Research article

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Empagliflozin in mice fed on a fast-food diet: Preliminary biochemical data

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ABSTRACT

Aim: Nonalcoholic fatty liver disease (NAFLD) is a chronic liver disease affecting a large proportion of the general population (25%). The aim of this study was to evaluate the effect of a sodium-glucose co-transporter 2 inhibitor, empagliflozin, on NAFLD and the preliminary data of week 8 are hereby presented.

Material and methods: Twenty-four C57Bl/6 mice of both genders were randomly assigned in three groups: 8 mice fed on chow diet (control group), 8 mice fed fast-food diet (FFD) and empagliflozin, and 8 mice fed FFD without empagliflozin for 8 weeks. Weight measurement, and blood sampling for the measurement of glucose, alanine aminotransferase, total cholesterol and triglycerides were performed at baseline and week 8.

Results: Between-group comparisons did not show significant differences between FFD groups with and without empagliflozin. The only between-group difference regarding empagliflozin group was in cholesterol at week 8, which was higher compared to control group (p=0.009). Within-group comparisons showed increases in total cholesterol in both FFD groups, ALT in FFD/empagliflozin group and glucose in FFD group. Despite the expected increase in body weight, no biochemical change was observed in the control group.

Conclusions: Empagliflozin did not show preventive benefits on body weight or metabolic biochemical parameters after 8 weeks of treating FFD mice, thus, possibly highlighting that 8 weeks may be short period for FFD to exert its adverse effects in full, as well as for empagliflozin to exert any potentially beneficial effect.

KEY WORDS: *Empagliflozin; fast food diet; fibrosis; nonalcoholic fatty liver disease; obesity; sodium-glucose co-transporter 2 inhibitor*

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a chronic liver disease with increasing prevalence, affecting about 25% of the general population, 70% of patients with type 2 diabetes mellitus (T2DM) and 90% of morbidly obese individuals[1,2]. Although NAFLD is a topic of intense

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research, no pharmaceutical treatment has specifically been approved yet[2,3].

Sodium glucose cotransporter-2 inhibitors (SGLT-2i) decrease blood glucose primarily by inhibiting renal glucose reabsorption, thereby inducing glycosuria, which in turn promotes weight loss. Some SGLT-2i have been approved for the treatment of T2DM and have also shown favorable cardiovascular and renal outcomes[4]. However, the effect of SGLT-2i on liver inflammation and especially fibrosis, the main prognostic histological factor of NAFLD, needs further investigation[4]. Limited data revealed a beneficial effect of empagliflozin, which is a SGLT-2i[3]. One of the studies, the E-LIFT trial ("Effect of Empagliflozin on Liver Fat in Patients With Type 2 Diabetes and Nonalcoholic Fatty Liver Disease"), which investigated the effect of empagliflozin on liver fat using magnetic resonance imaging - proton density fat fraction (MRI-PDFF), showed that empagliflozin reduced liver fat after 20 weeks of treatment, but liver histology was not assessed[5].

The aim of this study was to evaluate the effect of empagliflozin treatment on hepatic fibrosis (main), steatosis and inflammation (secondary) in C57BL/6 mice fed on a fast-food (high fat, high cholesterol, high fructose) diet after 24 weeks of treatment. Hereby, we present the 8-week preliminary data of this study, focusing on body weight and metabolic biochemical parameters.

MATERIAL AND METHODS

Male and female C57Bl/6 mice were kept in separately ventilated cages, under controlled conditions at 20±2°C temperature, 12h light/12h dark cycle and free access to water and diet. Twenty-four C57Bl/6 8-9 weeks-old mice, were randomly (stratified per sex) assigned into three groups (n=8, four males; four females) and received different diets over 6 months. Group 1 received chow diet, group 2 received fast-food diet (FFD; 0.21% cholesterol, providing 42% of energy as fat, 15% as protein and 43% as carbohydrates) with empagliflozin (10 mg/kg/d) and group 3 received FFD without empagliflozin. The mice on FFD also consumed fructose with their water (23.1 g/l) and glucose (18.9 g/l), as previously described[6]. Weight measurement and blood sampling from the submandibular vein were performed at baseline and every four weeks until the end of the study. The amount of food and water consumed was weighed every week. For the needs of this study, glucose, alanine aminotransferase (ALT), total cholesterol and triglycerides were measured at baseline and week 8 by standard laboratory methods.

Statistical analysis

Data are presented as mean ± standard deviation (SD) for continuous variables or number and/or percentages for categorical variables. Due to the small sample size, non-parametric tests were used. More specifically, Kruskal-Wallis test was used for comparisons between groups and Wilcoxon test was used for comparisons within groups. Bonferroni correction was used post-hoc to correct for multiple pairwise comparisons. A two-sided p-value <0.05 was considered statistically significant for all tests. Statistical analysis was performed with SPSS 27 for mac (IBM Corp., Armonk, NY, USA).

RESULTS

Comparative data within and between groups are presented in Table 1. Baseline characteristics were not different between groups, except for glucose, which showed a marginally significant trend. However, when pairwise comparisons with Bonferroni correction were conducted, there was no significant difference between groups. At week 8, there was a significant trend in ALT and total cholesterol. In pairwise comparisons, ALT was higher in group 1 than group 3 (p=0.007), and cholesterol was higher in group 2 than group 1 (p=0.009); cholesterol was marginally not significant (p=0.065) for the comparison between group 1 and 3. Regarding within-group comparisons, body weight increased in all groups, cholesterol increased in groups 2 and 3, ALT in group 2 and glucose in group 3. Triglycerides reduced in groups 2 and 3.

DISCUSSION

Analysis of the preliminary biochemical data at week 8 did not demonstrate any significant difference between mice receiving FFD with or without empagliflozin. Therefore, at this time point, this study could not indicate any potentially preventive effect of empagliflozin on body weight and metabolic biochemical parameters. Notably, no biochemical parameter increased within the control group, despite the increase in body weight[7]. These results should be cautiously interpreted, since 8 weeks may be short period for FFD to exert its adverse effects in full, as well as for empagliflozin to exert any potentially beneficial effect.

Compared with other studies, Petito-da-Silva et al. showed that empagliflozin reduced body mass and improved the metabolic profile of mice receiving treatment *vs.* untreated mice on a high-fat diet[8]. In accordance with our study, FFD increased body weight and glucose, while total cholesterol was higher compared to mice fed on a chow diet. The authors also demonstrated a significant increase in triglycerides and ALT in FFD group compared to chow group and triglycerides, cholesterol and ALT were lower in the empagliflozin group compared to the FFD group without empagliflozin. In another study, Kim et

TABLE 1. Comparative data within and between groups.

Variable	Time	Group 1	Group 2	Group 3	p value for trend (between groups)
Weight (gr)	Baseline	19.0±4.6	20.2±3.5	18.1±3.1	0.279
	Week 8	23.5±3.8	26.8±6.4	24.6±4.6	0.277
	p value (within groups)	**0.011**	**0.012**	**0.012**	
Glucose (mg/dl)	Baseline	91±47	125±12	101±22	**0.048**
	Week 8	119±11	135±27	126±20	0.670
	p value (within groups)	0.223	0.176	**0.017**	
ALT (U/l)	Baseline	46±17	35±9	37±16	0.300
	Week 8	53±8	47±14	37±6*	**0.007**
	p value (within groups)	0.310	**0.028**	0.833	
Total cholesterol (mg/dl)	Baseline	65±14	68±14	66±12	0.915
	Week 8	80±21	174±71*	150±63	**0.005**
	p value (within groups)	0.396	**0.012**	**0.012**	
Triglycerides (mg/dl)	Baseline	115 ±45	105±13	103±21	0.949
	Week 8	104±55	83±26	70±35	0.505
	p value (within groups)	0.204	**0.036**	**0.012**	

Data are presented as mean±standard deviation (SD).
*: p<0.05 compared with group 1 (Bonferroni correction for multiple pairwise comparisons).
Group 1 fed on chow diet; group 2 fed on FFD with empagliflozin; group 3 fed on FFD without empagliflozin.
ALT, alanine aminotransferase.

al. used a rat model of T2DM with characteristic features of obesity, hyperglycemia and hyperlipidemia[9]. It was shown that empagliflozin reduced body weight after 12 weeks of treatment, as well as ALT and triglycerides. Xu et al. used high-fat diet-induced obese mice receiving or not empagliflozin *per os* for 16 weeks, and showed that empagliflozin administration suppressed body weight gain, IR and hepatic steatosis[10]. Moreover, empagliflozin reduced ALT, with no effect on lipids. However, the duration of the above studies was rather longer than the duration of the preliminary data of our study. Other factors may also have contributed to the observed differences between studies, including the use of different animal models or different study designs. For example, Kim et al.[9] used a rat model known for T2DM, obesity, hyperglycemia, hyperlipidemia and diabetic complications, and they did not feed the animals on any type of high fat diet. On the other hand, Petito-da-Silva et al.[8] used C57BL/6 mice, but they kept the mice on high fat diet for a period of 10 weeks before the initiation of the treatment with empagliflozin, thus targeting to treat the already developed NAFLD rather than the prevention of NAFLD, as in our study.

In conclusion, empagliflozin did not show any preventive benefit on body weight and metabolic biochemical parameters after 8 weeks of treatment in mice fed on a FFD diet. However, 8 weeks may be short to demonstrate either a clearly adverse effect of FFD or any potentially preventive effect of empagliflozin. Importantly, the results of liver histology are expected to clarify whether empagliflozin has a potential therapeutic benefit on NAFLD.

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Declarations of interest

None.

Η εμπαγλιφλοζίνη σε μύες υπό διατροφή «ταχυφαγείου»: Προκαταρκτικά βιοχημικά δεδομένα

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Σκοπός: Η μη-αλκοολική λιπώδης νόσος του ήπατος (ΜΑΛΝΗ) είναι μια χρόνια ηπατική νόσος που επηρεάζει μεγάλο ποσοστό του γενικού πληθυσμού (25%). Ο στόχος αυτής της μελέτης ήταν να αξιολογήσει την επίδραση ενός αναστολέα του συμμεταφορέα νατρίου-γλυκόζης 2, της εμπαγλιφλοζίνης, σε πειραματικό μοντέλο μυός με ΜΑΛΝΗ, μετά από χορήγηση δίαιτας τύπου «ταχυφαγείου». Στην παρούσα μελέτη παρουσιάζονται τα προκαταρκτικά αποτελέσματα βιοχημικών παραμέτρων του μεταβολισμού κατά την 8η εβδομάδα.

Υλικά και μέθοδοι: Είκοσι τέσσερις μύες C57Bl/6 και των δύο φύλων χωρίστηκαν με τυχαιοποίηση σε τρεις ομάδες: 8 μύες έλαβαν κοινή τροφή (ομάδα ελέγχου), 8 δίαιτα «ταχυφαγείου» (fast-food diet, FFD) και εμπαγλιφλοζίνη, και 8 FFD χωρίς εμπαγλιφλοζίνη για 8 εβδομάδες. Κατά την έναρξη της μελέτης και στην 8η εβδομάδα, τα πειραματόζωα ζυγίστηκαν και πραγματοποιήθηκε αιμοληψία προς μέτρηση γλυκόζης, αμινοτρανσφεράσης της αλανίνης, ολικής χοληστερόλης και τριγλυκεριδίων ορού.

Αποτελέσματα: Οι συγκρίσεις μεταξύ των ομάδων δεν έδειξαν σημαντική διαφορά μεταξύ των ομάδων που έλαβαν FFD με ή χωρίς εμπαγλιφλοζίνη. Η μόνη σημαντική διαφορά που αφορούσε την ομάδα FFD με εμπαγλιφλοζίνη ήταν στη ολική χοληστερόλη της εβδομάδος 8, που ήταν υψηλότερη σε σύγκριση με την ομάδα ελέγχου (p=0,009). Οι συγκρίσεις εντός των ομάδων έδειξαν αύξηση της ολικής χοληστερόλης και στις δύο ομάδες υπό FFD, της αμινοτρανσφεράσης της αλανίνης στην ομάδα FFD με εμπαγλιφλοζίνη και της γλυκόζης στην ομάδα της FFD χωρίς εμπαγλιφλοζίνη. Παρά την αναμενόμενη αύξηση βάρους, δεν παρατηρήθηκε διαφορά σε καμία βιοχημική παράμετρο εντός της ομάδος των μαρτύρων.

Συμπεράσματα: Η εμπαγλιφλοζίνη δεν έδειξε προληπτική δράση επί του βάρους σώματος και των βιοχημικών μεταβολικών παραμέτρων μετά από 8 εβδομάδες θεραπείας σε μοντέλο μυός υπό διατροφή FFD. Επομένως, 8 εβδομάδες είναι πιθανώς μικρό χρονικό διάστημα, για να δειχθούν οι αρνητικές επιδράσεις της FFD, καθώς επίσης και οι πιθανώς ευεργετικές επιδράσεις της εμπαγλιφλοζίνης σε αυτό.

ΛΕΞΕΙΣ ΚΛΕΙΔΙΑ: *Αναστολέας του συμμεταφορέα νατρίου-γλυκόζης 2, διατροφή «ταχυφαγείου», εμπαγλιφλοζίνη, ίνωση, μη-αλκοολική λιπώδης νόσος του ήπατος*

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