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## Baseline Leptin Predicts Response to Metformin in Adolescents with Type 1 Diabetes and Increased BMI

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### Introduction

There is an increased prevalence of overweight and obesity among children and adults with type 1 diabetes (T1D) [1–4]. Further, the rate of overweight and obesity continues to be on the rise among people with T1D compared to general population trends [5]. This is problematic because obesity-induced insulin resistance increases exogenous insulin needs, with higher risk for hypoglycemia, weight gain, chronic inflammation, dyslipidemia, and long-term cardiovascular complications [4, 6].

Metformin is the first-line diabetes therapy in overweight and obese children and adults with type 2 diabetes (T2D). It improves insulin sensitivity and inhibits hepatic gluconeogenesis [7]. In a multicenter double-blind [8], placebo-controlled randomized clinical trial of 140 adolescents with overweight or obesity and T1D (NCT01881828) randomized to either metformin or placebo for 26 weeks, the use of metformin as an adjunct to insulin therapy failed to show a sustained effect on glycemic control after 26 weeks of treatment compared

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MJR and HMI conceptualized the study. HMI, BS, JW and MJR analyzed and interpreted the data, and wrote the manuscript. BS, JW, AS, MT, IL, FB and KD contributed to the design, interpreted the data and reviewed/edited the manuscript.

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to controls. Despite a small decrease in HbA<sub>1c</sub> at 13 weeks in the metformin group, mean HbA<sub>1c</sub> levels increased by 26 weeks in both groups. However, metformin therapy was associated with a reduction in weight, body mass index (BMI), %body fat (%BF), and total daily insulin dose (TDID).

Since the original trial did not show a sustained glycemic response and effect, as measured by HbA<sub>1c</sub>, we selected reduction in TDID, which was a positive outcome of the trial, to determine predictors of response. Here, we aimed to determine predictors of response to metformin therapy, in particular, we selected percent (%) change in TDID. We hypothesized that baseline phenotypic or metabolic predictors may help better identify youth with T1D with a higher reduction in % TDID in response to metformin therapy.

## Methods

We analyzed data from the T1D Exchange Clinic Network Metformin Randomized Clinical Trial [8] (NCT01881828). Detailed methods for this study were previously described [8]. For this analysis, data were extracted from the publicly available dataset as a text file, and Microsoft software used to convert the data tables to usable .xlsx files. The data were then cleaned, and random checks were performed to ensure accuracy of the data conversion and extraction.

Out of the 140 participants, 122 (61 in each arm) had available data on anthropometrics, physical examinations and laboratory evaluations at baseline, 13 and 26 weeks. Supplemental Figure 1 shows the analysis flow at each time point and the outcome measures of interest.

## Statistical analysis

Statistical analyses were conducted using MATLAB (<https://www.mathworks.com/>, V.R2019b) and R software (RStudio: Integrated Development for R). Between-group comparisons were made using a Student's t-test while within-group comparisons across multiple timepoints were done using a paired t-test. Correlation values were computed via the Spearman rank correlation method under the 'corrplot' package in R. Linear regression analyses were performed using the 'fitlm' function in MATLAB. TDID was quantified as units/kg of body weight/day. The primary outcome was the relative percentage change in TDID (% TDID) and was calculated as  $\% \Delta TDID = 100 * (TDID_{13 \text{ wks or } 26 \text{ wks}} - TDID_{baseline}) / TDID_{baseline}$  at 13 weeks and 26 weeks, respectively. The regression models adjusted for potential confounders included participant's age, diabetes duration, sex, race, BMI z-score, and %BF at baseline. P-values <0.05 were considered statistically significant. Comparisons for reduction in TDID were performed within each arm.

## Results

Supplemental Table 1 shows the baseline demographic and clinical data for individuals in each arm. We observed a statistically significant reduction in TDID at both 13 and 26 weeks compared to baseline (p<0.0001 at both time points) in the metformin arm. In addition, we

observed a reduction in TDID only at 26 weeks in the placebo arm ( $p < 0.001$ ) (Supplemental Figure 2 and Supplemental Table 2).

### Predictors of % TDID

Linear regression models were then used to identify the association between baseline prognostic factors (which included intervention type, age, sex, race, BMI z-score, diabetes duration, waist circumference, % body fat, leptin, adiponectin) and % TDID in the entire cohort from baseline to 13 weeks and 26 weeks. First, using pairwise Spearman correlation, we observed that no pair of factors had a correlation value greater than a pre-defined threshold of 0.8 and therefore, none were excluded from the linear regression analysis. We found that indeed, intervention type was significant ( $p = 0.004$ ), and the metformin group showed significantly more reduction in % TDID than the placebo group. Baseline leptin was the only significant prognostic factor associated with % TDID at 13 weeks and 26 weeks in the entire cohort ( $p < 0.0001$  and  $p = 0.0002$ , respectively), Supplemental Tables 3 and 4.

When assessing by intervention arm using multiple linear regression analysis to adjust for baseline variables, baseline leptin predicted larger negative % TDID (i.e., larger relative reduction) at 13 ( $p = 0.0012$ ) and 26 weeks ( $p = 0.007$ ) in the metformin arm (Table 1). In the placebo arm, baseline leptin was negatively associated with % TDID (i.e., larger relative reduction) at 13 ( $p = 0.043$ ) but not 26 weeks ( $p = 0.11$ ) (Supplemental Tables 5 and 6). Figures 1A and B show the regression slopes for association between baseline leptin and % TDID in the metformin (A) and placebo arms (B). To determine if simpler measures of insulin resistance could be used as predictors of % TDID in response to metformin, we used each of triglyceride levels (TG) and HDL cholesterol levels in univariate and multivariate regression models, with and without leptin in the multivariate models. We found the TG levels were not predictive in the univariate model whether at 13 or 26 weeks ( $p = 0.083$  and  $p = 0.146$ , respectively). HDL levels were also not predictive at either 13 or 26 weeks ( $p = 0.070$  and  $p = 0.865$ , respectively). Interestingly, we did observe that for the same value of HDL, a % reduction in TDID was slightly greater for the metformin arm than the placebo arm (effect = 0.6%,  $p = 0.014$ ). In multivariate regression models that included leptin, neither TG nor HDL levels were predictive at 13 or 26 weeks, while leptin levels remained strongly predictive of % reduction in TDID at both time points (effect = 1.1%,  $p < 0.0001$  and effect = 1.0%,  $p = 0.001$ , respectively). When leptin was removed from the regression models, only HDL was predictive at 13 weeks (effect = 0.3%,  $p = 0.039$ ). The results also suggest reduced explainability and predictive power if leptin is removed (drop in adjusted R-squared from 0.24 to 0.11 for 13 weeks, and from 0.15 to 0.05 for 26 weeks).

### Discussion

The goal of this analysis was to identify predictors of % TDID in response to metformin as adjuvant therapy in adolescents with T1D and obesity or overweight. We show that baseline leptin was a predictor of decrease in TDID in response to metformin at 13 and 26 weeks. In addition, baseline leptin was a prognostic factor of decrease in TDID in participants in the placebo arm, at 13 but not 26 weeks.

Leptin is an adipokine that is secreted by the adipose tissue to regulate obesity induced inflammation [9], food intake, BMI and lipolysis, among other actions. It has been shown to be an indicator of insulin resistance, independent of body fat mass [10, 11]. Therefore, it is possible that higher baseline leptin levels reflect higher insulin resistance and thus, predict more pronounced reduction in TDID in response to metformin. It is likely that the reduction in TDID is secondary to improved insulin sensitivity. Indeed, in a subsequent study that included hyperinsulinemic euglycemic clamps in a subgroup of participants from the original trial by Libman et al, [8], there was improved insulin sensitivity in those who received metformin [12]. We additionally performed an analysis to evaluate if baseline leptin is positively correlated with reduction in HbA1c. We found a weak significant relationship at 13 weeks between baseline leptin and reduction in HbA1c ( $p=0.047$ ) that was not sustained at 26 weeks (data not shown).

We assessed whether other simpler measures of insulin resistance, such as HDL and TG levels, could be used as predictors of response to metformin. We found that when including leptin in the multivariate models that also contained TG and HDL, leptin remained a strong predictor at both 13 and 26 weeks. Removing leptin reduced the explainability and predictive power of the model, and HDL was no longer a significant predictor of % TDID at 26 weeks. This strongly suggests that leptin provides additional value compared to simpler measures such as TG and HDL and that perhaps baseline HDL may be used to complement rather than replace baseline leptin to predict % TDID.

We did not observe a predictive value for baseline adiponectin on % TDID, whether in the univariate or multivariate models, and whether we performed the analysis in the entire cohort or by study arm. Since lower adiponectin levels could reflect higher insulin resistance, we assessed for correlations between levels of adiponectin levels and available measures of insulin resistance and found a significant positive correlation of adiponectin with HDL levels and negative correlation with triglyceride levels (data not shown). However, in our predictive models using TG or HDL as surrogate measures for insulin resistance, we did not observe a predictive effect of these measures on % TDID. Therefore, it appears that % TDID is not affected by insulin resistance and therefore would not be influenced by adiponectin levels. Thus, it appears that leptin may have a predictive effect independent of insulin resistance.

This analysis has some limitations, including the relatively smaller sample size, although whether in the entire cohort or in each arm separately, univariate and multiple regression analyses appeared to show the same predictive and prognostic value of baseline leptin. Additionally, while we did not see a predictive effect of baseline %BF on % TDID, and since leptin has been positively correlated with obesity and %BF, it is still possible that leptin as a predictor and independent of adiposity, has an effect on response to metformin, and that this effect is mediated by insulin sensitivity.

Our study shows that baseline leptin may be a useful predictor of response to metformin therapy. Future larger studies are needed to verify whether leptin can serve as an adequate biomarker for treatment response in youth with T1D.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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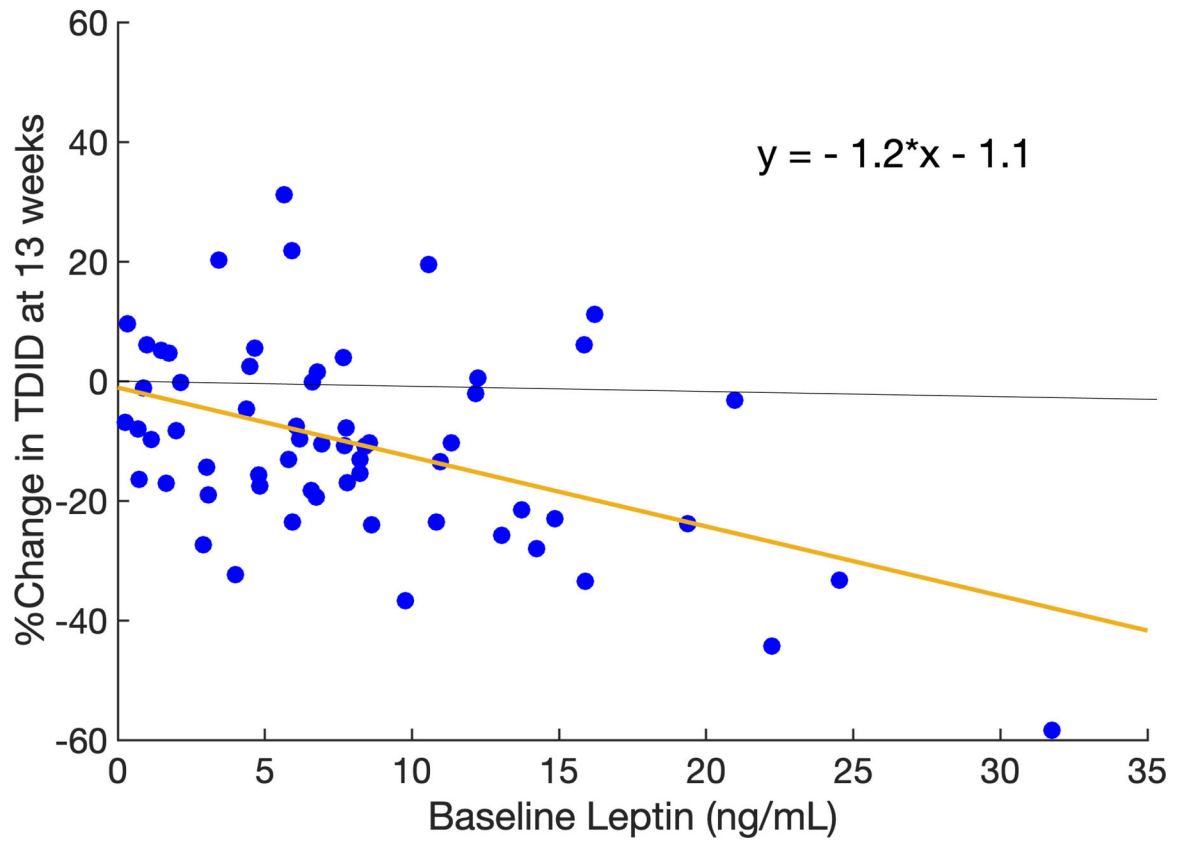
## Data Availability:

The data were analyzed and generated from a publicly available dataset and is available on request from the authors.

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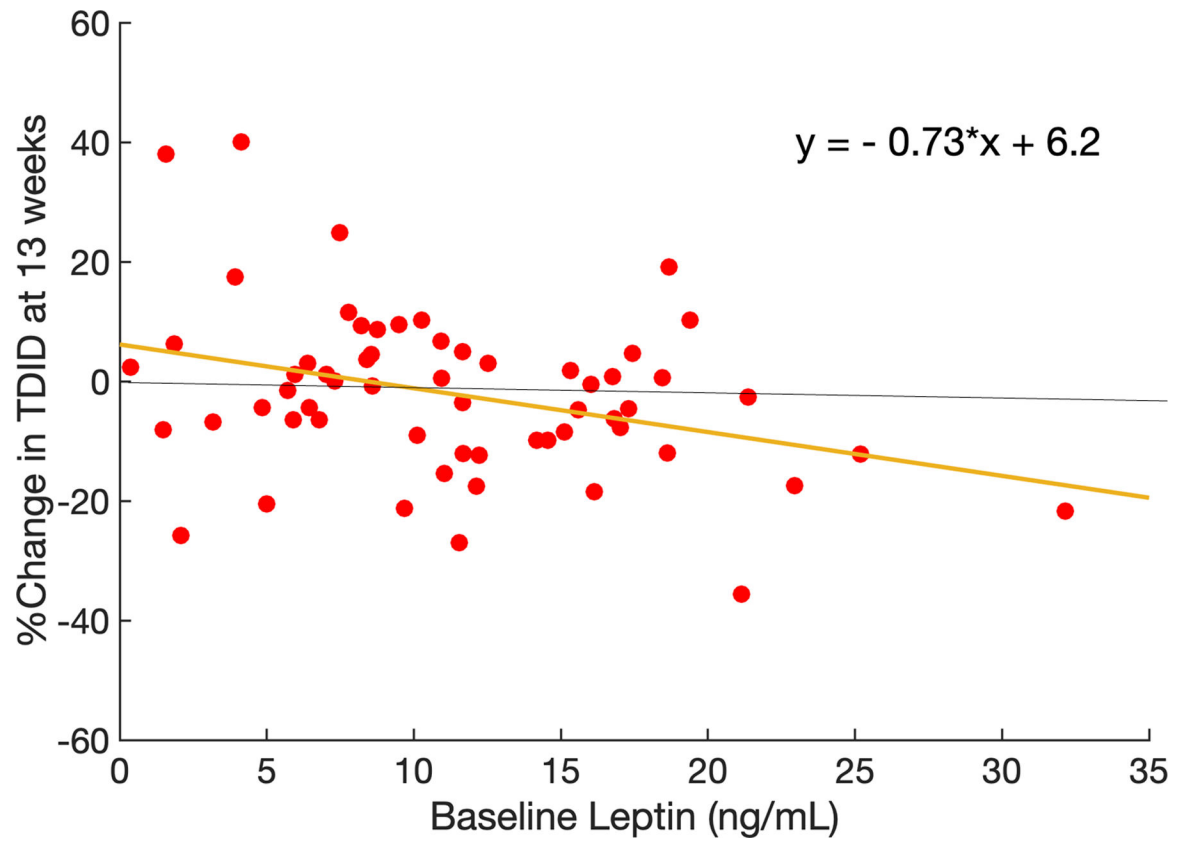


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**Figure 1:** Univariate linear regression showing the relationship between Leptin vs. % TDID in the metformin group (A) and the placebo group (B)

Multiple linear regression results [Predict relative (%) change in TDID from baseline to 13 weeks and baseline to 26 weeks] for the metformin group

**Table 1:**

Predictor	13 weeks				26 weeks			
	Regression coefficient	Std. error	t-statistic	p-value	Regression coefficient	Std. error	t-statistic	p-value
Intercept	1.59	30.05	0.05	0.96	3.18	33.93	0.09	0.93
Age	1.52	1.45	1.05	0.30	1.06	1.64	0.65	0.52
Sex (ref: Female) – Male	-12.02	6.39	-1.88	0.07	-7.52	7.21	-1.04	0.30
Duration of T1D	-0.69	0.70	-0.98	0.33	-1.18	0.79	-1.50	0.14
Race/Ethnicity (ref: White)								
– Hispanic	-0.55	6.06	-0.09	0.93	-5.79	6.84	-0.85	0.40
– African-American	-7.80	9.75	-0.80	0.43	-10.3	11.01	-0.93	0.36
– Other	-0.05	7.73	-0.01	0.99	-8.30	8.73	-0.95	0.35
BMI z-score	2.14	11.41	0.19	0.85	-5.72	12.89	-0.44	0.66
Waist circumference	0.02	0.32	0.06	0.95	0.21	0.36	0.58	0.56
%Body fat	-0.19	0.42	-0.45	0.65	0.22	0.47	0.46	0.65
Leptin	-1.55	0.45	-3.45	0.0012*	-1.44	0.51	-2.83	0.007*
Adiponectin	-0.32	0.38	-0.85	0.40	-0.54	0.42	-1.27	0.21
Baseline TDID	-6.45	11.02	-0.59	0.56	-16.51	12.45	-1.33	0.19

Key for p-values

\*, p<0.01