



## RESEARCH ARTICLE

# Acipimox in Type 1 Diabetes: fatty acids as a potential, but challenging, therapeutic target

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OPEN ACCESS

**PUBLISHED**

31 December 2024

**CITATION**

Kaput, KL, Lyon, E., et al., 2024. Acipimox in Type 1 Diabetes: fatty acids as a potential, but challenging, therapeutic target. Medical Research Archives, [online] 12(12).  
<https://doi.org/10.18103/mra.v12i12.6170>

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**DOI**

<https://doi.org/10.18103/mra.v12i12.6170>

**ISSN**

2375-1924

**ABSTRACT**

**Objective:** To examine the effect of acute, isolated non-esterified fatty acid lowering, on insulin action, arterial stiffness and cardiac autonomic function in type 1 diabetes.

**Methods:** Randomized, double-blind, crossover trial of acipimox versus placebo in participants with and without type 1 diabetes (n=9 and 8, respectively).

**Results:** Participants with type 1 diabetes were more insulin resistant than controls and exhibited increased arterial stiffness (pulse wave velocity and augmentation index), autonomic dysregulation (heart rate variability), cardiac diastolic dysfunction (E:A ratio), and increased cardiac contractility (fractional shortening) with preserved cardiac output. Acipimox treatment was confounded by a pronounced early rebound effect. However, independent of acipimox, within-subject higher pretest fatty acid levels corresponded to greater insulin resistance, higher mean tachycardic heart rates at rest, and possibly increased cardiac output.

**Conclusions:** These findings confirm and expand upon previous observations that individuals with type 1 diabetes are more insulin resistant and exhibit cardiovascular changes consistent with increased sympathetic tone. The correlation of poor peripheral glucose utilization and cardiovascular changes with elevated fatty acid levels suggests that lowering fatty acid levels may improve insulin sensitivity and decrease sympathetic tone. In the future, a more durable or targeted method of fatty acid lowering could represent a novel cardioprotective target of intervention in people with type 1 diabetes.

## Introduction:

Cardiovascular disease (CVD) remains the leading cause of death in participants with type 1 diabetes (T1D). The overall risk of CVD in participants with T1D compared to participants without diabetes is two to threefold higher in men and three to fivefold higher in women.<sup>1</sup> This risk persists despite management of conventional CVD risk factors such as blood pressure, dyslipidemia, smoking and intensive glycemic control. Prediction models for determining CVD risk are currently available, but grossly underestimate CVD risk in persons with T1D.<sup>2</sup> Multiple studies now indicate that risks outweigh benefits of glycemic control that is more intensive than current guidelines in both type 1 and type 2 diabetes.<sup>3,4</sup> Furthermore, while a link between elevated hemoglobin A1C (HbA1c) and CVD was initially suggested by the Pittsburgh diabetes control and complications trial/epidemiology of diabetes interventions and complications study (DCCT/EDIC), this has since been discounted.<sup>4-6</sup> These discrepancies in the current literature highlight a significant gap in our therapeutic approach to CVD risk in participants with T1D and provide the rationale for an investigation of other therapeutic targets which may be unaddressed in this high-risk population.

Insulin resistance, a known correlate of CVD risk in T2D has also been demonstrated and linked to CVD in T1D.<sup>7-9</sup> A 10-year-follow up from the Pittsburgh EDIC study in T1D, identified insulin resistance-related factors, rather than glycemia and standard CVD risk factors, as the most significant predictor of coronary artery disease endpoints.<sup>5</sup> Similarly, a cohort of participants with T1D and well-matched controls without diabetes were followed for six years to assess coronary artery calcification (CAC) and CVD outcomes in the CACTI study. Participants with T1D were found to be more insulin resistant than persons without diabetes, despite adjustment for age, sex, BMI and fasting glucose. Further, the insulin resistance that was observed in this cohort did not correlate with glycemic control, but was associated with CAC volume and predicted presence and progression of CAC suggesting that insulin resistance may play a significant role in CVD risk in participants with T1D.<sup>10</sup> Similarly, other studies, including the Paris Prospective Study have further demonstrated that hyperinsulinemia, presumably due to insulin resistance, is an independent predictor of cardiovascular death.<sup>9</sup>

To date, the exact mechanism of insulin resistance in T1D is not well understood. Acute non-esterified fatty acid (NEFA) elevation has been shown in multiple studies to induce insulin resistance in lean and obese persons without diabetes.<sup>11-18</sup> Conversely, isolated NEFA lowering with acipimox, a nicotinic acid analog approved for the treatment of lipid disorders in Europe, has been shown to improve insulin resistance and endothelial and mitochondrial function in people with and without T2D.<sup>19-26</sup> In the CACTI study, Schauer et al. demonstrated that defective insulin-mediated suppression of NEFA is present in T1D as well.<sup>10</sup> If NEFA concentrations prove to be a significant contributor to insulin resistance in T1D, then this could represent a novel target of intervention. Therefore, we proposed to test the hypothesis that sub-acute, isolated NEFA lowering with acipimox, would improve

insulin action, as well as vascular correlates of insulin resistance in participants with T1D.

## Methods:

**Study population:** Participants with type 1 diabetes (T1D, n=9) and controls without diabetes (non-DM, n=8) were recruited from the CACTI cohort, by campus emailing by flyer posting, and through clinical relationships of the PI. Inclusion criteria were age 25 to 59, HbA1c 6-9.5% (T1D only), and willingness to commit to 14 days of prescribed diet and study medication and two 44-hour inpatient stays. Participants were excluded for tobacco use, pregnancy, steroid use, scheduled physical activity > 3 days a week, angina or any other cardiovascular or pulmonary symptoms, a history of COPD or asthma, uncontrolled hypertension, proteinuria >200 g/day or a creatinine >153 µmol/L, proliferative retinopathy, any comorbid condition associated with inflammation, insulin resistance or dyslipidemia (including type 2 diabetes), niacin treatment, history of peptic ulcers, history of known hereditary angioedema or C1 esterase deficiency, uncontrolled thyroid disease, or anemia.

**Study design:** The hypothesis was tested in a double-blind, placebo-controlled, random order, crossover design study. Participants took acipimox 250 mg PO QID or matching placebo for one week each in random order with a >two-week wash-out period between phases. An investigation of new drug approval from the FDA was obtained to use acipimox to investigate the effect of NEFA lowering on insulin action in individuals with T1D (IND#107649). Acipimox was purchased from <sup>TM</sup>Pfizer, and blinded capsules of acipimox and placebo were prepared by the University of Colorado Research Pharmacy. Written and informed consent was obtained from each participant prior to the initiation of the study.

**Continuous glucose monitoring:** Participants with T1D underwent blinded continuous glucose monitoring (CGM, (Dexcom 7plus system) for three days prior to the inpatient hyperinsulinemic-euglycemic clamp visit. Reported CGM measures included: mean glucose, percentage of hypoglycemic events (glucose < 3.9 mmol/L), percentage of hyperglycemic events (glucose > 9.9 mmol/L) and glycemic variability within days. Modification of insulin could be made to allow for improved safety and avoid changes in glycemic control that might occur during the acipimox treatment.

**Physical activity monitoring:** Participants were provided with an Actigraph accelerometer to wear during each week of the study drug and were prescribed a standardized diet (40% carbohydrate, 35% fat, 25% protein) for two x 3 days before their inpatient baseline studies.

**Blood collection and sampling:** Laboratory assessment for glucose, insulin levels, triglycerides, glycerol, lactate and NEFA levels were drawn during study visits every 1-3 hours for a total of 24 hours and once for the measurement of endothelial, inflammatory (endothelin 1, interleukin 6, TNF- α, and hsCRP) and prothrombotic (PAI-1) markers. Blood samples to determine baseline hormone function and substrate (insulin, glucose, C-peptide, NEFA, glycerol and lactate) concentrations were also drawn the morning before the initiation of the clamp.

**Hyperinsulinemic-euglycemic clamp:** Participants were admitted to the clinical translational research center (CTRC) on the evening of day six for a 24-hour period of frequent metabolic labs while on the prescribed diet with usual insulin dosing. At the end of the 24-hour period, participants fasted for > 12 hours and those with type 1 diabetes were transitioned to intravenous insulin with blood sugar monitoring and insulin infusion adjustments to achieve euglycemia by morning and clear residual subcutaneous insulin. In the morning, hyperinsulinemic-euglycemic clamp was performed using the method of deFronzo et al, as referenced in the CACTI study, but omitting the lowest insulin stage (4 mU/m<sup>2</sup>/min) to complete a three hour, two-stage clamp with 8 and 40 mU/m<sup>2</sup>/min insulin infusion.<sup>10,27</sup> The rate of glucose infusion was adjusted based on arterialized blood specimens drawn every five minutes with plasma glucose quantified using a hand-held glucose analyzer (Precision Xceed Pro) at the bedside. Blood samples were also obtained for the determination of free fatty acids, triglycerides, insulin, and glycerol at the end of each stage of the clamp. Glucose disposal (M-value) is expressed as milligrams of glucose per kilogram body mass per minute and after normalization for final serum insulin concentration.

**Arterial stiffness:** Arterial stiffness was assessed, noninvasively, by <sup>TM</sup>Sphygmocor BPAS 1 device (Atcor Medical) applanation tonometry measurement of augmentation index (AIx) and pulse wave velocity (PWV) as described elsewhere.<sup>28,29</sup>

**Autonomic function:** Cardiac autonomic function was measured by heart rate variability (HRV) using standard methods including variation in R-R interval with cycled deep breathing to a metronome, R-R variation with the Valsalva maneuver, and orthostatic heart rate and blood pressure as previously described.<sup>30-32</sup>

**Echocardiography:** Resting two-dimensional echocardiography was performed using Vivid 7 and Echopac (BTO6 V6.1.3; General Electric, Milwaukee, WI) as previously described.<sup>30</sup>

**Statistical Analysis:** This study was intended as a proof of concept, mechanistic study and was powered to detect acipimox-mediated improvement in insulin sensitivity within the diabetes cohort. Ten participants with diabetes were expected to provide 80% power to detect one standard deviation (SD) improvement in the final glucose infusion rate (GIR). Data for the study was collected from each subject, verified and entered into a computer database. A group t-test was used to compare participants with and without type 1 diabetes. Paired t-testing was used to compare placebo and acipimox values for both primary outcome and secondary outcomes. In addition, data were sorted by pretest fatty acid levels and outcomes were compared for high versus low pretest fatty acid levels by paired t-test. All data are presented as a mean±SD. Two-sided P values < 0.05 were interpreted as an indication of statistical significance.

## Results:

**Baseline characteristics:** Baseline characteristics for all participants are shown in Table 1. Seventeen participants met the criteria for randomization (9=T1D and 8=non-DM) and underwent both phases of the intervention. Participants were well matched for body mass index (BMI), blood pressure, % body fat, renal function, liver function, inflammatory markers, and baseline lipid profile. As expected, HbA1c and fasting glucose were higher in the participants with T1D compared to controls. Consistent with previous reports adiponectin concentrations were also higher in participants with T1D.<sup>10</sup> Participants with T1D were older, predominantly female (66.7%), and more likely to be on a statin and have a documented diagnosis of hypertension, than the individuals without diabetes.

**Table 1:** Baseline characteristics for participants with and without type 1 diabetes (T1D)

	T1D	Non-DM	P Value
Male	2:9	3:8	N/A
Female	7:9	5:8	N/A
Age	48 ±8	39 ±12	<b>0.01</b>
Creatinine (µmol/L)	76 ±23	69 ±8	0.22
AST (ukat/L)	0.40 ±0.31	0.37 ±0.12	0.80
ALT (ukat/L)	0.35 ±0.18	0.33 ±0.13	0.95
A1C (%)	7.2 ±0.9	5.1 ±0.2	<b>&lt; 0.01</b>
Serum Glucose (mmol/L)	9.55 ±3.55	4.36 ±0.56	<b>&lt; 0.01</b>
Adiponectin (µg/mL)	13.2 ±9	6.4±3	<b>&lt; 0.01</b>
HDL (mmol/L)	1.3 ±0.3	1.3 ±0.3	0.92
Systolic BP (mmHg)	102 ±9	101 ±11	0.93
Diastolic BP (mmHg)	67 ±6	69 ±8	0.62
Triglycerides (mmol/L)	0.6 ±0.5	0.7 ±0.3	0.45
TNF-α (pg/mL)	1.8 ±0.9	1.4 ±0.8	0.31
IL-6 (pg/mL)	3.5 ±2.6	3.1 ±2	0.60
CRP (nmol/L)	24.8 ±28.6	14.3 ±9.5	0.20
Height (cm)	170 ±10	175 ±9	0.50
Body fat (%)	32 ±6	29 ±12	0.50
BMI (kg/m <sup>2</sup> )	27 ±4	26 ±5	0.60
Statin Treatment	3:7	0:8	N/A
History of Hypertension	2:9	0:8	N/A

\*Data are presented as mean±SD

**Participants with T1D differ from those without DM in insulin sensitivity and cardiovascular measures:**

**Insulin Sensitivity:** Participants with T1D were more insulin resistant by hyperinsulinemic-euglycemic clamp than

those without diabetes (Table 2). These findings were even more pronounced after adjustment for final insulin clamp concentration which was higher in participants with T1D compared those without.

**Table 2:** Insulin sensitivity by Glucose Infusion Rate (GIR) in stage 1 and by M-value and M-value/Insulin ratio in stage 2 of the 2-stage hyperinsulinemic-euglycemic clamp performed for the placebo phase

	<b>T1D</b>	<b>Non-DM</b>	<b>P value</b>
Final clamp glucose (mmol/L)	5.1 ±0.5	4.9 ±0.2	0.30
Final clamp insulin concentration (µIU/mL)	102 ±43	59 ±22	<b>0.03</b>
GIR Stage 1 (mg glucose/kg/min)	0.64 ±0.37	1.7 ±0.6	<b>&lt;0.001</b>
M-value Stage 2 (mg glucose/kg/min)	3.7 ±1.5	9.0 ±2.9	<b>&lt; 0.001</b>
M/I Stage 2 (mg glucose/kg/min/IU/ml) x100	4.1 ±2.1	19.4 ±13.9	<b>0.008</b>

\*Data are presented as mean±SD

**Vascular Compliance:** Compared to participants without T1D, participants with T1D exhibited increased arterial stiffness based on increased HR-adjusted Augmentation

Index (Aix) and faster pulse wave velocity (PWV) despite similar systolic blood pressure (Table 3).

**Table 3:** <sup>TM</sup>Sphygmocor BPAS 1 device applanation tonometry measurement of Aix @ HR 75 bpm and PWV

	<b>T1D</b>	<b>Non-DM</b>	<b>P value</b>
Aix (%)	20.5 ± 9	11.75 ±12	<b>0.03</b>
PWV (m/sec)	9.6 ±2.8	7.2 ±1.3	<b>0.005</b>
Systolic BP (mmHg)	102 ±9	101 ±11	0.93

\*Data are presented as mean±SD

**Cardiac autonomic function:** Participants with T1D exhibited evidence of autonomic dysfunction, specifically sympathetic dominance, based on heart rate variability measurements. Mean tachycardic and bradycardic extremes during deep respiration were higher in T1D participants than in non-DM controls (Table 4). However, the difference in maximum and minimum heart rate with

respiratory variation was not significantly different between participants with and without T1D. In contrast, during Valsalva maneuver participants with T1D had both significantly higher heart rates and decreased tachycardia:bradycardia ratio (Table 4). Heart rates were also faster in T1D participants during rest in both the standing and the supine positions (Table 4).

**Table 4:** Sympathetic and parasympathetic balance measured via heart rate variability (HRV) by standard methods

	<b>T1D</b>	<b>Non-DM</b>	<b>P Value</b>
Mean tachycardia during cycled breathing (bpm)	74 ±6	70 ±6	<b>0.04</b>
Mean bradycardia during cycled breathing (bpm)	61 ±7	53 ±9	<b>0.010</b>
Average difference with cycled breathing	13 ±7	17 ±7	0.20
Standing HR	80 ±11	67 ±11	<b>0.002</b>
Supine HR	66 ±7	56 ±9	<b>0.002</b>
Valsalva Tachycardia (bpm)	93 ±9	87 ±10	0.06
Valsalva bradycardia (bpm)	67 ±9	54 ±7	<b>&lt; 0.001</b>
Valsalva tachy:brady ratio	1.4 ±0.1	1.6 ±0.2	<b>0.002</b>

**Cardiac function:** Participants with T1D again exhibited increased heart rate in addition to diastolic dysfunction based on reduced mitral valve E:A ratio, increased contractility based on fractional shortening, and

preserved overall cardiac output (Table 5). Stroke volume was decreased and ejection fraction increased, though these did not achieve statistical significance.

**Table 5:** Cardiac parameters by echocardiography

	<b>T1D</b>	<b>Non-DM</b>	<b>P-value</b>
Heart rate on echo	69 ± 8	57 ± 11	<b>0.005</b>
MV E:A ratio	1.26 ± 0.35	1.82 ± 0.68	<b>0.02</b>
% fractional shortening	42 ± 11	29 ± 6	<b>0.003</b>
Ejection fraction	72.0 ± 4.6	69.4 ± 5.1	0.07
Stroke volume	68 ± 9	74 ± 7	0.11
Cardiac Output (L/min)	4.7 ± 0.8	5.6 ± 3.8	0.39

**Effects of acipimox:**

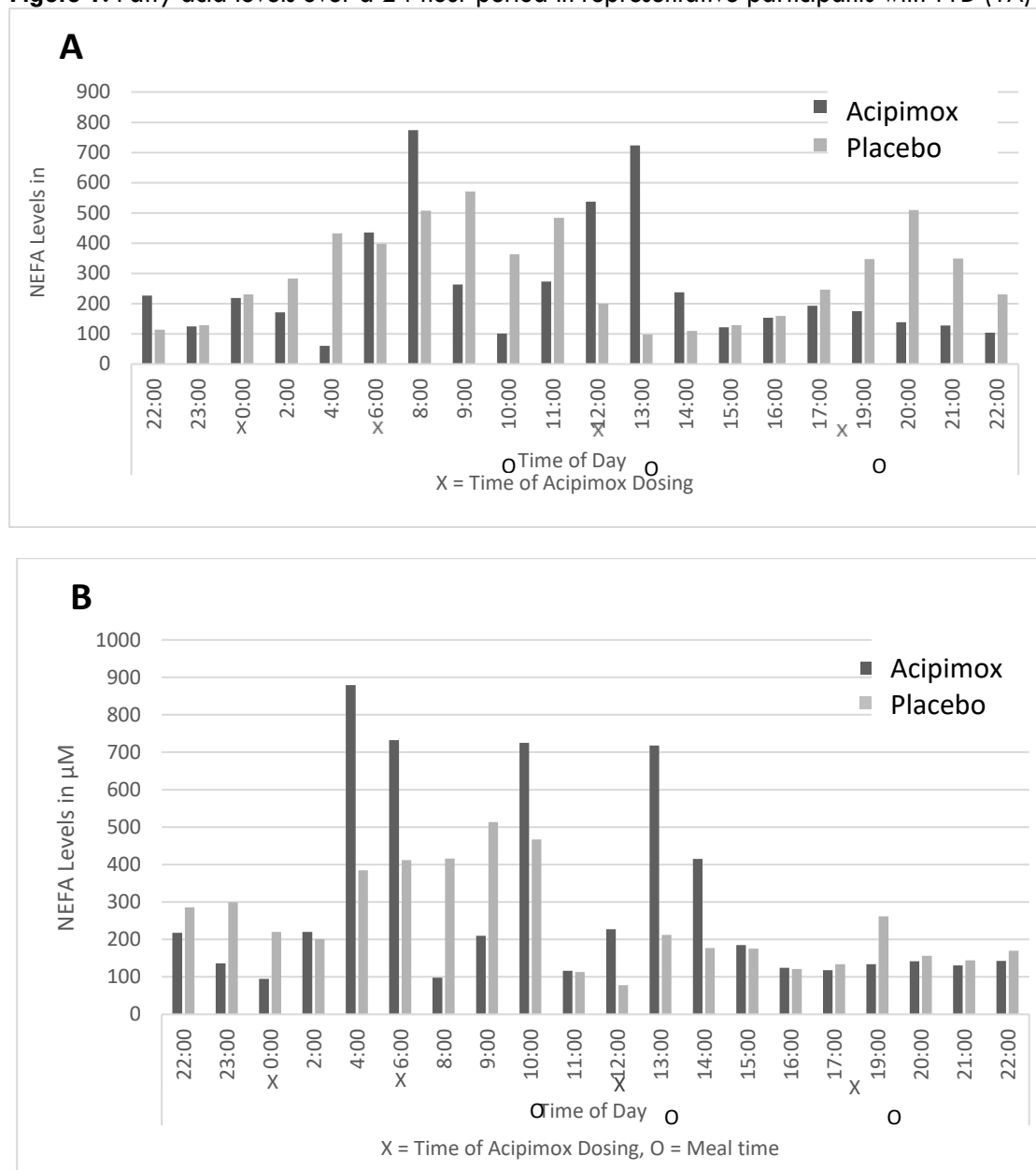
**Non-esterified fatty acid levels:** Unfortunately, acipimox did not significantly lower mean 24-hour NEFA levels in T1D after one week of treatment as expected (343

±122 µM in the acipimox group vs. 297 ±56 µM in the placebo group, p=0.28). Very similar results were seen in the nonDM population. This was due largely to an earlier than expected and profound rebound effect.

Figures 1 and 2 illustrate the 24-hour pattern of NEFA concentrations relative to acipimox dosing and meals/insulin for two representative participants, one with T1D (Figure 1A) and one without T1D (Figure 1B). Fatty acid levels increased significantly prior to and just

after acipimox dosing, particularly overnight and in the morning. There was then a one-to-two-hour lag before NEFA lowering occurred following dosing. Mealtimes and insulin administration further confounded the acipimox effect.

**Figure 1:** Fatty acid levels over a 24-hour period in representative participants with T1D (1A) and without T1D (1B)



**Effect of acipimox on glycaemic control:** Acipimox did not significantly improve glucose control over the seven days of study drug. During continuous glucose monitoring (CGM), there were no significant hypoglycemic or hyperglycemic events prior to inpatient clamp visit (T1D only), and glycaemic control remained well matched between the two treatment phases (Table 6). Participants with T1D did appear to spend more time in the glycaemic

target range during acipimox treatment though this did not achieve statistical significance. They did have significantly lower average preclamp overnight glucose levels. In addition, average total daily insulin dose during the week of treatment and total preclamp overnight insulin infusion appeared lower with acipimox, but also did not reach statistical significance (Table 6).

**Table 6:** CGM as an assessment of glycaemic control in participants with T1D

	Acipimox	Placebo	P Value
Average 7-day glucose (mmol/L)	8.8 ± 1.2	9.1 ± 0.7	0.7
Standard Deviation Glucose	3.64 ± 0.92	3.64 ± 0.66	0.86
Average total daily insulin	62.4 ± 13.5	73.8 ± 15.0	0.06
% Hyperglycemic time	12 ± 8	12 ± 6	0.72
% Hypoglycemic time	3.7 ± 2.8	2.8 ± 4.1	0.86
% Target blood glucose	45 ± 13	37 ± 9	0.23
Average preclamp overnight glucose	7.7 ± 1.2	8.6 ± 1.2	<b>0.02</b>
total preclamp overnight insulin	9.0 ± 4.1	10.4 ± 2.5	0.14

**Preclamp NEFA levels acutely affect insulin sensitivity:**

Despite the overall failure of acipimox to consistently lower NEFA levels, preclamp NEFA levels did vary between the two study phases, especially in participants with T1D. When clamp results were sorted based on high

versus low preclamp serum NEFA levels, M-value was significantly higher (greater insulin sensitivity) when preclamp NEFA levels were lower. This relationship was consistent and statistically significant in T1D, but not in participants without diabetes (Table 7).

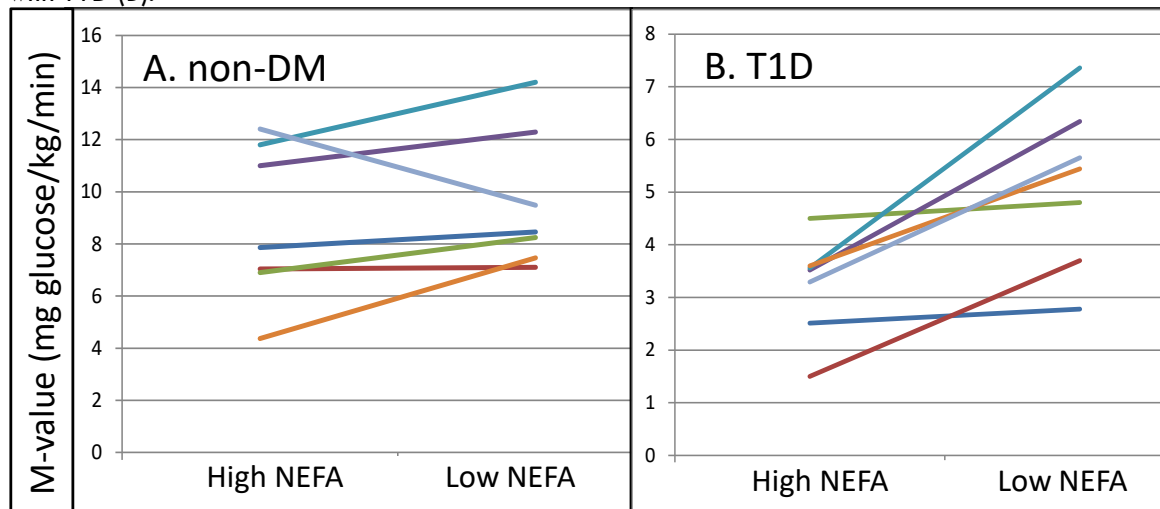
**Table 7:** Correlation of within participant M-value vs preclamp NEFA levels

		NEFA levels in $\mu\text{M}$	M-value Stage II (mg glucose/kg/min)	M/insulin x 100
T1D	High	735 $\pm$ 606	3.2 $\pm$ 1.0	3.7 $\pm$ 1.9
	Low	256 $\pm$ 113	5.2 $\pm$ 1.6	5.6 $\pm$ 2.8
	P value	<b>0.048</b>	<b>0.007</b>	<b>0.021</b>
nonDM	High	427 $\pm$ 174	8.8 $\pm$ 3.0	13.7 $\pm$ 5.4
	Low	237 $\pm$ 152	9.6 $\pm$ 2.7	19.4 $\pm$ 13.3
	p-value	0.05	0.30	0.31

Furthermore, when individual insulin-corrected M-values were against preclamp high vs low NEFA levels, most participants with T1D exhibited significantly greater insulin sensitivity after lower preclamp NEFA levels

(Figure 2). A similar trend, but much lower magnitude was seen in the non-DM participants where the difference in preclamp NEFA levels was smaller.

**Figure 2:** Hyperinsulinemic euglycemic clamp M-value versus high/low NEFA levels for participants without T1D (A) and with T1D (B).



**Preclamp NEFA levels acutely affect cardiac autonomic input, but not vascular stiffness:** higher pretest NEFA levels also correlated with increased mean tachycardic heart

rates, with a trend towards an increase in mean bradycardic heart rates, but no statistically significant effect on Alx @ HR 75 bpm or PWV (Table 8).

**Table 8:** Correlation of Mean Tachycardia and Mean Bradycardia Ratios with NEFA levels

	Pretest NEFA levels ( $\mu\text{M}$ )	Mean Tachycardia (bpm)	Mean Bradycardia (bpm)	Mean Alx	Mean PWV
High	494 $\pm$ 151	73 $\pm$ 7	59 $\pm$ 9	18.8 $\pm$ 10.4	10.1 $\pm$ 3.2
Low	177 $\pm$ 119	70 $\pm$ 6	57 $\pm$ 8	21.0 $\pm$ 19.0	9.7 $\pm$ 2.8
P value	< 0.001	0.02	0.08	0.39	0.75

**Study termination:** The study was terminated slightly prematurely (after n=9 T1D participants) due to an unexpected serious adverse event. One participant had recurrent mild hypoglycemia (50-70 mg/dL) during the overnight stay prior to the hyperinsulinemic euglycemia clamp. This was corrected appropriately but recurred in the AM around the time of his preclamp acipimox dose. He was again provided with glucose to correct the hypoglycemia. Over the next hour he had recurrent vasovagal symptoms and became increasingly bradycardic and hypotensive. He denied chest pain or shortness of breath. He was placed in fully recumbent position with legs slightly elevated and the saline infusion was opened fully. Repeat BP was 90s/50s. Soon after he again became nauseated and presyncopal, and now

noted some chest pain. BP was now 60s/40s, and the medical response team alert was called. EKG showed a RBBB and some T-wave inversions, and heart rate dropped as low as the 30s. He demonstrated some seizure activity, and a short period of unresponsiveness, and a code blue was called. He was given a dose of atropine, started on a dopamine drip, and his blood pressure and clinical picture stabilized, though his heart rate remained in the 50s. Emergency unblinding revealed that he was on the active study drug, not placebo. He was moved to the cardiac ICU and then to the cath lab where angiography showed no occlusive disease. Prior to going to the cath lab the participant was stable and comfortable with a heart rate in the 80s and blood pressure of 104/68 on dopamine at 5 mcg/hr. Peak

symptoms occurred during the time when the NEFA lowering from the acipimox was presumably at its peak and following a period of mild hypoglycemia potentially leaving the myocardium with inadequate metabolic fuel. Therefore, due to concern that this event was not just a prolonged vasovagal event, the study was terminated.

## Discussion:

Our results again demonstrate that, despite similar baseline characteristics and increased adiponectin serum concentrations, participants with T1D are more insulin resistant than well matched non-DM controls. They also exhibit markers of increased arterial stiffness, diastolic dysfunction, and reduced heart rate variability indicative of autonomic dysfunction, all of which are known risk factors for cardiovascular disease and/or heart failure. These findings strengthen and confirm previous observations of increased insulin resistance in T1D, seen in the CACTI cohort<sup>10</sup> and further highlight the importance of examining mechanisms of insulin resistance within this population. Insulin resistance may represent a novel CVD risk-modifying therapeutic target in this high-risk population.

Studies in type 2 diabetes and other insulin resistant populations have demonstrated that even acute NEFA lowering with acipimox improves insulin sensitivity.<sup>20,33</sup> Our original objective was to use acipimox to suppress lipolysis and explore the effects of NEFA lowering in T1D. However, due to an early and pronounced rebound effect, acipimox did not stably reduce NEFA concentrations. This observation is consistent with the previously described rebound effect of acipimox.<sup>34</sup> However, at one week, this effect occurred earlier and much more rapidly in our study than has previously been reported. Therefore, we are unable to conclude that the participants' NEFA levels decreased over the course of the acipimox treatment intervention. Despite this, observed trends from the final week CGM data and from preclamp overnight glucose and insulin values suggest, but do not prove, improved insulin sensitivity with acipimox vs placebo in the T1D participants. Finally, there were no significant differences between acipimox and placebo treatment with regards to the surrogate cardiovascular risk measures.

Despite this, the study design did provide two parallel study visits in each subject. Since NEFA levels were quite different at the two visits, we were able to examine the within-subject relationship between NEFA level and insulin resistance and cardiovascular measures. When analyzed based on pretest NEFA levels, we were able to demonstrate an inverse correlation between elevated preclamp NEFA levels and insulin sensitivity and a positive correlation between elevated pretest NEFA levels and measures of sympathetic dominance in vascular autonomic tone. These findings indicate that NEFA lowering may improve insulin resistance and potentially CVD risk in T1D. However, the precise mechanism by which higher NEFA levels lead to insulin resistance and the possible effects on sustained NEFA reduction on surrogates of cardiovascular dysfunction in T1D remain unknown. More recent studies suggest that certain proinflammatory NEFA may be responsible for this (reviewed in Diaz Ludovico et al 2023).<sup>35</sup>

To date, no durable pharmacologic method of NEFA lowering exists. As previously described and further confirmed here, acipimox, does not represent a clinically viable NEFA-lowering intervention. Diet, lifestyle interventions, and exercise are interventions which are known to improve insulin resistance, but are difficult to sustain lifelong. Thiazolidinediones (TZDs) have been shown to lower NEFA levels, but have not been extensively studied in T1D, especially in terms of cardiovascular effects.<sup>36</sup> In addition, the pleiotropic effects of TZDs and recent cardiovascular studies in Type 2 diabetes have raised concerns regarding their side effect profiles, including cardiovascular side effects. A selective, partial agonist of the A1 adenosine receptor may represent an effective therapy for participants with T2D and dyslipidemia via free fatty acid lowering which is more sustained than acipimox.<sup>37</sup> However, more recent work has suggested that deletion of this receptor improves glucose control and insulin sensitivity in a rodent model, suggesting a more complex interaction there as well.<sup>38</sup> Other possibilities being pursued in the literature include more selective efforts to decrease levels of particular proinflammatory fatty acids rather than universally lowering NEFA levels.<sup>39,40</sup>

Finally, our serious adverse event raises the possibility that lowering NEFA levels may increase the potential harm from hypoglycemia. Our adverse event may have been partly a vasovagal response, but the prolonged hypotension and bradycardia argue against this as the only cause. Fatty acids are a preferred substrate for myocardial metabolism, and lowering both glucose and NEFA has the potential to lead to bradycardia and impaired contractility.

This study had several significant limitations. First was the failure of the study drug to have its intended effect on NEFA levels. However, as described above, the study still provided valuable insights into the effects of NEFA levels on insulin sensitivity and autonomic function. Second, our cohort with T1D was older than our cohort without diabetes. This may have confounded our comparison of participants with and without T1D, contributing to the defects in insulin sensitivity and vascular compliance in our T1D cohort. However, other differences between the cohorts (higher statin use and higher adiponectin in the T1D cohort) would be expected to have the opposite effects on insulin sensitivity and cardiovascular age and may have balanced out the effect of age. Finally, the study was small in size and may have been underpowered to detect effects of NEFA on several of our outcomes.

## Conclusion:

Insulin resistance is a clinically under recognized factor and possible contributor to increased CVD risk in participants with T1D. These results confirm and expand upon the presence of insulin resistance in individuals with T1D which was seen in the previously reported NIH-CACTI study. Although elevated NEFA levels have been shown to impair insulin action and vascular function and compliance in persons with T2D, the role of NEFA elevation and effects of NEFA lowering in individuals with T1D is not understood. Our results suggest that NEFA lowering could have insulin sensitizing and cardiovascular

benefit in T1D. Current treatments which acutely lower NEFA concentrations are available, but none that induce sustained lowering of NEFA levels are clinically available. In the future, a more durable or targeted method of NEFA lowering could improve insulin resistance and impact cardiovascular outcomes in participants with T1D.

**Conflict of Interest:** None of the authors have any conflicts of interest to declare.

**Funding statement:** Funding sources for this study include the American Diabetes Association (ADA 7-11-JF-

42) and the Eastern Colorado Geriatric Research, Education, and Clinical Center.

### **Acknowledgments:**

Author contributions: K.K. performed the analysis and wrote the manuscript, E.L recruited and scheduled participants, ran study visits, and provided data, I.E.S. designed the study, obtained funding, obtained IRB and FDA approvals, provided data, and extensively edited the manuscript.



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