Digitally-Supported Continuous Care Intervention Including Individualized Nutritional Ketosis Significantly Improves Surrogate Markers of Non-Alcoholic Fatty Liver Disease (NAFLD) and Liver Fibrosis in Patients with Type 2 Diabetes: An Open Label, Non-Randomized, Controlled Study

Eduardo Vilar-Gomez[1], Shaminie J. Athinarayanan[2], Sarah J. Hallberg[2,3], Nasir H. Bhanpuri[2], Amy L. McKenzie[2], Wayne W. Campbell[4], James P. McCarter[2,5], Stephen D. Phinney[2], Jeff S. Volek[2,6], Naga Chalasani[1].

[1] Division of Gastroenterology and Hepatology, Indiana University School of Medicine, Indianapolis, IN, USA

[2] Virta Health, 535 Mission Street, San Francisco, CA, USA

[3] Indiana University Health Arnett, Lafayette, IN

[4] Department of Nutrition Science, Purdue University, West Lafayette, IN, USA

[5] Department of Genetics, Washington University School of Medicine, St. Louis, MO, USA

[6] Department of Human Sciences, The Ohio State University, Columbus, OH, USA

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Correspondence:

Naga P Chalasani, MD

Division of Gastroenterology and Hepatology

Indiana University School Medicine

[redacted]

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Author contributions

E.V.G, S.J.A, J.P.M and N.P.C wrote the manuscript. A.L.M, N.H.B, S.J.H and S.J.A participated in data acquisition. E.V.G and S.J.A analyzed the data. N.P.C, S.J.H, N.H.B, A.L.M, W.W.C, J.P.M, S.D.P and J.S.V supervised this particular analysis and edited the manuscript. All authors approved the final version of the manuscript.

Abbreviations:

CCI, continuous care intervention; **UC**, usual care; **NAFLD**, non-alcoholic fatty liver disease;; **N-LFS**, non-alcoholic liver fatty score; **NFS**, non-alcoholic fatty liver disease fibrosis score; **CLD**, chronic liver disease; **HCC**, hepatocellular carcinoma; **T2D**, type 2 diabetes; **NASH**, non-alcoholic steatohepatitis; **BMI**, body mass index; **LCHF**, low-carb high-fat; **ALT**, alanine aminotransferase; **AST**, aspartate aminotransferase; **IHLC**, intrahepatic lipid content; **ADA**, American Diabetes Association; **BHB**, beta-hydroxybutyrate; **CLIA**, clinical laboratory improvement amendments; **ITT**, intention-to-treat; **MIM**, multiple imputation methods; **WL**, weight loss; **EOT**, end-of-treatment; **HDL**, high density lipoprotein; **RCT**, randomized controlled trial; **LCD**, low-carb diet; **KD**, ketogenic diet; **HFD**, high-fat diet.

ABSTRACT

Aims: One-year of comprehensive continuous care intervention (CCI) through nutritional ketosis significantly improves HbA1c, body weight and liver enzymes among type 2 diabetes (T2D) patients. Here, we report the effect of CCI on surrogate scores of non-alcoholic fatty liver disease (NAFLD) and liver fibrosis.

Materials and methods: This was a non-randomized longitudinal study, including adults with T2D who were self-enrolled to CCI (n=262) or to receive usual care (UC, n=87) during 1 year. The NAFLD liver fat score [N-LFS] > -0.640 defined the presence of fatty liver. The NAFLD fibrosis score [NFS] of > 0.675 identified subjects with advanced fibrosis. Changes in N-LFS and NFS at one year were the main endpoints.

Results: At baseline, NAFLD was present in 95% of patients in the CCI and 90% of patients in the UC. At one year, weight loss of ≥ 5% was achieved in 79% of patients with CCI vs. 16% of patients with UC (P<.01). N-LFS mean score levels were significantly reduced in the CCI (-1.85 ± 0.33) compared with UC (+0.19 ± 0.64) (P<.01). NFS was significantly reduced in the CCI group (-.370 ± 0.10, P<0.01) whereas it increased in the UC group (.256 ± 0.20, P=0.21) (P<.01 between two groups). In the CCI group, the presence of advanced fibrosis was reduced from 27% at baseline to 17% at 1 year (P<.01).

Conclusions: One-year of a digitally supported CCI significantly improved surrogates of NAFLD and advanced fibrosis in type 2 diabetes patients. (**Word count**: 245)

Key words: Type 2 diabetes, very-low carb diet, ketogenic diet, non-alcoholic fatty liver disease, liver fibrosis, weight loss.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is an important cause of chronic liver disease (CLD), hepatocellular carcinoma (HCC) and liver transplant worldwide, and is associated with increased risk of heart disease, diabetes, chronic kidney disease and malignancies [1-4]. NAFLD is highly prevalent (~70%) among patients with obesity and type 2 diabetes (T2D) [5]. T2D is usually associated with the more aggressive form of NAFLD, including non-alcoholic steatohepatitis (NASH, indicating significant hepatocellular injury) and advanced fibrosis [6] and is linked with high risk for all-cause and liver-related mortality [7-9]. Currently, there are no approved pharmacological interventions for NASH. Weight loss via lifestyle changes including dietary modification and exercise is the first-line intervention used in treating and improving NAFLD/NASH [10, 11]. However, the majority of patients do not achieve or sustain targeted weight loss goals [10, 12]. Previous studies show a close relationship between the degree of weight reduction and improvements in most of the NASH-related features, including steatosis, inflammation, fibrosis, insulin resistance and elevated liver enzymes, irrespective of the type of diet consumed [12-21]. However, there is an intense debate about what types of diet are most effective for treating NASH and, to date, the optimal degree of energy restriction and macronutrient composition of dietary interventions in subjects with NASH and T2D are not well defined [11].

Low-carbohydrate, high-fat (LCHF) and ketogenic diets have demonstrated a superior weight loss effect to low-fat, high-carbohydrate (LFHC) diets in adults with overweight and obesity [22-25] and short-term interventions with very low-carbohydrate diets are associated with improved insulin sensitivity and glycemic control [26, 27]. Lower consumption of carbohydrate,

LCHF and ketogenic diets improve appetite control, satiety and/or reduce daily food intake helping to limit dietary energy consumption while maintaining patient-perceived vigor [28]. In patients with NAFLD, the beneficial effects of LCHF diets on liver enzymes and intrahepatic lipid content (IHLC) were explored with contradictory results. Some studies reported a significant reduction of aminotransferases [15, 29-31], while others did not report significant changes in these enzymes[16, 32, 33], even though the studies varied in their carbohydrate intake. A recent meta-analysis of pooled data from 10 clinical trials reported that low carbohydrate diet (LCD) in NAFLD patients led to a significant reduction in IHLC[34].

We recently demonstrated that 1 year of a telemedicine and lifestyle behavioral change based comprehensive continuous care intervention (CCI) was able to significantly reduce HbA1c and medication intake in patients with T2D [35]. One of the crucial benefits of the CCI relies on maintained restriction of carbohydrate intake, and monitoring compliance with the dietary regimen by assessing the patient's nutritional ketosis state by blood tests over the year. We also demonstrated that one year of the CCI was effective in improving liver enzymes, where mean alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) was significantly reduced by 29%, 20% and 13% , all $P<.01$, respectively. These findings not only highlights the beneficial effect of the CCI on diabetes management, but also in ameliorating the liver-related injury. In the current post-hoc analysis, we explore the effect of the CCI on changes of non-invasive liver markers of steatosis (NAFLD liver fat score) and fibrosis (NAFLD fibrosis score); and further assess the effectiveness of the CCI on liver- and non-liver related markers in the subgroup of patients with abnormal ALT at baseline (ALT levels of > 30 U/L in men and >19 U/L in women) [36].

METHODS

The design and primary results of this study were previously published, and the current data are based on 1-year post-hoc analysis using the data collected from the same cohort in that clinical study (*Clinical trials.gov identifier: NCT02519309*) [35]. A brief description of the study design, participants and interventions are listed in the **supplementary appendix (Methods section)**. Briefly, this was a non-randomized and open-label controlled longitudinal study, including patients between 21 to 65 years old with a diagnosis of T2D and a BMI > 25 kg/m^2. Further, patients were excluded if they had significant alcohol intake (average consumption of 3 or more alcohol-containing beverages daily or consumption of more than 14 standard drinks per week), presence of any other cause of liver disease or secondary causes of NAFLD and decompensated cirrhosis.

Patients on CCI had access to a remote care team consisting of a personal health coach and medical providers (physician or nurse practitioner). The participants in the CCI self-selected between two different educational modes; either via on-site education classes (n=136, CCI-onsite) or via web-based educational contents (n=126, CCI-virtual). The CCI patients were routinely assessed for nutritional ketosis based on blood beta-hydroxybutyrate (BHB) concentrations. The on-site and virtual patients were grouped together for analyses since no significant differences were observed in biochemical markers between these two modes of educational delivery [35]. We also recruited and followed a cohort of patients with T2D (n=87) who were categorized as UC [35]. This group of patients received a standard diabetes care treatment from their primary care physician or endocrinologist without modification. These patients were aware of the intervention cohort and could participate in that group if they chose.

Diagnosis of NAFLD and liver fibrosis by non-invasive biomarkers

NAFLD liver fat score (N-LFS) is a surrogate marker of fatty liver which includes the presence of the metabolic syndrome, type 2 diabetes, fasting serum insulin, AST and the AST/ALT ratio. N-LFS cutoff of > -0.640 predicts liver fat (> 5.56 % of hepatocytes) with a sensitivity of 86% and specificity of 71% [38, 39]. NAFLD fibrosis score (NFS) is a widely validated biomarker for identifying patients at different risks of fibrosis severity. NFS is derived from age, BMI, hyperglycemia, the AST/ALT ratio, platelet and albumin. The NFS threshold of < -1.455 can reliably exclude patients with advanced fibrosis (negative predictive value ≈ 92%) and > 0.675 can accurately detect subjects with advanced fibrosis (positive predictive value ≈ 85%)[40-42]. The equations for calculating both scores are displayed in the **supplementary appendix (Methods section)**.

Definition of outcomes

This study was designed to explore 2-years effectiveness of the CCI on glycemic status, medication use, body weight and metabolic- features in patients with type 2 diabetes. In the current report, we evaluated 1-year effectiveness of the CCI on mean changes from baseline to 1 year in: (1) non-invasive biomarkers of steatosis (NAFLD liver fat score), (2) non-invasive biomarkers of fibrosis (NAFLD fibrosis score) and (3) abnormal alanine aminotransferase (ALT).

Statistical analysis

Statistical analyses were performed using STATA software, release 14 (StataCorp, LP, College Station, TX). The primary study endpoints were changes from baseline in non-invasive liver biomarkers of steatosis and fibrosis for both CCI and UC. The secondary endpoint was to

evaluate the effect of the CCI on metabolic parameters and liver markers in a subset of patients with increased (> 30 U/L in men and >19 U/L in women) [36] ALT at baseline (195 of 347; 157 CCI and 38 UC). Data were expressed in percentage of patients for categorical variables and as means and SDs for continuous variables. Variables with skewed distributions were log-transformed prior to analysis. Within-group comparisons were performed with paired-sample Student *t* test or McNemar's test (categorical data) to detect differences from baseline for each group. Between-groups differences were compared using Wilcoxon signed-rank (non-normally distributed data without transformation), Student *t* test (normally distributed data) or χ^2 test (categorical data) when appropriate. One-way ANCOVA analyses while controlling by the baseline value of the dependent covariates such as age, gender, BMI at baseline, diabetes duration, and medications for type 2 diabetes and hyperlipidemia were applied for comparisons between different cutoffs of weight loss (%) and HbA1c. Sequential Bonferroni corrections ($P<0.01$) were used for multiple comparisons. Unadjusted Pearson´s or Spearman´s correlations, when appropriate, were performed to identify relationships between changes from baseline in ALT levels and changes in metabolic- and lipid-related parameters. Adjusted correlations were also performed while controlling by the baseline values of dependent covariates, duration of diabetes, and medications for type 2 diabetes and hyperlipidemia. Both intention-to-treat (ITT) and per-protocol (PP) approaches were conducted for endpoint analyses. Baseline and 1-year missing data were imputed by multiple imputation methods (MIM) in which missing data were replaced with a set of plausible values. ITT analyses were computed on the datasets with imputed missing values whereas PP analyses were performed on the datasets including patients who completed 1 year of intervention (completers). No missing data for covariates and endpoints

were recorded in the dataset with patients who completed 1 year of the trial. All confidence intervals, significance tests, and resulting *P* values were two-sided, with an alpha level of 0.05

RESULTS

Baseline features of participants

Recruitment and baseline data were published previously [35]. Briefly, between August 2015 and April 2016, 262 and 87 patients were enrolled in CCI and UC groups, respectively. **Supplemental Figure 1** shows the flow of patients through the study. Brief baseline characteristics and adverse events of the participants are listed in **supplementary appendix (Results section).**

Influence of intervention on 1-year study endpoints

Change in body weight.

At 1 year, mean BMI change from baseline was -4.9 ± 4.1 kg/m^2 in the CCI as compared to +0.9 ± 5.1 kg/m^2 in UC (P<.01) (**Table 1**). The CCI also showed mean BHB increase from 0.12 ± 0.16 to 0.26 ± 0.31 mmol/L (P<.01), without any significant change in the UC. The majority of patients in the CCI (207, 79%) lost more than 5% of body weight and 140 (53%) lost more than 10%. In contrast, the proportion of patients losing more than 5% (14, 16%) or 10% (4, 5%) of body weight was lower in UC (**Supplementary Figure 2**). In CCI, there was a trend toward greater mean percentage weight loss (WL) by higher baseline BMI classification (**Supplementary Table 1**).

Change in non-invasive markers of steatosis (N-LFS) and NAFLD fibrosis (NFS)

The CCI showed significant reductions in N-LFS (-1.85 ± 0.33) and NFS (-0.370 ± 0.10) as compared with baseline values, all P<0.01. Notably, the proportion of patients with suspected steatosis (N-LFS > -0.640) was reduced from 95% to 70% at 1 year in the CCI whereas no change from baseline was observed in UC (**Table 1**). At 1 year, the proportion of patients with advanced fibrosis (NFS > 0.675) was reduced from 27% to 17% in CCI, P<.01. Among UC, NFS increased from -0.485 to -0.229 at 1 year, P<.01; however, no major change in the number of patients with advanced fibrosis was observed. Among patients with abnormal ALT levels at baseline, improvements from baseline in N-LFS and NFS were also observed in CCI versus UC, P<.01 (**Table 2**). Similar to the full cohort, the proportion of patients with suspected steatosis (N-LFS>-0.640) was reduced from 97% to 71%, P<.01 and with advanced fibrosis (NFS>0.675) was reduced from 21% to 11%, P<.01 among CCI patients with abnormal ALT levels (**Table 2**).

Change in glucose and lipid-related markers in abnormal ALT patients

At 1 year, beneficial changes previously reported [35, 43] for all CCI patients were also seen among patients with abnormal ALT levels at baseline (**Table 2**). Significant and favorable 1-year changes in HbA1c (CCI: -1.14 ± 0.13% vs. UC: 0.18 ± 0.35%), triglycerides (CCI: -43.5 ± 14.2 vs. UC: 16.8 ± 31.1), HDL cholesterol (CCI: 6.7 ± 1.7 vs. UC: -3.8 ± 2.8) and insulin resistance (CCI: -5.4 ± 3.1 vs. UC: 2.3 ± 3.02) were seen in CCI vs. UC patients, all P<.01 (**Table 2**).

Associations between weight loss and changes in liver enzymes

In the full cohort, statistically significant correlations were found between change in ALT (r=0.23, P<.01), AST (r=0.21, P<.01) and ALP (r=0.30, P<.01) and change in body weight at 1 year of the CCI, even after controlling for BMI and individual values for each covariate at baseline. Stronger correlations with change in body weight were observed among CCI patients

with abnormal ALT levels at baseline (change in ALT, r=0.35, P<.01 and AST, r=0.31, P<.01). By multiple linear regression, change in body weight (%) at 1 year among CCI patients (*full cohort*) was positively and independently associated with decrease in ALT (β = 0.16, 95% CI: 0.13-0.34, P<.01), AST (β = 0.13, 95% CI: 0.08-0.22, P<.01) and ALP (β = 0.28, 95% CI: 0.18-0.38, P<.01) after adjustments for baseline BMI and other potential confounders. Among patients with increased ALT levels at baseline, 86 (55%) of 157 participants enrolled in the CCI vs. 9 (24%) of 38 patients in UC had ALT normalization at 1 year (**Table 2**).

Associations between weight loss degree and study outcomes

As shown in **Table 3**, there were relationships between the degree of 1-year of WL (%) and changes in liver, metabolic and non-invasive markers of steatosis and fibrosis among CCI participants. Liver enzymes improved in patients achieving WL ≥ 5%, however, maximum benefits were observed among subjects who lost ≥ 10%. At 1 year, patients who achieved WL ≥ 10% showed highest reductions of N-LFS (-2.64 ± 4.3) and NFS (-0.669 ± 0.78), whereas no statistically significant differences were found comparing patients with WL between 5%-10% versus <5%. Similarly, patients who achieved WL ≥ 10% also showed decreases of HbA1c (-1.51 ± 1.36), triglycerides (-65.9 ± 79.5) and ALP (-11.5 ± 14.6). The one-year probability of suspected fatty liver (N-LFS >-0.64) was lower (54%) among patients with WL ≥ 10% as compared with the remaining WL groups (<5% [91%] and 5%-10% [87%]). However, the proportion of patients with advanced fibrosis at 1 year was not different between patients achieving 5%-10% (15%) versus ≥ 10% (14%) of WL.

Correlations between changes from baseline in ALT and lipid- and glucose-related markers

Change in HbA1c from baseline to 1 year was linked to change in ALT levels in the CCI (r=0.26, P<.01), and this correlation was stronger among patients with abnormal levels of ALT at baseline (r=0.42, P<0.01) (**Table 4**). **Figures 1A and 1C** (full CCI cohort) and **Figures 1B and 1D** (CCI cohort with increased ALT levels at baseline) display 1-year associations between change in HbA1c and normalization of ALT levels. In the overall CCI, 130 (72%) of 180 patients with HbA1c reductions of ≥ 0.5% at 1 year had normal ALT levels (**Figure 1A**). Among CCI patients with increased ALT levels at baseline, 71 (65%) of 109 patients with a reduction of ≥ 0.5% in HbA1c showed normalization of ALT levels (**Figure 1B**). One-year reduction of ≥ 0.5% in HbA1c increased the odds of ALT normalization 3.2 fold (95% CI: 1.5-6.8) after controlling for baseline levels of HbA1c, BMI, ALT, diabetes duration, antidiabetic medication use and weight loss (%) at 1 year. Given that weight reductions (≥ 5%) can be associated with changes in HbA1c level, we sought to explore whether a reduction of ≥ 0.5% in HbA1c was still associated with ALT normalization, independent of weight loss (≥ 5%) (**Figures 1C-D**). Interestingly, a reduction of ≥ 0.5% in HbA1c was associated with higher rates of ALT normalization, regardless of whether or not 5% weight loss was achieved, P<.01. Changes in other lipid markers did not correlate with changes in ALT levels (**Table 4**).

DISCUSSION

The findings of the current analysis show that one year of a digitally-supported CCI reduced risk of fatty liver and advanced liver fibrosis in overweight and obese adults with T2D. Improvements were concurrent with improved glycemic status, reduction in cardiovascular risk factors and decreased use of medications for diabetes and hypertension [35,43]. The beneficial

effects extended to patients with increased levels of aminotransferase and those at high risk of advanced fibrosis at baseline, thus indicating that remote care medically-supervised ketosis is also effective in patients at risk of liver disease progression. In addition, our data support previous findings that a relationship exists between the degree of weight loss and improvement of liver enzymes, glucose control and insulin sensitivity [12, 21, 44, 45] with more drastic and positive changes in patients who achieve WL $\geq$ 10%. The influence of carbohydrate restriction and nutritional ketosis on liver histology of patients with biopsy-proven NASH remains largely unexplored in the context of a well-designed RCT. A pilot study including five patients with biopsy-proven NASH showed that 6-months of KD (less than 20 grams per day of carbohydrate) induced significant WL (mean of 13 kg) and four of five patients reduced liver fat, inflammation, and fibrosis. The current study provides evidence that a remote-care medically-supervised KD can improve NASH and even fibrosis [46]. A recent meta-analysis of ten studies reported the effects of LCD on liver function tests in NAFLD patients, and concluded that LCD significantly reduced IHLC, but did not improve liver enzymes [34], although a significant heterogeneity among NAFLD populations and interventions were observed across the included studies. Relative to prior outpatient interventions, the current study is unusual in the degree of health coach and physician support, the degree of prescribed carbohydrate restriction and the use of BHB as a blood biomarker of dietary adherence. These attributes may contribute to superior outcomes.

CCI participants also showed close correlations between the improvements of HbA1c, fasting glucose and insulin sensitivity with ALT changes, even after controlling for WL and changes in diabetes medications. Among subjects with abnormal levels at baseline, a reduction of $\geq$ 0.5% in HbA1c was associated with increased rates of ALT normalization. This finding

suggests that liver enzyme improvements may be related to improvements in glycaemic control and insulin levels in addition to weight loss. Importantly, few studies have directly compared the metabolic advantages of different diets for the treatment of NAFLD [14, 31, 47], and the impact of dietary macronutrient composition remains largely unknown. Three studies have shown that low-carb and low-fat diets reduced liver fat, transaminases and insulin resistance to a similar degree [14, 20, 47], whereas another study reported that a moderate hypocaloric low-carb diet in insulin resistant patients improved ALT levels more than a hypocaloric low-fat diet, despite equal weight loss [47]. Among T2D patients, a "low-carb" modified Mediterranean diet (35% carbohydrates, 45% high monounsaturated fat) showed greater ALT reductions than two other hypocaloric diets including the 2003 recommended ADA or low glycaemic index diets [48].

Our data also demonstrated that non-invasive risk scores for fatty liver and fibrosis were significantly improved in patients who underwent CCI as compared to the UC control, and greater reductions were observed in patients with the largest reductions in body weight (≥ 10%). Our results are consistent with previous studies reporting that LCD reduce intrahepatic lipid accumulation [14, 15, 20, 31, 46]. Likewise, 1-year liver fibrosis as assessed by NFS significantly improved in the CCI group, and the proportion of patients at risk of advanced fibrosis was reduced from 27% to 17% at 1 year of intervention. Similar to previous studies addressing the impact of WL on NASH-related fibrosis [12, 49], we showed a relationship between the degree of weight loss and improvements in NFS.

LCD or KD have been proposed as the most effective dietary intervention in reducing all features of the metabolic syndrome, which is present in approximately 80% of NAFLD patients [50, 51]; however, the physiological mechanisms are not fully established [52-54]. In line with our

findings, Holland et al.[55] showed that irrespective of physical exercise, rats fed a ketogenic formulation had lower liver triglycerides and lower activation of the pro-inflammatory NF-kB pathway compared to rats fed Western and standard chow diets. Likewise, a recent human study using a two-week isocaloric carbohydrate restricted diet, not only demonstrated a drastic reduction of hepatic steatosis, but a shift in lipid metabolism pathway from de-novo lipogenesis to ß-oxidation and increased BHB production[56]. This shift in the lipid homeostasis following a short-term ketogenic diet occurred in conjunction with a shift in gut microbia towards increased folate production as well as decreased expression of key serum inflammatory markers [56].

Strengths and weaknesses of this clinical trial have been previously described [35]. Some strengths of this study include a large cohort of T2D patients with high suspicion of NAFLD, an intervention with one-year of digitally-supported continuous care including monitored adherence to nutritional ketosis, and a control group of T2D patients provided usual care with standard nutritional recommendations [35].

Some weaknesses of this study include the absence of imaging- or biopsy-proven NAFLD or NASH diagnosis and lack of random allocation to assign patients to intervention and control groups. Food was not provided for participants so dietary macronutrient (e.g. carbohydrate) and micronutrient (e.g. omega 3) composition was not strictly controlled.

In conclusion, one year of a digitally-supported continuous care intervention including individualized nutritional ketosis led to significant improvement in non-invasive markers of liver fat and fibrosis together with sustained weight loss in overweight and obese type 2 diabetes patients. A relationship was observed between the degree of weight loss and improvements in liver- and non-liver-related outcomes with greater benefits in patients losing more than 10% of

body weight. A reduction of ≥ 0.5% in HbA1c was independently associated with ALT normalization even after controlling for weight loss. Medical interventions incorporating ketogenic diets appear effective for improving NAFLD, and therefore, may play an important role in reversing the natural history of NAFLD progression, although further studies are needed to confirm potential beneficial effect in patients with biopsy-confirmed NASH.

FIGURES LEGENDS

Figure 1. Association between reduction in HbA1c (%) and normalization of ALT* levels at 1 year of intervention in CCI group.

(A) Full CCI cohort (n=262)

Higher proportion of patients with ALT normalization were observed in HbA1c (%) reduction categories 0.5-1%; 74% and >1%; 72%.

(B) CCI patients with increased levels of ALT at baseline (n=157)

Higher proportion of patients with ALT normalization were observed in HbA1c (%) reduction categories 0.5-1%; 68% and >1%; 64%. Adjusted OR for change in HbA1c > 0.5% = 3.2 (95% CI: 1.5-6.8), P<0.01

(C) CCI patients with weight loss ≥ 5% (n=207).

Among patients with weight loss ≥ 5%, higher levels of ALT normalization (74%) were observed in patients with HbA1c (%) reduction of >0.5%.

(D) CCI patients with increased levels of ALT at baseline and weight loss ≥ 5% (n=130).

Among patients with weight loss ≥ 5% and abnormal ALT levels at baseline, higher levels of ALT normalization (69%) were observed in patients with HbA1c (%) reduction of >0.5%.

* ALT levels ≤ 19 in women and ≤ 30 in men.

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TABLES

Table 1. Patients' baseline and 1-year characteristics. Intention-to-treat analysis.

Variables	Continuous Care Intervention, n=262				Usual Care, n=87				
	Baseline	**1 year**	**Change**	**P value***	**Baseline**	**1 year**	**Change**	**P value***	**Between-groups P values***
Age, y	53.8 ± 8.4	-	-		52.3 ± 9.5	-	-		0.19
Female, n (%)	175 (67%)	-	-		51 (59%)	-	-		0.17
BMI (Kg/m^2)	40.4 ± 8.9	35.5 ± 8.1	-4.9 ± 4.1	<.01	36.8 ± 7.2	37.7 ± 8.4	0.9 ± 5.1	.13	<0.01
Liver-related parameters									
Abnormal ALT, n (%) †	157 (60%)	88 (34%)	-	<.01	38 (44%)	41 (47%)	-	.83	<0.01
Albumin (g/dl)	4.27 ± 0.30	4.24 ± 0.27	-.03 ± 0.22	.04	4.45 ± 0.37	4.44 ± 0.38	-.01 ± 0.25	.64	0.91
Platelet (x 10^9)	251 ± 60	233 ± 58	-18 ± 33	<.01	254 ± 79	244 ± 75	-10 ± 31	<.01	0.03
Non-invasive biomarkers									
NAFLD-LFS	2.99 ± 3.7	1.14 ± 3.9	-1.85 ± 0.33	<.01	2.91 ± 3.8	3.1 ± 4.6	0.19 ± 0.64	.76	<0.01
> -0.640	249 (95%)	184 (70%)	-	<.01	78 (90%)	76 (87%)	-	.61	<0.01
NAFLD fibrosis score	-.010 ± 1.29	-.380 ± 1.17	-.370 ± 0.10	<.01	-.485 ± 1.33	-.229 ± 1.39	.256 ± 0.20	.21	<0.01
Cut-offs				<.01				.79	<0.01
< -1.455	32 (12%)	51 (19%)	-		19 (22%)	16 (18%)	-		
-1.455 to 0.675	159 (61%)	166 (63%)	-		48 (55%)	48 (55%)	-		

> 0.675	71 (27%)	45 (17%)	-	20 (23%)	23 (26%)	-	

Abbreviations: BMI, body mass index; **ALT,** alanine aminotransferase; **NAFLD**, nonalcoholic fatty liver disease; **LFS**, liver fat score.

NAFLD-LFS cutoff > -0.640 for detecting liver fat > 5.56 % (sensitivity 86% and specificity 71%).

NAFLD fibrosis score < -1.455 corresponds with low probability of advanced fibrosis (NPV ≈ 92%) and > 0.675 indicates high probability of advanced fibrosis (PPV ≈ 85%).

* McNemar's or paired T test when appropriated. Chi-square or Wilcoxon signed-rank tests when appropriated.

† > 19 U/L for women and > 30 U/L for men.

Table 2. Patients' baseline and 1-year characteristics. Sub-analysis in patients (n=195) with abnormal ALT levels at baseline*.

Variables	Continuous Care Intervention, n=157				Usual care, n=38				Between-groups P values†
	Baseline	1 year	Change	P value†	Baseline	1 year	Change	P value†	
BMI (Kg/m2)	39.4 ± 7.2	34.3 ± 6.5	-5.1 ± 3.9	<.01	37.6 ± 6.9	38.2 ± 8.2	0.6 ± 1.7	.72	<0.01
Metabolic parameters									
HbA1c (%)	7.45 ± 1.31	6.31 ± 1.06	-1.14 ± 0.13	<.01	7.31 ± 1.48	7.49 ± 1.61	0.18 ± 0.35	.60	<0.01
Fasting glucose (mg/dl)	156.7 ± 53.8	126.1 ± 36.5	-30.6 ± 5.2	<.01	142.4 ± 9.78	158.6 ± 13.6	16.2 ± 16.4	.32	<0.01
Fasting insulin (m/Ul)	30.3 ± 25.2	19.1 ± 15.8	-11.2 ± 2.4	<.01	31.5 ± 23.8	33.9 ± 34.5	2.4 ± 6.8	.72	<0.01
HOMA-IR	12.2 ± 13.9	6.8 ± 8.5	-5.4 ± 3.1	<.01	11.6 ± 9.4	13.9 ± 14.3	2.3 ± 3.02	.45	<0.01
Triglycerides (mg/dl)	196.3 ± 120.2	152.8 ± 130.6	-43.5 ± 14.2	<.01	197.4 ± 100.5	214.2 ± 149.8	16.8 ± 31.1	.59	<0.01
Cholesterol (mg/dl)	176 ± 40.9	184.6 ± 44.7	8.6 ± 4.8	.07	184.5 ± 46.7	168.3 ± 51.3	-16.2 ± 12.1	.18	<0.01
HDL cholesterol (mg/dl)	43.2 ± 13.8	49.9 ± 16.5	6.7 ± 1.7	<.01	41 ± 11.3	37.2 ± 11.4	-3.8 ± 2.8	.19	<0.01
LDL cholesterol (mg/dl)	94.8 ± 34.1	105.2 ± 37.7	10.4 ± 4.1	<.01	96.1 ± 31.4	83.8 ± 29.7	-12.3 ± 7.9	.12	0.06
Liver-related tests									
ALT (U/L)	39.2 ± 25.4	25.8 ± 15.4	-13.4 ± 21.9	<.01	40.7 ± 23.4	38.6 ± 24.9	-2.1 ± 5.5	.70	<0.01
Normal ALT, n (%)	-	86 (55%)	-	<.01	-	9 (24%)	-	<.01	<0.01
AST (U/L)	28.5 ± 17.6	20.7 ± 8.5	-7.8 ± 12.2	<.01	32.7 ± 26.1	30.7 ± 20.5	-2 ± 5.4	.71	<0.01
ALP (U/L)	74.1 ± 24.1	66.1 ± 22.6	-8 ± 9.8	<.01	80.6 ± 28.2	82.6 ± 30.2	2 ± 7	.77	<0.01

Albumin (g/dl)	4.33 ± 0.27	4.29 ± 0.26	-.04 ± 0.03	.21	4.53 ± 0.31	4.50 ± 0.37	-.03 ± 0.02	.71	0.52
Platelet (x 10^9)	244 ± 58	227 ± 56	-17 ± 6	<.01	253 ± 92	248 ± 88	-5 ± 20	.78	<0.01
Non-invasive biomarkers									
NAFLD-LFS	3.61 ± 3.9	1.06 ± 2.7	-2.55 ± 0.38	<.01	3.84 ± 3.9	4.15 ± 5.2	0.31 ± 1.05	.77	<0.01
> -0.640	153 (97%)	112 (71%)	-	<.01	35 (92%)	37 (97%)	-	.49	<0.01
NAFLD fibrosis score	-.149 ± 1.18	-.556 ± 1.02	-.407 ± 0.12	<.01	-.591 ± 1.44	-.377 ± 1.56	.214 ± 0.34	.53	<0.01
Cut-offs				<.01				.51	<0.01
< -1.455	22 (14%)	34 (22%)	-		9 (24%)	8 (21%)	-		
-1.455 to 0.675	98 (62%)	106 (68%)	-		21 (55%)	21 (55%)	-		
> 0.675	8 (21%)	17 (11%)	-		8 (21%)	9 (24%)	-		
Kidney function tests									
Creatinine (mg/dl)	0.86 ± 0.20	0.82 ± 0.18	-.04 ± 0.02	<.01	0.83 ± 0.14	.84 ± 0.17	.01 ± 0.02	.57	<0.01
eGFR (CKD-EPI)	85.9 ± 17.2	89.2 ± 16.7	3.3 ± 11.5	<.01	86.5 ± 15.4	85.2 ± 18.1	-1.3 ± 10.4	.44	<0.01
Cut-offs				.67				.82	0.24
< 60 mL/min/1.73m2	12 (8%)	9 (6%)	-		2 (5%)	3 (8%)	-		
60-89 mL/min/1.73m2	74 (47%)	61 (39%)	-		20 (53%)	20 (53%)	-		
> 90 mL/min/1.73m2	71 (45%)	87 (55%)	-		16 (42%)	15 (39%)	-		
Other parameters									
CRP (mg/dl)	8.16 ± 17.1	5.9 ± 7.4	-2.25 ± 14.8	.05	10.15 ± 8.2	10.17 ± 8.7	0.02 ± 6.3	.98	<0.01
BHB (mmol/l)	0.17 ± 0.16	0.29 ± 0.31	0.12 ± 0.34	<.01	0.14 ± 0.10	0.12 ± 0.05	-0.02 ± 0.10	.41	<0.01

Abbreviations: ALT, alanine aminotransferase; **AST,** aspartate aminotransferase; **ALP**, alkaline phosphatase, **HbA1c,** Glycosylated hemoglobin; **HDL**, high-density lipoprotein; **LDL**, low-density lipoprotein; **eGFR**, estimated glomerular filtration rates; **CKD-EPI**, chronic kidney disease-epidemiological collaboration equation; **BHB**, beta-hydroxybutyrate; **NAFLD**, nonalcoholic fatty liver disease; **LFS**, liver fat score.

NAFLD-LFS cutoff > -0.640 for detecting liver fat > 5.56 % (sensitivity 86% and specificity 71%).

NAFLD fibrosis score < -1.455 corresponds with low probability of advanced fibrosis (NPV ≈ 92%) and > 0.675 indicates high probability of advanced fibrosis (PPV ≈ 85%).

* Abnormal ALT refers to >19 U/L for women and 30 U/L for men.

† McNemar's or paired T test when appropriated. Chi-square or Wilcoxon signed-rank tests when appropriated.

Table 3. One-year associations between weight loss (%) and changes in liver- and diabetes-related variables. Intention-to-treat analysis.

Variables	CCI cohort, n=262			
	≤ 5%	**5-10%**	**>10%**	
	N=55	**N=67**	**N=140**	**P values***
Liver-related parameters				
Δ ALT (U/L)	-2.1 ± 14.4	-7.7 ± 14.6*	-10.4 ± 26.5	0.02
Δ Platelet (x 10^9)	-10 ± 32	-12 ± 29	-23 ± 34	<0.01
Δ ALP (U/L)	-1.9 ± 8.3	-6.5 ± 12.7*	-11.5 ± 14.6**	<0.01
Metabolic-related parameters				
Δ HbA1c (%)	-0.59 ± 0.99	-0.87 ± 1.11*	-1.51 ± 1.36**	<0.01
Δ Triglycerides (mg/dl)	4.6 ± 128.3	-24.3 ± 121*	-65.9 ± 79.5**	<0.01
Δ Cholesterol (mg/dl)	-4.5 ± 33.2	4.6 ± 40.4	11.4 ± 34.1	0.21
Δ HDL cholesterol (mg/dl)	-0.29 ± 6.7	1.73 ± 6.6*	9.88 ± 10.5**	<0.01
Δ LDL cholesterol (mg/dl)	0.6 ± 23.4	1.76 ± 29.4	11.77 ± 31.9	0.04
Kidney function parameters				
Δ Creatinine (mg/dl)	-0.03 ± 0.11	-0.007 ± 0.10	-0.06 ± 0.17	0.03
Δ eGFR (CKD-EPI)	1.62 ± 9.3	1.23 ± 10.2	4.16 ± 11.6	0.05
Non-invasive biomarkers				
Δ NAFLD-LFS	-0.56 ± 2.8	-1.25 ± 3.1	-2.64 ± 4.3**	<0.01
> -0.640	50 (91%)	58 (87%)	76 (54%)	<0.01

Δ NAFLD fibrosis score	0.123 ± 0.57	-0.153 ± 0.59	-0.669 ± 0.78**	<0.01
Cut-offs				<0.01
< -1.455	7 (13%)	9 (13%)	35 (25%)	
-1.455 to 0.675	33 (60%)	48 (72%)	85 (61%)	
> 0.675	15 (27%)	10 (15%)	20 (14%)	
Other parameters				
Δ CRP (mg/dl)	-0.89 ± 6.2	-0.03 ± 7.5	-4.10 ± 15.1	<0.01
Δ BHB (mmol/l)	0.02 ± 0.31	0.05 ± 0.30*	0.20 ± 0.37**	<0.01

Abbreviations: ALT, alanine aminotransferase; **ALP**, alkaline phosphatase, **HbA1c,** Glycosylated hemoglobin; **HDL**, high density lipoprotein; **LDL**, low density lipoprotein; **eGFR**, estimated glomerular filtration rates; **CKD-EPI**, chronic kidney disease-epidemiological collaboration equation; **BHB**, beta-hydroxybutyrate; **NAFLD**, nonalcoholic fatty liver disease; **LFS**, liver fat score.

Δ means change from baseline.

For categorical variables, P value for the Mantel-Haenszel $_{X}^{2}$ test for trend; and for continuous variables, analysis of covariance (ANCOVA) while controlling by age, gender, BMI at baseline, concurrent medications for type 2 diabetes and hyperlipidemia. * Significant difference (P<0.01) between WL 5-10% as compared with WL < 5%. ** Significant difference (P<0.01) between WL >10% as compared with WL 5-10%. All ANCOVA analyses were adjusted by Bonferroni test for multiple comparisons (P<0.01)

Table 4. Correlations* between improvement in ALT and changes in metabolic parameters.

Variable	*Full CCI cohort N=262*				*CCI cohort with abnormal baseline ALT levels N=157†*			
	Unadjusted *r*	*P value**	Adjusted *r*	*P value**	Unadjusted *r*	*P value**	Adjusted *r*	*P value**
Δ Body weight (%)	0.17	<.01	0.19	<.01	0.22	<.01	0.27	<.01
Δ Fasting glucose (mg/dl)	0.19	<.01	0.24	<.01	0.28	<.01	0.42	<.01
Δ HbA1c (%)	0.25	<.01	0.26	<.01	0.35	<.01	0.44	<.01
Δ Triglycerides (mg/dl)	0.06	.32	0.09	.15	0.08	.34	0.13	.09
Δ Cholesterol (mg/dl)	-0.09	.21	-0.10	.16	-0.04	.62	-0.12	.18
Δ HDL cholesterol (mg/dl)	-0.13	.04	-0.10	.12	-0.14	.09	-0.18	.02
Δ LDL cholesterol (mg/dl)	-0.11	.08	-0.11	.07	-0.08	.33	-0.16	.05

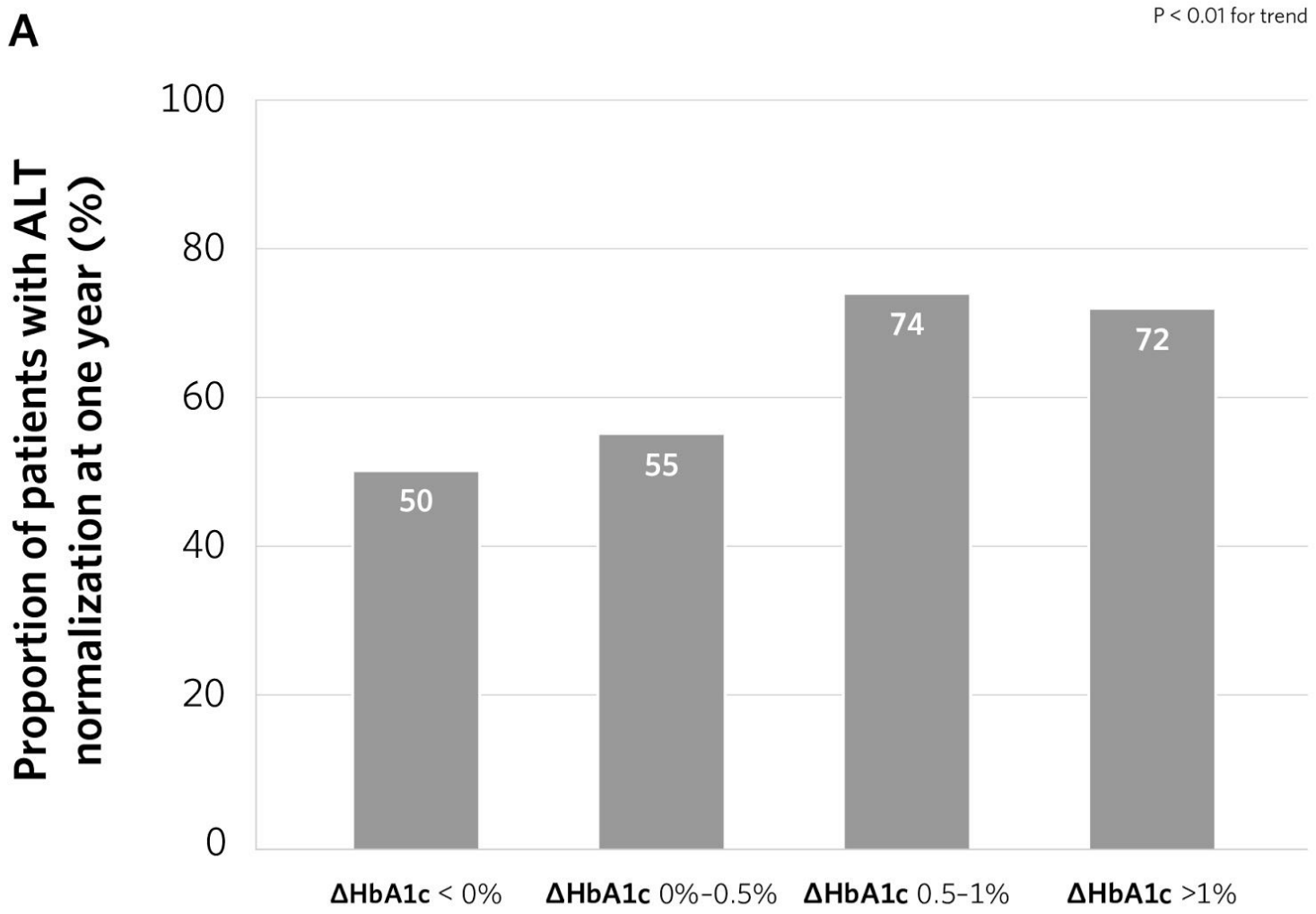
Abbreviations: ALT, alanine aminotransferase; **HbA1c,** glycosylated hemoglobin; **HDL**, high-density lipoprotein; **LDL**, low-density lipoprotein.

* Unadjusted and adjusted Pearson's correlations. Adjustments while controlling for baseline HbA1c, ALT, BMI and lowering-lipid and anti-diabetic medications.

† ALT levels > 19 in women and > 30 in men.

Δ means change from baseline.

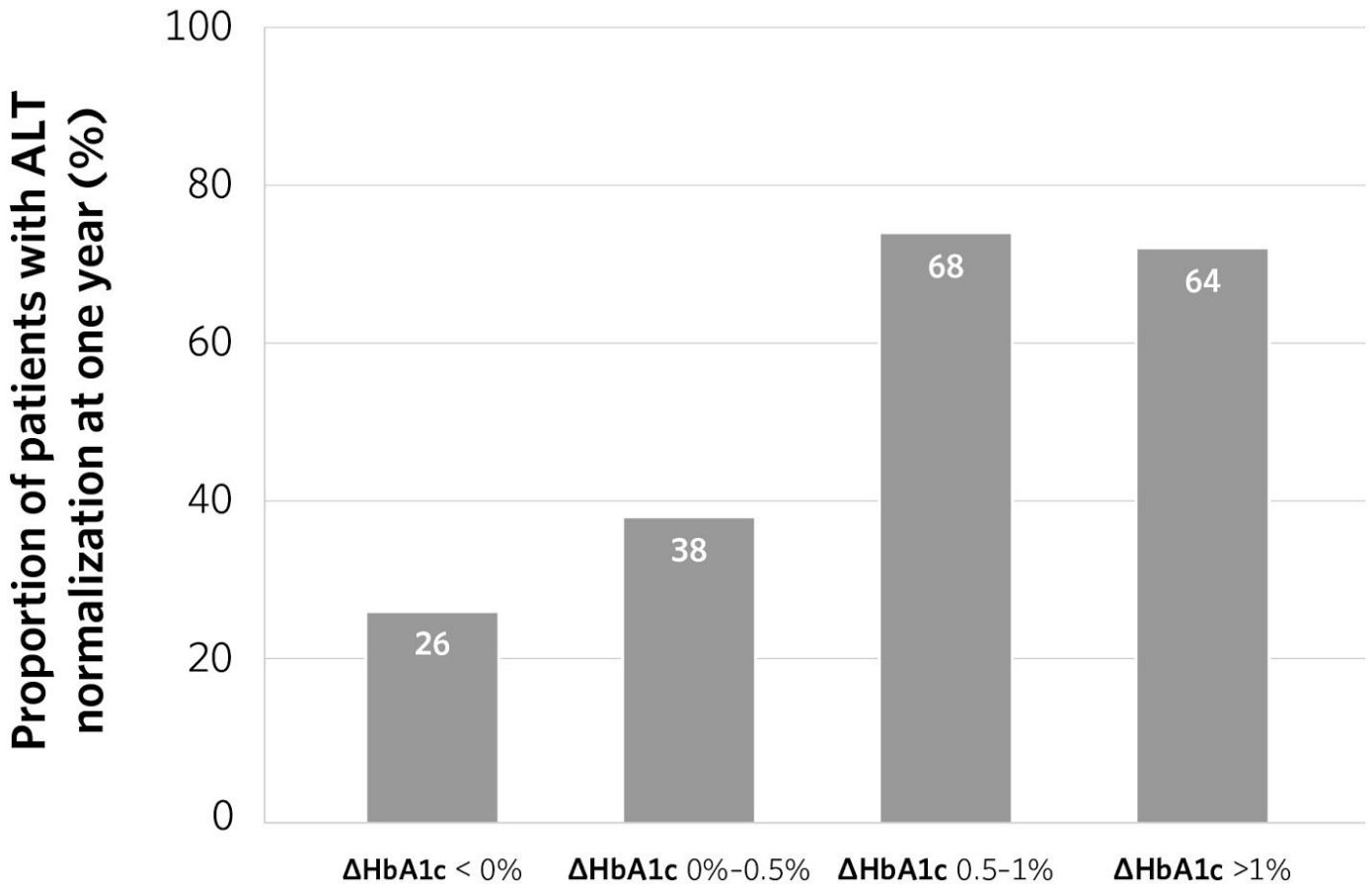
A



Mean WL (%)	**6.3% ± 5.8**	**7.7% ± 6.8**	**12% ± 7.5**	**18.9% ± 8.2**

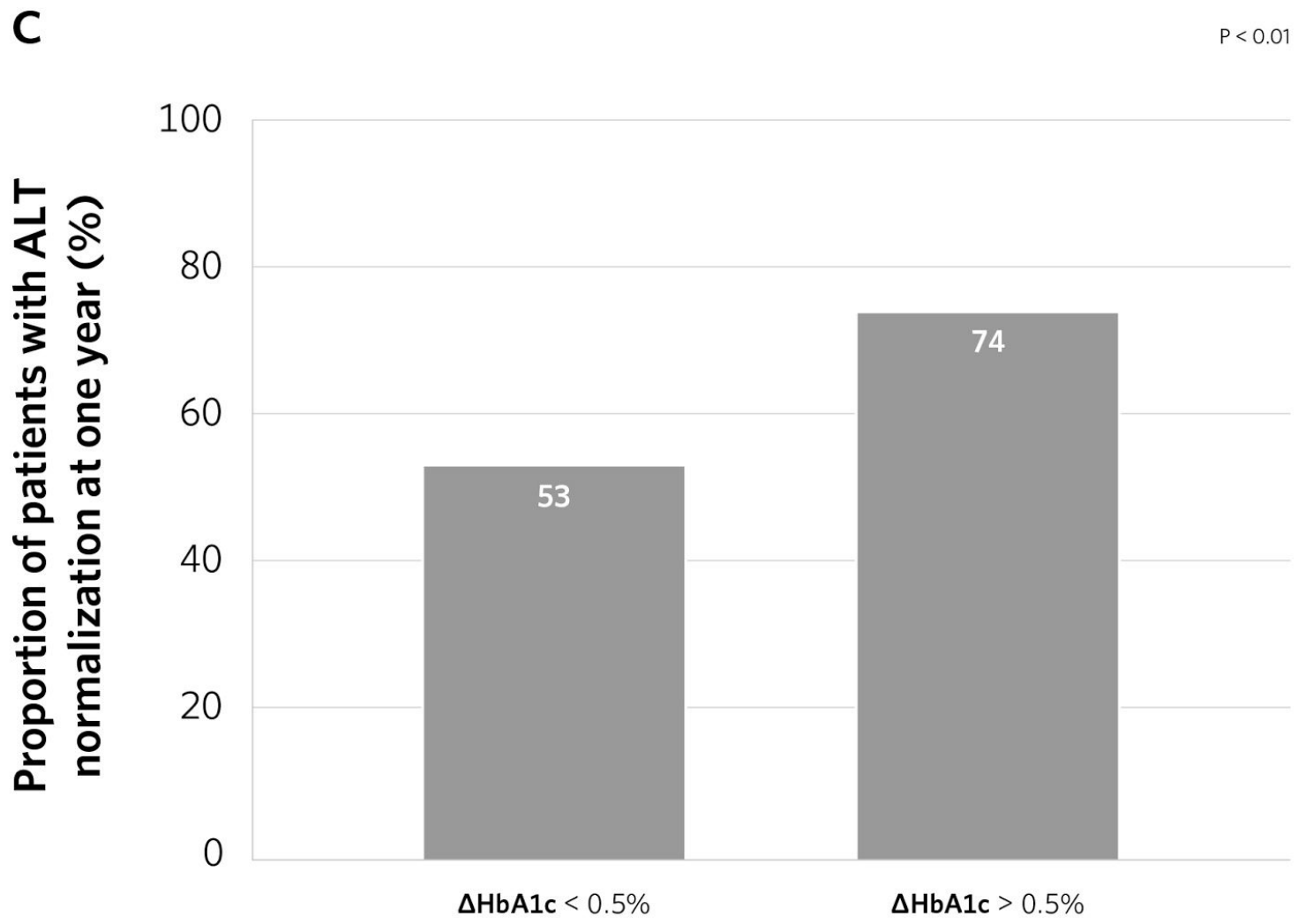
B

P < 0.01 for trend



Mean WL (%)	**6.7% ± 5.9**	**10.2% ± 7.7**	**11.9% ± 7.1**	**15.5% ± 7.4**

C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Proportion of patients with ALT normalization at one year (%)
100
80
60
40
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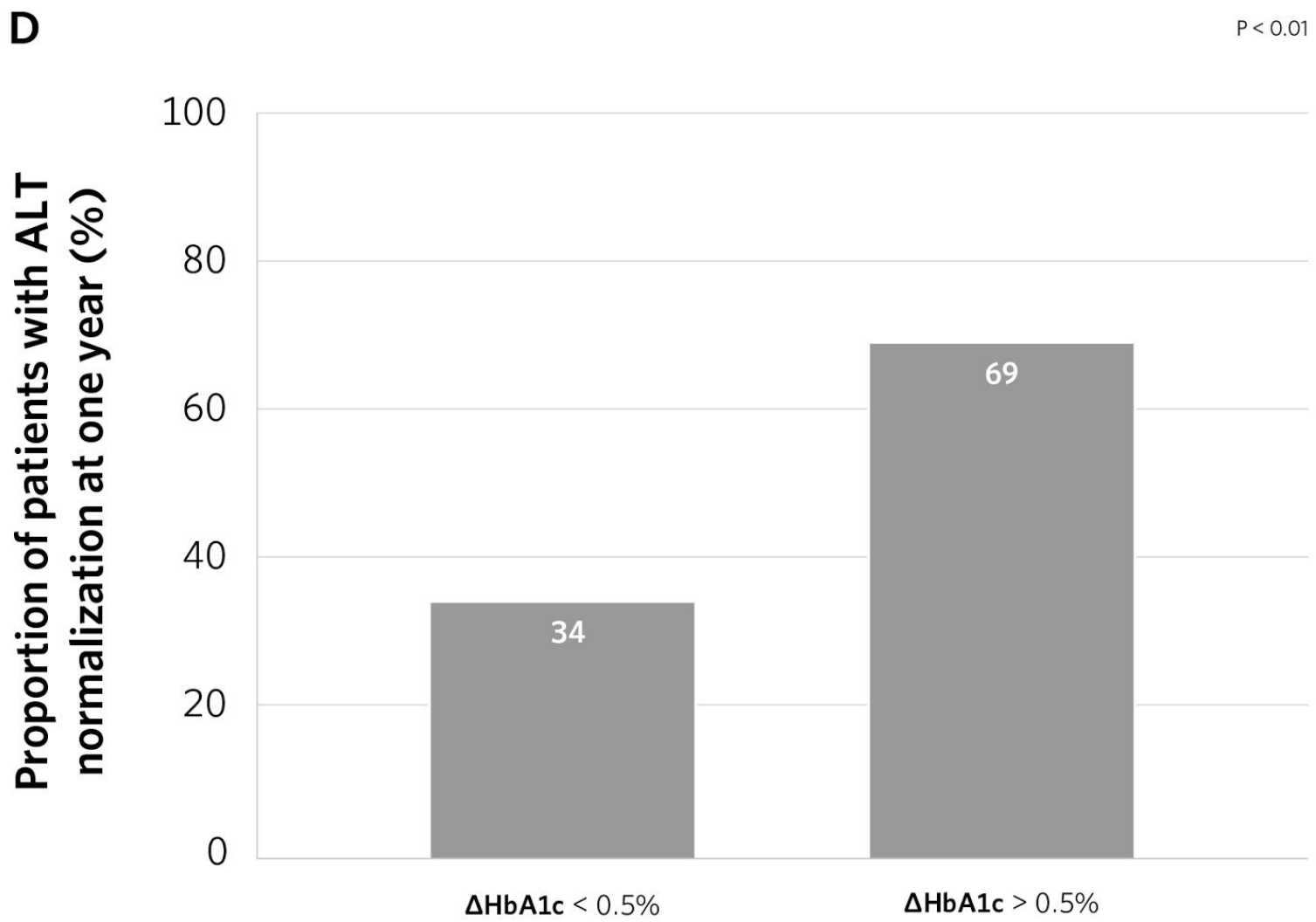
53
74
ΔHbA1c < 0.5%
ΔHbA1c > 0.5%


D
P < 0.01
Proportion of patients with ALT normalization at one year (%)
100
80
60
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20
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34
69
ΔHbA1c < 0.5%
ΔHbA1c > 0.5%