 33.86	0.53
NAFLD/fibrosis scores							
FLI	30	0.87	-8.66 to 10.39	0.85	1.87	-8.06 to 11.81	0.70
FIB4	30	0.11	-0.09 to 0.32	0.27	0.12	-0.09 to 0.34	0.25

EXE= exenatide, PLAC= placebo, DAPA= dapagliflozin, n= number, SD= standard deviation, BMI= body mass index, Cf.= circumference, BP= blood pressure, sys.= systolic, dia.= diastolic, HOMA IR= Homeostasis Model Assessment for Insulin Resistance, HCL= hepatocellular lipids, VAT= visceral adipose tissue, SAT= subcutaneous adipose tissue, AST= aspartate aminotransferase, ALT= alanine

aminotransferase, GGT= gamma-glutamyl transpeptidase, LDL-C= low-density lipoprotein cholesterol, HDL-C= high-density lipoprotein cholesterol, FLI=fatty liver index, FIB4= fibrosis 4 score

Table 3: Within group changes after 24 weeks of treatment in the total group and in the combined EXE&DAPA and PLAC&DAPA

	Total changes – before after analysis				**Before- after EXE&DAPA**				**Before- after PLAC&DAPA**		
	Mean reduction (BL to EOS)	**95% CI**	**P for change from baseline to week 24**	**N**	**Mean reduction (BL to EOS)**	**95% CI**	**p**	**N**	**Mean reduction (BL to EOS)**	**95% CI**	**p**
Lipid distribution											
HCL absolute %	-4.14	-6.30 to -1.99	<0.001	16	-4.41	-8.16 to -0.67	0.024	14	-3.87	-6.04 to -1.69	0.002
VAT mm²	-1.04	-1.49 to -0.59	<0.001	14	-1.09	-1.77 to -0.41	0.004	12	-0.99	-1.63 to -0.34	0.006
SAT mm²	-2.54	-3.48 to -1.59	<0.001	14	-3.00	-4.38 to -1.62	<0.001	12	-2.08	-3.56 to -0.65	0.009
Glycemic parameters											
HbA1c %	-1.13	-1.49 to -0.77	<0.001	16	-1.63	-2.27 to -0.98	<0.001	14	-0.63	-0.96 to -0.30	0.001
Fasting glucose mg/dl	-50.62	-71.83 to -29.41	<0.001	16	-66.31	-102.80 to -29.82	0.002	14	-34.93	-57.17 to -12.69	0.005
Fasting insulin U/L	-2.19	-9.71 to 5.33	0.56	16	1.27	-6.71 to 9.24	0.74	14	-2.97	-6.48 to 0.63	0.09
HOMA IR	-2.13	-3.70 to -0.56	<0.01	16	-2.17	-4.68 to -0.35	0.09	14	-2.10	-4.11 tp -0.09	0.04
Anthropometrics											
Weight kg	-5.96	-7.75 to -4.17	<0.001	16	-7.34	-9.87 to -4.82	<0.001	14	-4.57	-7.36 to -1.79	0.004
BMI kg/m²	-1.97	-2.57 to -1.38	<0.001	16	-2.43	-3.26 to -1.60	<0.001	14	-1.52	-2.45 to -0.59	0.004
Waist cf. cm	-3.56	-5.56 to -2.36	<0.001	16	-4.23	-6.82 to -1.63	0.003	14	-3.69	-5.67 to -1.71	0.001
Hip cf. cm	-4.91	-6.77 to -3.05	<0.001	16	-5.00	-7.59 to – 2.41	0.001	14	-4.81	-7.74 to -1.89	0.004
Liver function parameters											
AST U/L	-7.97	-12.01 to -3.92	<0.001	16	-6.88	-13.39 to -0.36	0.04	14	-9.07	-14.17 to -3.97	0.002
ALT U/L	-10.85	-17.20 to -4.49	0.002	16	-10.13	-16.94 to -3.31	0.006	14	-11.57	-23.55 to 0.41	0.057
AST:ALT ratio	0.11	-0.20 to 0.42	0.46	16	0.02	-0.15 to 0.10	0.68	14	0.01	-0.13 to 0.13	0.99

GGT U/L	-13.23	-22.17 to -4.30	0.005	16	-12.25	-26.50 to 1.99	0.087	14	-14.21	-25.74 to -2.69	0.019
Lipids											
LDL-C mg/dl	-7.63	-19.62 to 4.56	0.20	16	-11.98	-31.41 to 7.56	0.21	14	-2.67	-18.12 to 12.77	0.72
HDL-C mg/dl	2.30	0.19 to 4.41	0.03	16	1.38	-1.12 to 3.87	0.26	14	3.36	-0.56 to 7.18	0.08
Triglyceride mg/dl	-31.83	-48.94 to -14.73	0.001	16	-22.63	-47.19 to 1.94	0.07	14	-42.36	-68.20 to -16.51	0.004
NAFLD/fibrosis scores											
FLI	-12.81	-17.54 to -8.07	<0.001	16	-12.37	-19.23 to -5.52	0.002	14	-13.30	-20.77 to -5.83	0.002
FIB4	-0.22	-0.39 to -0.05	0.014	16	-0.18	-0.45 to 0.09	0.18	14	-0.26	-0.49 to -0.03	0.028

EXE= exenatide, PLAC= placebo, DAPA= dapagliflozin, n= number, SD= standard deviation, BMI= body mass index, Cf.= circumference, BP= blood pressure, sys.= systolic, dia.= diastolic, HOMA IR= Homeostasis Model Assessment for Insulin Resistance, HCL= hepatocellular lipids, VAT= visceral adipose tissue, SAT= subcutaneous adipose tissue, AST= aspartate aminotransferase, ALT= alanine aminotransferase, GGT= gamma-glutamyl transpeptidase, LDL-C= low-density lipoprotein cholesterol, HDL-C= high-density lipoprotein cholesterol, FLI= fatty liver index, FIB4= fibrosis 4 score.

Figure 1: Enrolment and group allocation chart

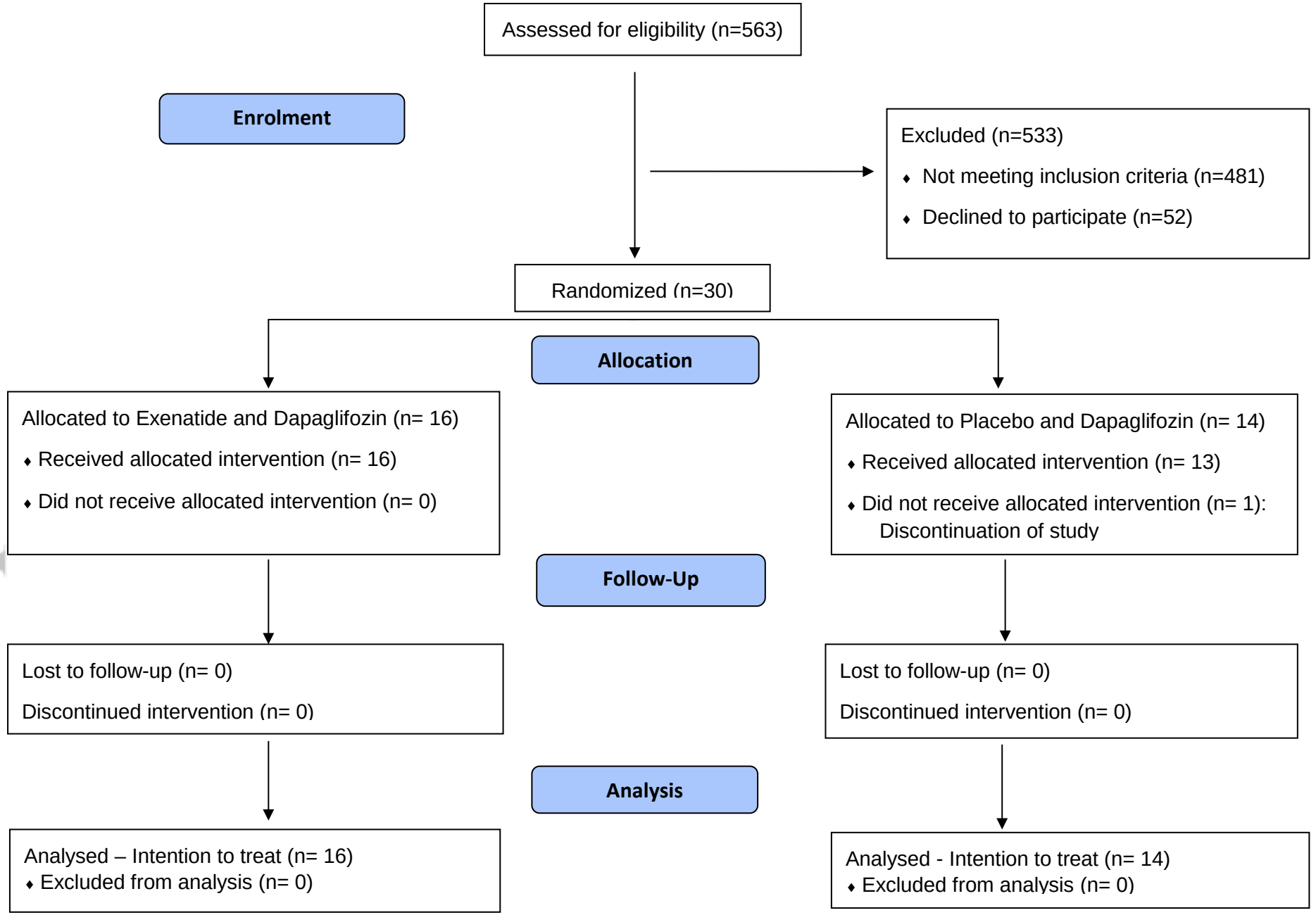
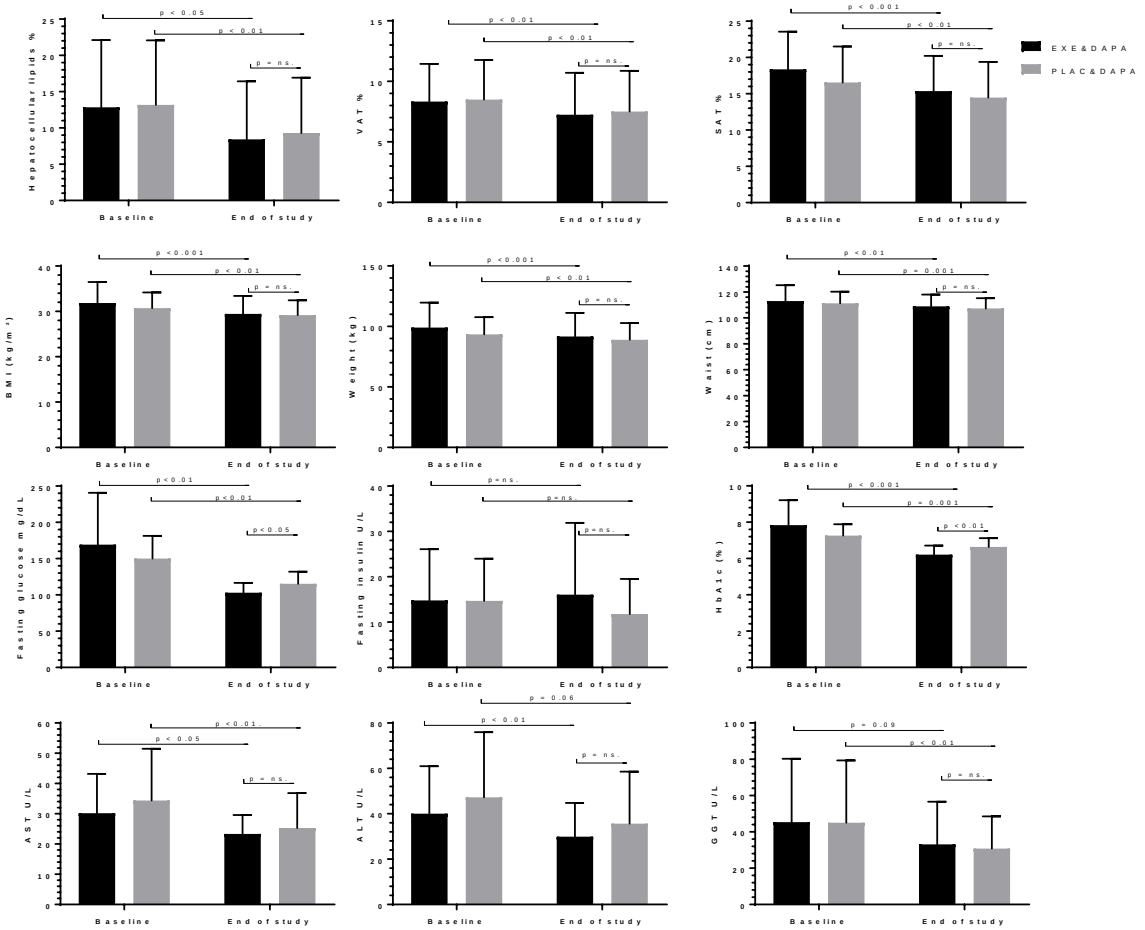


Figure 2: Ectopic lipids, visceral and subcutaneous fat, glycaemic, anthropometric parameters and liver function parameters at baseline and 24 weeks after treatment for EXE&DAPA and PLAC&DAPA.



EXE= exenatide, PLAC= placebo, DAPA= dapagliflozin, BMI= body mass index, VAT= visceral adipose tissue, SAT= subcutaneous adipose tissue, AST= aspartate aminotransferase, ALT= alanine aminotransferase, GGT= gamma-glutamyl transpeptidase, ns= not significant.