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Oral Azelaic Acid Ester Decreases Markers of Insulin Resistance in Overweight Human Male Subjects

ROBERT T. STREEPER[1*], CHRISTOPHER LOUDEN[2] and ELZBIETA IZBICKA[1*]

[1]*New Frontier Labs L.L.C., San Antonio, TX, U.S.A.;*
[2]*Louden Consulting, Bandera, TX, U.S.A.*

Abstract. *Background/Aim: Insulin resistance (IR) is linked to increased risk of cardiovascular disease and cancer. We examined safety and efficacy of the natural product diethyl azelate (DEA) in overweight males with a varying degree of IR. Patients and Methods: Seventeen subjects [age 18-42, hemoglobin A1c (A1c) of 5.2-6.2%] received orally 1 mg/kg DEA daily for 21 days. Blood plasma glucose, insulin and lipid levels were assessed before and after treatment. Results: DEA was well tolerated without hypoglycemia or adverse effects except transient diarrhea (n=1). DEA significantly reduced fasting glucose by 6.06 mg/dl (n=8) and insulin by 37.8% (n=8) in subjects with IR and/or A1c ≥5.6%. Furthermore, it improved cholesterol/HDL, LDL/HDL, and non-cholesterol HDL/HDL by 5.4, 6.5, and 6.6%, respectively in all subjects, and by 8.0, 9.8, and 9.8%, respectively in 9 subjects with A1c ≥5.6%. Conclusion: DEA efficacy correlates with the degree of IR. DEA holds promise as a novel treatment for the management of IR.*

Azelaic acid and its esters, azelates, occur naturally in plants, animals, and humans. We discovered that the naturally occurring fatty acid ester, diethyl azelate (DEA) (1), can be used for the treatment of diet- and ethanol-induced insulin resistance (IR), the hallmark of metabolic syndrome, prediabetes and Type 2 diabetes (T2D). A number of studies (2-4) have shown a correlation of metabolic diseases with increased risk of cancer, especially liver, pancreatic and endometrial (5-7).

The Western diet combined with a sedentary lifestyle results in chronic metabolic inflammation (8, 9). A diet consisting of ~50% carbohydrates with high levels of fructose has been shown to induce IR in healthy non-obese men within 2-7 days (10). The detrimental health effects of dietary fructose are similar to those of ethanol (11). The diabetogenic effects of ethanol consumption, either acute (12) or chronic (13), strongly correlate with the development of IR in a dose-dependent manner (14, 15).

Current T2D treatments do not reduce the incidence of or cure T2D and have side effects that range from mild to life-threatening, in some cases warranting 'Black Box' warnings mandated by the Food and Drug Administration of the United States of America (US FDA). Therapies available to patients with type T2D after metformin failure have been shown to induce weight gain, cause hypoglycemia or show poor long-term efficacy (16). No T2D drugs address the progressive nature of the disease and the underlying causes of IR. There is a need for agents with prolonged efficacy, superior disease modification power, and improved safety.

DEA and other azelates are metabolic products occurring naturally in humans and other mammals (17, 18). Azelates are also present in grains and grain-derived products including liquors (19), and in fermented foods due to bacterial degradation of acyl glycerol fatty acids and esterification of the resulting medium chain fatty acids (20). Fermentation of olives by Lactobacilli to render them edible has been practiced for at least 6 millennia in the Mediterranean basin (21). The Lactobacilli destroy bitter alkaloids contained in olive fruits, converting them to table olives (22). In addition, the Lactobacilli ferment some of the oleic acid contained in the olives into azelaic acid and azelates. The rind of olives also contains appreciable quantities of azelaic acid. Fermented soybean products, produced by humans for over 3 millennia (23), may help prevent or attenuate the progression of T2D (24). Notably, nonfermented soybean products have no effect on IR (24). Azelaic acid and azelate ethyl esters are present in douchi, a fermented black bean product (25). Although not currently used as drugs, azelates and similar fatty acid esters are used as food additives, lubricants and plasticizers. DEA is approved as a flavoring additive in the European Union (26, 27) and diethylhexyl azelate is approved for food contact

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*These Authors contributed equally to this work.

Correspondence to: Elzbieta Izbicka, New Frontier Labs L.L.C., 9901 IH10 West, suite 800, San Antonio, TX 78230, U.S.A. Tel: +1 2107256868, e-mail: eizbicka.g4@gmail.com

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packaging in the United States. A closely related ester, diethyl sebacate, which differs from DEA in that sebacic acid is one methylene unit longer than azelaic acid, is on the list of generally regarded as safe (GRAS) compounds (28) and the inactive ingredients list (29) of the US FDA.

The purpose of our study was to evaluate the effects of DEA on certain surrogate markers of IR, namely, blood plasma glucose, insulin and lipids (30), when administered orally to overweight or obese adult male volunteers. The cohort spanned from normal to prediabetic subjects based on the levels of the blood marker glycated hemoglobin A1c (A1c), which is considered a longer-term gauge of blood glucose control (31). The American Diabetes Association defines prediabetes as an A1c of 5.7%-6.4%, but also states that patients with an A1c just below the 5.7% threshold are at risk of developing diabetes (32). Our study has demonstrated that DEA can significantly improve the condition of subjects with IR.

Patients and Methods

DEA was synthesized from azelaic acid and ethyl alcohol using the standard acid-catalyzed esterification followed by fractional distillation to 99% purity as determined by gas chromatography-mass spectrometry (GC-MS). The human studies were performed with the approval of the Institutional Review Board at IntegReview (Austin, TX, USA). Consent was obtained from study subjects following the informed consent protocol EP20160001. The Board was constituted and operated in accordance with the ethical rules of the Helsinki Declaration and requirements as described in the US Code of Federal Regulations 21 CFR Part 56.

Seventeen subjects were recruited by sampling a large population at risk for T2D according to convenience sample; a statistical method of drawing representative data (33) to measure the changes in glucose, lipid and insulin measurements after an oral glucose tolerance test (OGTT) after the subjects had been treated for 21 days.

The subjects were overweight to obese males with body mass indices (BMI) ranging from 27.2 to 43.6 kg/m^2, glycated hemoglobin A1c (HbA1c) of 5.0-6.2% and insulin levels of 8.8-52 μU/ml. The study was conducted by Clinical Trials of Texas, Inc. in San Antonio, TX, USA. The cohort represented a population at risk for the development of T2D. The study was restricted to male participants to control for the variability of insulin sensitivity associated with the menstrual cycle (34). The subjects received 21 daily oral doses (q1d) of 1 mg/kg DEA. A fasting OGTT was performed on Day 0 and again on Day 21, and glucose measurements were performed at –30, –5 and 0 min, insulin levels at –30 and 0 min and both glucose and insulin levels at 30, 60, 90, 120 and 180 min. The 180 min time point was selected to gain an early insight into the possible drug action (35). Blood lipid levels (triglycerides, cholesterol, HDL, non-cholesterol HDL and LDL) were measured before the onset of treatment on Day 0 and again on Day 21. The error of the assays was <5% (36).

The results of the various marker measurements at Day 0 and Day 21 were compared using both the paired Students *t*-test and the Wilcoxon signed rank test. The results of both calculations are provided; the *p*-value from the paired Student *t*-test first, followed by the *p*-value from the Wilcoxon signed rank test. Generalized Estimating Equations and bootstrapping were used to verify the results generated with other methods. Fasting glucose was calculated as the average of the –30, –5 and 0 min measurements and fasting insulin was calculated as the average of the –30 and 0 min measurements. Spearman's correlation coefficient was calculated for the relationship between A1c and pre-treatment fasting plasma glucose *versus* post-treatment fasting plasma glucose. Area under the curve (AUC) was calculated over the 180 min time span of the OGTT. All analyses were performed using the open language engine R 3.4.4. Statistical significance was set at the α=0.05 level.

Results

Daily oral DEA was well tolerated by all study subjects; and only one individual experienced transient mild diarrhea in the first week of treatment. No other adverse effects were reported. Specific effects of DEA on examined endpoints are summarized in Table I and presented in detail below.

Glucose: The levels of A1c were measured to assess the effects of oral antidiabetic agents on glucose control with the drug activity becoming apparent within the first 4 to 6 months (37). We did not expect a measurable effect on A1c in this short-term study. Instead, the pre-treatment A1c levels were examined to assess the relative state of IR in the subjects. Then, the cohort was sorted by descending A1c values (Figure 1A), 3 subjects with A1c's of 6.2, 6.1 and 6.0% were classified as prediabetic and 6 subjects with A1c's of 5.6-5.7% as having an increased risk for T2D. This subgroup of 9 subjects with A1c ≥5.6% is referred to as 'high A1c' herein. The remaining 8 subjects with A1c's of 5.0-5.4% having a lower risk for T2D were referred to as 'low A1c'. Stratification by fasting plasma glucose levels (Figure 1B) showed that 9 subjects had ≥100 mg/dl ('high glucose') and 8 subjects were below the threshold of 100 mg/ml ('low glucose').

For measuring the effect of DEA on blood glucose, we relied on assessment of fasting plasma glucose levels, a measure that is commonly used as an indication that a subject may be diabetic. A level under 100 mg/dl is considered clinically normal (38) while the range between 100 and 125 mg/dl is indicative of prediabetes (39). At the threshold of 100 mg/dl the human body begins to have a compromised IR to glucose shock (40). We used the oral glucose tolerance test (OGTT) whereby a standard dose of glucose is ingested by mouth and blood samples are taken afterward for measurements of blood glucose as a means of understanding the pharmacodynamic effects of DEA.

When the entire cohort of 17 subjects was analyzed as a group, post-treatment fasting glucose increased slightly yet insignificantly by 0.11 mg/dl (p=0.962; p=0.96). However, fasting glucose decreased in subjects both in the high glucose and high A1c groups. For those with an HbA1c≥5.6%, the

Table I. *Variables (geometric mean and 95% confidence limits) determined during a 21-day's study of diethylazelate in overweight male subjects.*

Variable	All (n=17)		Low A1c (n=8)		High A1c (n=9)		FPG <100 mg/dl (n=8)		FPG>100 mg/dl (n=9)	
	D0	D21	D0	D21	D0	D21	D0	D21	D0	D21
Fasting plasma glucose (mg/dl)	101.662 (90.113, 113.211)	99.732 (92.462, 107.002)	97.731 (86.515, 108.947)	99.138 (92.916, 105.359)	105.156 (93.863, 116.448)	100.261 (91.825, 108.697)	92.769 (85.484, 100.053)	97.25 (90.903, 103.597)	109.567 (101.143, 117.99)	**101.939**** (94.269, 109.609)
Glucose 180 min (mg/dl)	100.315 (66.998, 133.631)	91.579 (62.923, 120.236)	90.569 (72.025, 109.113)	87.588 (72.575, 102.6)	108.978 (67.269, 150.687)	95.128 (57.51, 132.745)	89.919 (71.388, 108.45)	86.494 (71.181, 101.807)	109.556 (68.139, 150.972)	96.1 (58.839, 133.361)
AUC Glucose	25.826 (20.758, 30.894)	25.356 (20.152, 30.559)	24.153 (21.644, 26.663)	24.756 (23.040, 26.471)	27.313 (20.944, 33.682)	25.889 (18.754, 33.023)	23.53 (21.166, 25.894)	24.475 (19.643, 29.307)	27.867 (21.824, 33.910)	26.138 (20.459, 31.818)
Fasting insulin (µU/ml)	26.082 (10.065, 42.1)	25.894 (3.064, 48.724)	21.212 (12.172, 30.253)	22.512 (10.835, 34.19)	30.411 (10.492, 50.331)	28.9 (–1.125, 58.925)	20.212 (8.336, 32.089)	20.95 (8.242, 33.658)	31.3 (13.283, 49.317)	30.289 (1.05, 59.528)
AUC insulin	24.963 (15.288, 34.637)	26.834 (14.646, 39.021)	22.609 (14.285, 30.932)	27.785 (14.299, 41.271)	27.055 (16.280, 37.830)	25.988 (14.315, 37.660)	22.215 (11.805, 32.625)	27.461 (12.317, 42.605)	27.405 (18.567, 36.243)	26.275 (16.494, 36.056)
Cholesterol, total (mg/dl)	150.118 (104.379, 195.856)	148.882 (101.074, 196.69)	129.5 (86.608, 172.392)	125.5 (72.38, 178.62)	168.444 (126.358, 210.531)	169.667 (136.963, 202.37)	154.875 (98.406, 211.344)	146.125 (81.035, 211.215)	145.889 (109.131, 182.646)	151.333 (122.183, 180.484)
LDL cholesterol (mg/dl)	93.765 (61.047, 126.482)	89.765 (57.062, 122.467)	81.125 (54.698, 107.552)	74.375 (45.045, 103.705)	105 (69.957, 140.043)	103.444 (72.805, 134.084)	101 (62.344, 139.656)	90.75 (49.127, 132.373)	105 (69.957, 140.043)	103.444 (72.805, 134.084)
HDL cholesterol (mg/dl)	32.765 (22.273, 43.256)	34.412 (22.653, 46.17)	27 (16.033, 37.967)	26.75 (13.858, 39.642)	37.889 (30.67, 45.108)	41.222 (36.728, 45.716)	31.25 (17.847, 44.653)	31.5 (14.887, 48.113)	34.111 (26.455, 41.767)	37 (32.641, 41.359)
Non-cholesterol HDL (mg/dl)	117.412 (80.052, 154.772)	114.471 (76.013, 152.929)	102.5 (67.958, 137.042)	98.75 (55.996, 141.504)	130.667 (94.232, 167.102)	128.444 (98.582, 158.307)	111.778 (80.892, 142.664)	114.333 (87.278, 141.389)	123.75 (78.88, 168.62)	114.625 (64.187, 165.063)
Triglycerides (mg/dl)	118.588 (77.926, 159.25)	124 (73.094, 174.906)	106.875 (57.756, 155.994)	122.625 (49.036, 196.214)	129 (98.398, 159.602)	125.222 (104.229, 146.215)	122.889 (89.197, 156.58)	127.667 (87.911, 167.422)	113.75 (64.441, 163.059)	119.875 (55.999, 183.751)
Cholesterol, total/HDL	4.806 (3.68, 5.931)	**4.553*** (3.426, 5.68)	5.2 (3.746, 6.654)	5.062 (3.672, 6.453)	4.456 (3.831, 5.08)	**4.1*** (3.502, 4.698)	4.311 (3.621, 5.001)	4.089 (3.358, 4.819)	5.362 (4.065, 6.66)	5.075 (3.769, 6.381)
LDL/HDL	3.442 (2.481, 4.403)	**3.201*** (2.31, 4.092)	3.744 (2.543, 4.944)	3.582 (2.543, 4.621)	3.173 (2.531, 3.816)	**2.862**** (2.255, 3.47)	3.082 (2.383, 3.78)	2.892 (2.224, 3.559)	3.847 (2.752, 4.942)	3.549 (2.527, 4.571)
LDL/ triglycerides	0.998 (0.562, 1.434)	0.893 (0.619, 1.166)	1.048 (0.471, 1.624)	0.804 (0.556, 1.051)	0.954 (0.664, 1.244)	0.972 (0.687, 1.256)	0.861 (0.669, 1.054)	0.887 (0.612, 1.161)	1.152 (0.568, 1.735)	0.9 (0.608, 1.191)
Non-cholesterol HDL/HDL	3.799 (2.68, 4.917)	**3.549*** (2.434, 4.664)	4.196 (2.758, 5.633)	4.046 (2.666, 5.426)	3.446 (2.816, 4.077)	3.108 (2.512, 3.704)	3.309 (2.624, 3.995)	3.098 (2.376, 3.819)	4.349 (3.058, 5.641)	4.057 (2.757, 5.357)
Triglycerides/ HDL	3.761 (2.399, 5.123)	3.907 (2.227, 5.588)	4.076 (2.299, 5.854)	4.82 (2.816, 6.824)	3.481 (2.611, 4.35)	3.096 (2.35, 3.843)	3.693 (2.618, 4.769)	3.521 (2.135, 4.907)	3.837 (2.133, 5.541)	4.342 (2.38, 6.304)

D0: Day 0, pre-treatment values; D21: Day 21, post-treatment values; AUC: area under the curve; FPG: fasting plasma glucose; LDL: low density lipoprotein; HDL: high density lipoprotein; Values without paretheses: mean; Values in parentheses: 95% confidence intervals. *p<0.05; bold type. **p<0.01; bold type.

average decrease was 4.25 mg/dl (p=0.128; p=0.22). The largest decrease occurred in the 8 subjects with a fasting glucose ≥100 mg/dl in whom the fasting glucose decreased by an average 6.06 mg/dl (p=0.033; p=0.06), see also Figure 2A. The decrease in fasting glucose after treatment was moderately correlated with the pre-treatment A1c (p=–0.551) and strongly correlated with the fasting plasma glucose pre-treatment (p=–0.755) (Figure 2B and 2C).

Modulation of postprandial glucose level is of interest for drug development (41) given that even transient hyperglycemia

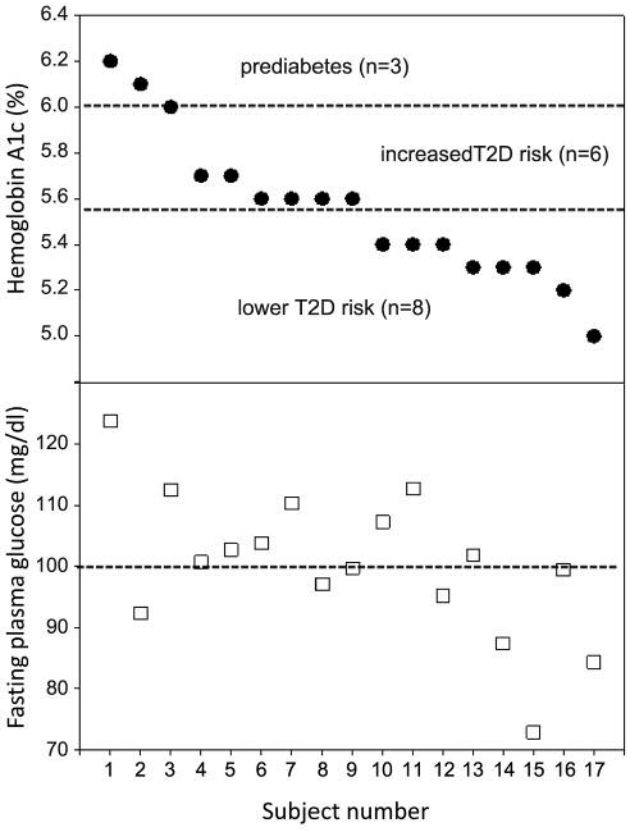

Figure 1. *Stratification of the study cohort of 17 subjects by glucose markers. A; stratification by descending hemoglobin A1c levels, filled circles. B; corresponding fasting plasma glucose levels; open squares.*

has long-term impact on cardiovascular and kidney diseases, neuropathy and retinopathy (42, 43). Figure 3A shows the effects of DEA on glucose at 180 min in the high and low A1c groups. In a subset of 12 subjects, DEA decreased glucose levels at 180 min, compared to the average pre-OGTT glucose levels on Day 21, by 2.4% to 31.5% with an average decrease of 21.7% ($p<0.001$; median decrease 25.3%). For the entire cohort, the average decrease at 180 min was not significant (9.14%; $p=0.136$; 0.057) due to a single outlier (subject #1) who showed a 58.6% increase. This particular subject had an average fasting insulin of 77.45 μU/ml and may have been leptin resistant (44) which may interfere with the putative mechanism of action of DEA (unpublished data). Excluding that subject, the remaining 16 subjects exhibited a decrease in 180 min plasma glucose of 13.5% after treatment ($p=0.002$; 0.003).

The effects of DEA can be appreciated by the analysis of three individual prediabetic cases. As shown in Figure 3B, the glucose disposal profile of subject #1 (A1c 6.2%) increased post-treatment but the fasting and 180 min glucose levels decreased from 123.8 to 116.3 mg/dl and from 200.0 to 184.5 mg/dl, respectively. Subjects #2 (A1c 6.1%) and #3 (A1c 6.0%) experienced improvement in glucose clearance rates at 180 min (from 88.3 to 69 mg/dl and from 146 to 119 mg/dl, respectively).

Insulin: In the prediabetic state and more so in T2D, the body does not respond to insulin properly leading to IR. Subjects with IR show elevated blood glucose and insulin levels. In our study, fasting insulin spanned mostly normal ranges of <25 μU/ml before and after the treatment in the high and low A1c groups (Figure 4A) and the inter-group differences were not significant. An outlier was a single subject (#1) in the high A1c group (Figure 4B) whose pre-treatment average fasting insulin increased post-treatment from 77.45 μU/ml to 96.15 μU/ml. The remaining 16 subjects experienced a decrease of fasting insulin of 13.4% ($p=0.007$; 0.009)

In a subset of 8 subjects (#2-4, 8-11, and 13) from both high and low (≥5.3%) A1c groups, DEA treatment significantly ($p=0.004$, $p=0.008$) decreased mean fasting insulin by 37.8% (a median decrease of 42.5%). The apparent non-responders including the outlier (subject #1) had otherwise either normal pre-treatment levels of fasting insulin, plasma glucose, and/or lipid markers. Considering all 17 subjects, the decrease was 0.7 μU/ml ($p=0.916$; $p=0.963$). In the high fasting plasma glucose group, the decrease was 2.97 μU/ml ($p=0.752$; $p=0.855$) and in the high A1c group, the decrease was 0.84 μU/ml ($p=0.916$; $p=0.963$).

The effects of treatment on individual insulin profiles in 3 prediabetic subjects (Figure 4B) parallel their glucose response (Figure 3B) and suggest that in cases such as subject #1 with advanced prediabetes, the dose and/or duration of the treatment need to be further optimized.

The median insulin AUC decreased by 1663.5 in the high A1c group but increased by 3380.25 in the low A1c group. Neither change was statistically significant. The glucose and insulin responses to DEA were correlated for the entire cohort. Overall, DEA increased the correlation between AUCs for glucose and insulin from 0.229 pre-treatment to 0.523 post-treatment (data not shown).

Lipid panel: When the lipid data were analyzed for the entire cohort, DEA did not exert statistically significant effects on any endpoint considered singly: total cholesterol, LDL, HDL, non-cholesterol HDL, and triglycerides (Table I). However, the pharmacological effects of DEA become noticeable between the high and low A1c groups (Figure 5A-E). Abnormal total cholesterol (>200 mg/dl) in two subjects in the high A1c group decreased or returned to normal levels. The median total cholesterol decreased by 1 mg/dl in the high A1c group but increased by 9 mg/dl in the low A1c group (Figure 5A). LDL showed a decreasing trend toward normal values of <100 mg/dl in the high A1c group but less so in the low A1c group (Figure 5B). HDL and non- cholesterol HDL were within the normal range (>40 mg/dl and <130 mg/dl) in all subjects and were non-significantly affected by the

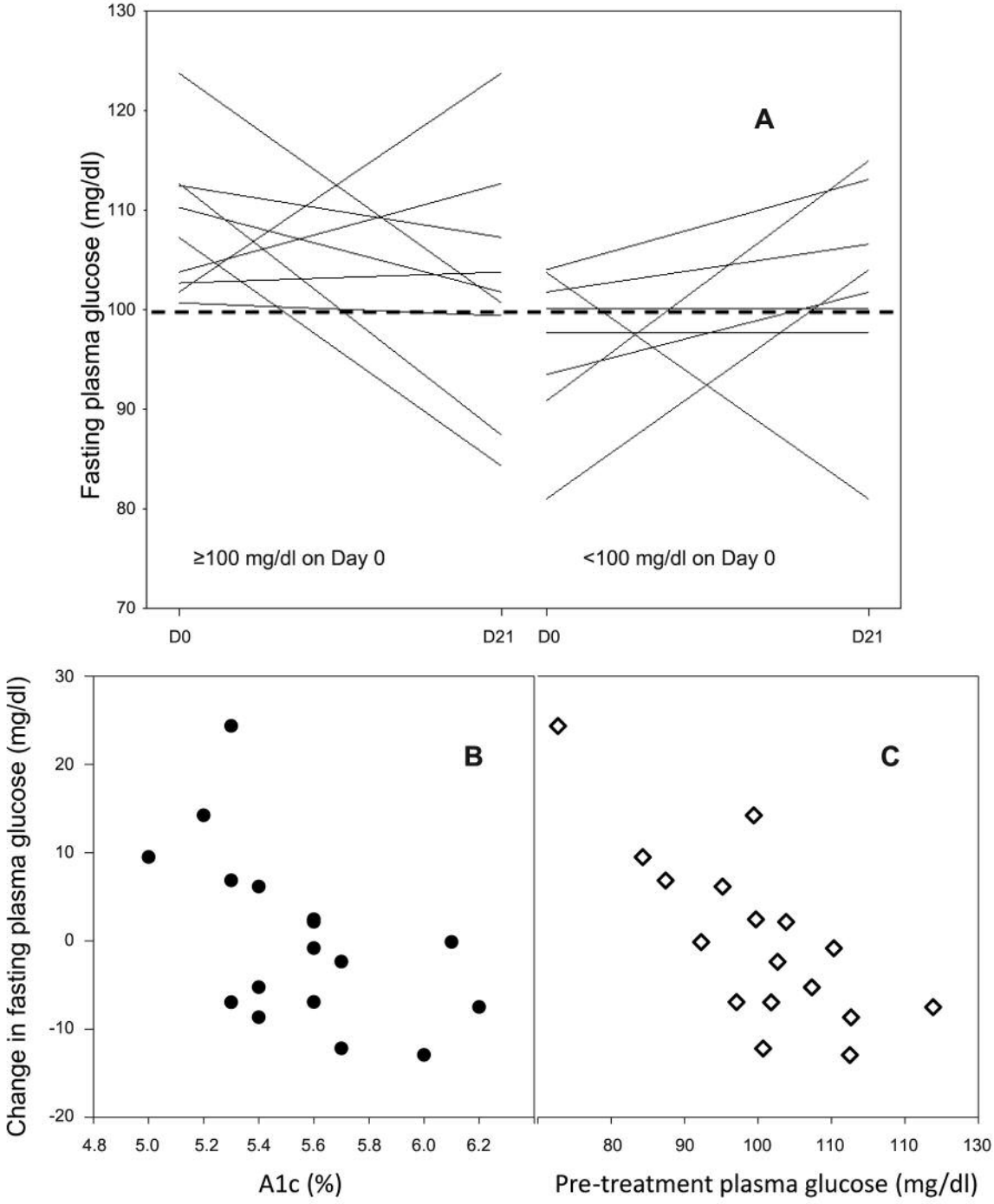

Figure 2. *Effects of DEA on fasting plasma glucose. A; glucose levels in the subgroup of subjects with ≥100 mg/dl and <100 mg/dl pre-treatment. B; correlation of glucose levels after treatment with the fasting plasma glucose pre-treatment and C; with hemoglobin A1c levels.*

treatment (Figure 5C, D). Elevated triglycerides decreased post-treatment to normal levels in 8 subjects including two subjects with abnormal triglycerides of >150 mg before treatment in the high A1c group (Figure 5E).

In contrast, substantial differences were observed in the lipid ratios. While the total cholesterol remained largely unaffected by DEA treatment, the ratio of total cholesterol/HDL decreased significantly by 5.36% (p=0.025;

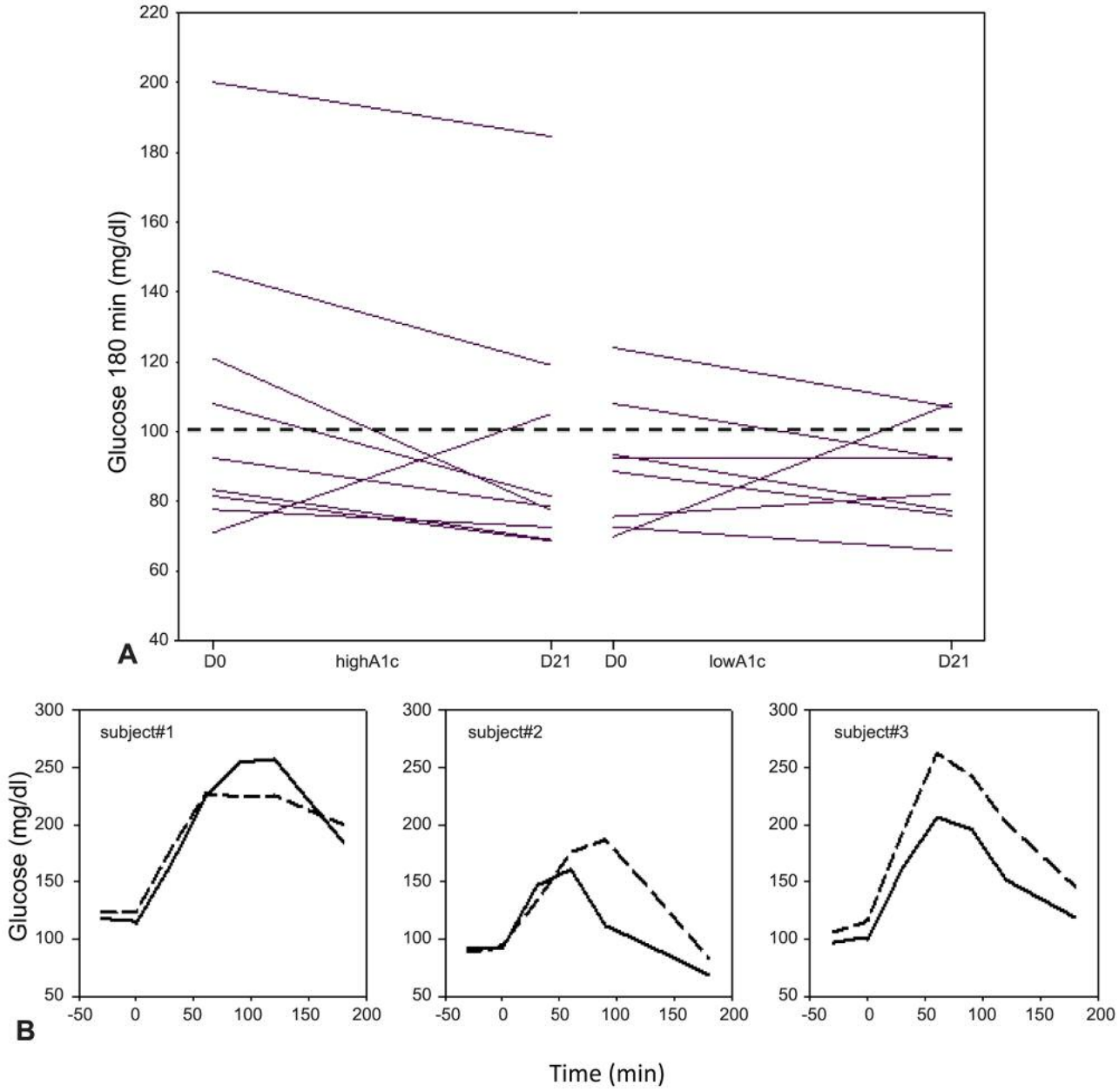

Figure 3. *Effect of DEA on glucose levels in the oral glucose tolerance test (OGTT). A; comparison of the DEA effect at 180 min in the high and low A1c subgroups. A horizontal line at 100 mg/dl marks the border between normal and abnormal ranges for glucose. B; OGTT glucose profiles of 3 prediabetic subjects. Day 0; dashed lines, Day 21; solid lines.*

p=0.041). This decrease was primarily driven by the high A1c group which exhibited a 7.99% decrease (p=0.017; p=0.068); see also Figure 6A. Likewise, LDL/HDL decreased in all 17 subjects by 6.46% (p=0.011; p=0.02). Among the high A1c subjects, this decrease was 9.8% (p=0.008; p=0.02); see also Figure 6B. The ratios of LDL/triglycerides and triglycerides/HDL did not differ significantly between the high and low A1c groups but several individuals experienced clear improvement (Figure 6C, E). Of interest is the effect of treatment on the ratio of triglycerides/HDL, a predictor of cardiovascular disease (39), which increased by 15% in the low A1c (from 3.9 to 4.6 post-treatment) but decreased by 11% (from 3.4 to 3.0) in the high A1c group. A significant improvement was also observed in the non-cholesterol HDL/HDL ratio, a predictor of onset of non-alcoholic fatty liver disease (NAFLD) (45), which decreased by 6.6% in the

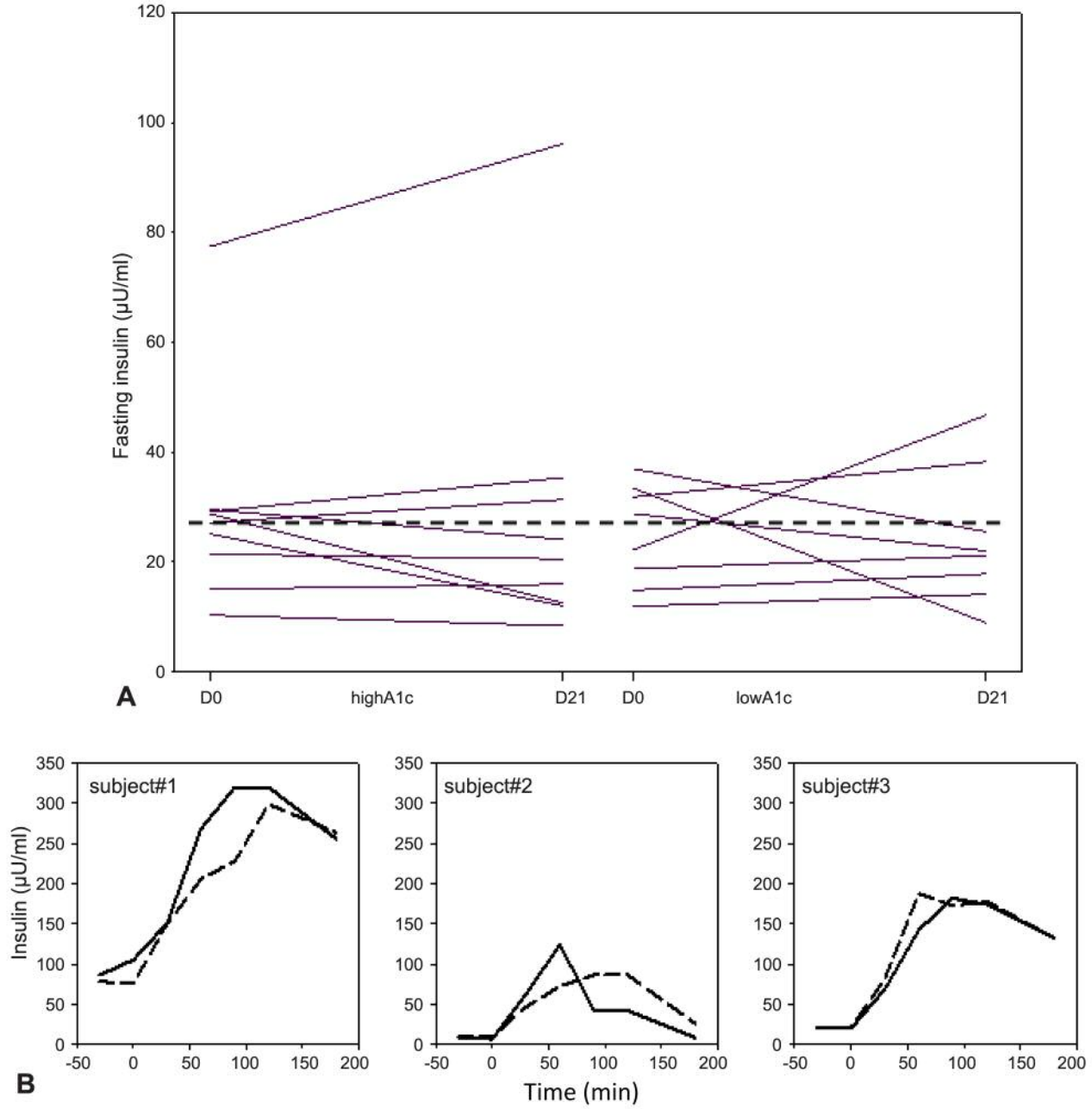

Figure 4. *Correlation of the DEA effect on fasting insulin and the A1c levels. A; fasting insulin in the cohort stratified by A1c levels into the high and low A1c subgroups. B; insulin profiles in 3 prediabetic subjects over 180 min. A horizontal line at 25 µU/ml marks the border between normal and abnormal ranges for insulin. D0; dashed lines, D21; solid lines.*

entire cohort (p=0.025; p=0.057) and by 9.8% in the high A1c group (p=0.025; p=0.074); see also Figure 6D.

Figure 7 illustrates the lipid panel results for the entire cohort and both the low and high A1c groups. Large differences between the A1c subgroups are evident for HDL/LDL, total cholesterol/HDL and triglycerides. Overall, the lipid panel differences between the high and low A1c groups suggest an adaptive response to DEA.

Discussion

Data mining using several statistical analytic methods confirmed the statistical significance of DEA effects on markers of IR; glucose, insulin and lipids. For fasting plasma glucose, the DEA effects were significant in prediabetic subjects and those with elevated risk for T2D (the high A1c subgroup and the high fasting plasma glucose group). The

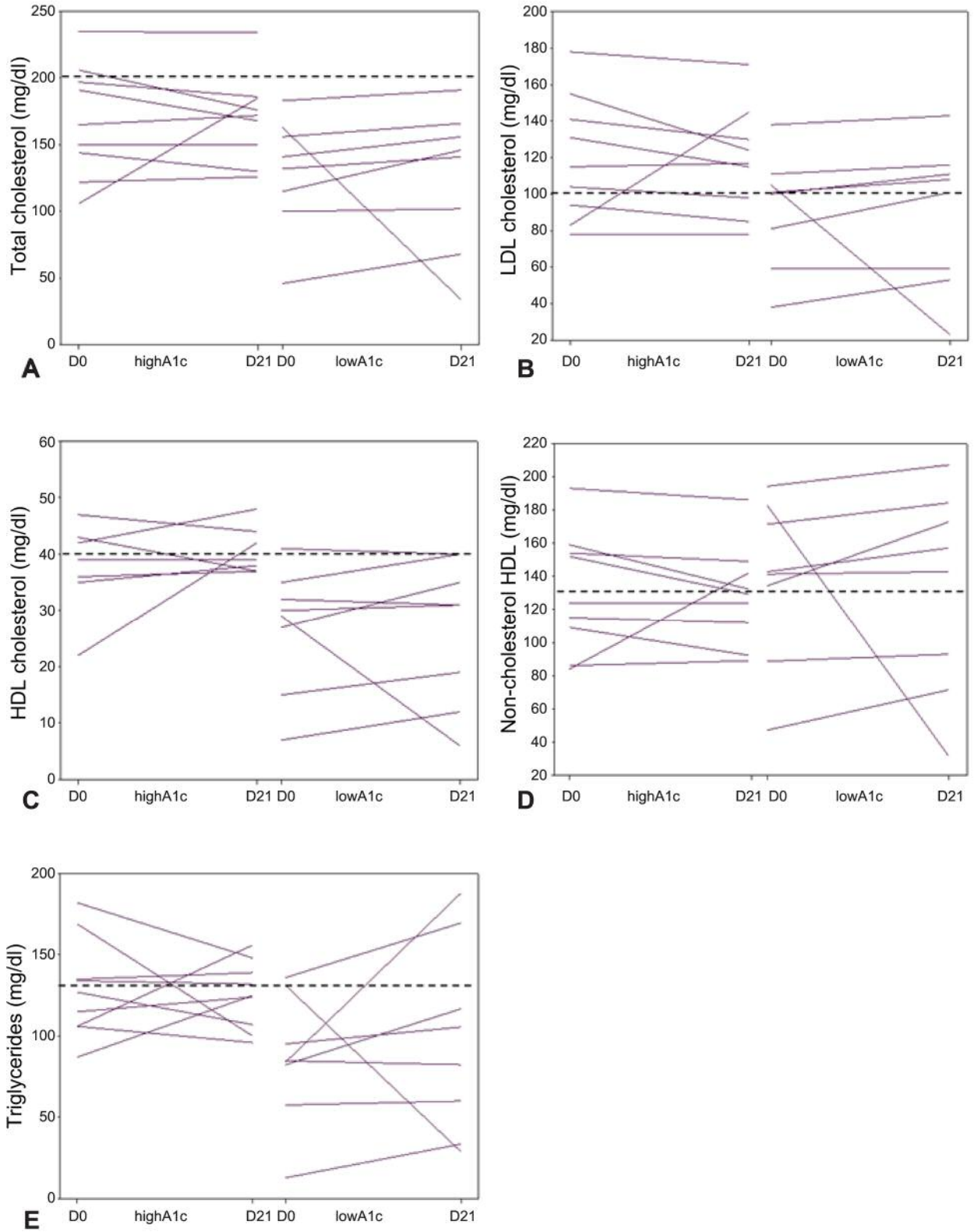

Figure 5. *Effect of DEA on single lipid markers. A; total cholesterol, B; LDL cholesterol, C; HDL cholesterol, D; non-cholesterol HDL, E; triglycerides. In all cases, the cohort was stratified by A1c levels into the high and low A1c subgroups. Horizontal dashed lines mark borders between normal and abnormal ranges for the measured endpoints.*

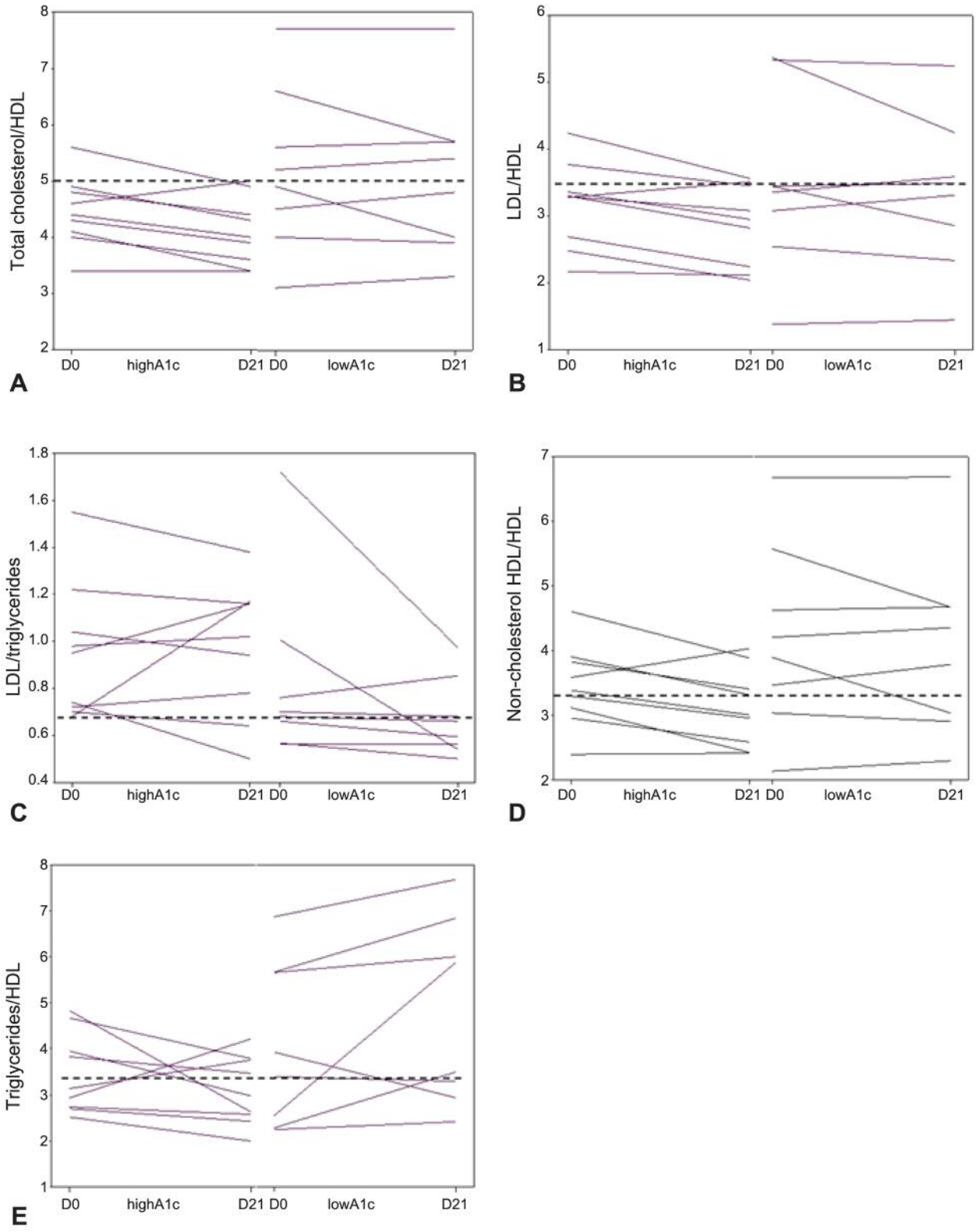

Figure 6. *Effect of DEA on the ratios of lipid markers. A; total cholesterol/HDL, B; LDL/HDL, C; LDL/triglycerides, D; non-cholesterol HDL/HDL, E; triglycerides/HDL. In all cases, the cohort was stratified by A1c levels into the high and low A1c subgroups. Horizontal dashed lines mark borders between normal and abnormal ranges for the measured endpoints.*

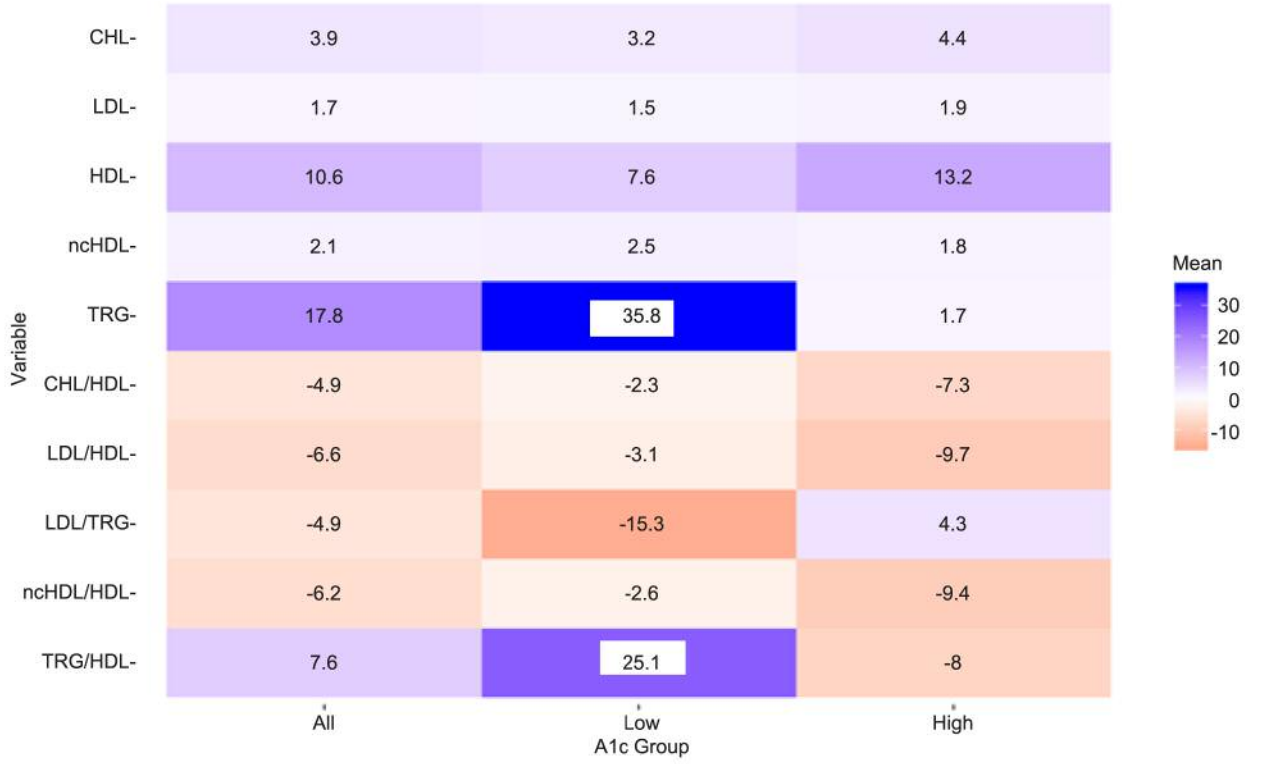

Figure 7. *DEA effects on lipid markers in the entire cohort, the high A1c and low A1c subgroups. Mean percentage changes in the levels of the endpoints are presented in the color scale and the numerical values are shown for all endpoints. The blue range corresponds to increased values post-treatment and the yellow/orange range corresponds to the decreased values.*

apparent non-responders did not have clinical indicators of T2D or prediabetes and as such would not be considered a population needful of antidiabetic therapy. The discordant responses in the test group suggest that normal subjects do not benefit from DEA and that those with signs of IR show improvements in their clinical indices of IR in response to DEA treatment. The individuals with higher IR experienced even greater benefit from DEA treatment. Subjects classified in the range of T2D risk or prediabetes evidenced improvement not only in the plasma glucose but also insulin levels. These results suggest that upon DEA treatment the pancreas functions less hard in producing insulin and is less likely to 'burn out' as seen in late T2D (46).

A review of the literature on metformin revealed many similarities and a possible advantage of DEA over metformin based on our data (Table II). For example, in a 28-day study in 16 subjects with T2D, metformin reduced fasting glucose but had no effect on insulin (47). In a meta-analysis of 4750 prediabetic subjects in randomized trials of at least 8 weeks, metformin reduced fasting glucose (-4.5%), fasting insulin (-14.4%), and LDL (-5.6%) and increased HDL (5.0%) compared to placebo or no treatment (48). In our 21-day study, fasting plasma glucose decreased by 5.9% and fasting insulin decreased by 38%. In a 15-year study, metformin reduced the incidence of diabetes compared to placebo by 17% and the subset that benefited most included subjects with higher baseline plasma glucose or A1c (49). Our data illustrate a similar trend and we have supporting evidence (50) that DEA will be even more effective in more advanced diabetic pathology.

Neither metformin (51) nor DEA induced hypoglycemia. The effects of DEA on the lipid levels were also qualitatively similar to metformin (47). In particular, DEA significantly improved the LDL/HDL ratio and the decrease of 9.8% that was achieved in 3 weeks is comparable with an 11.7% decrease reported after 1 year of treatment with metformin in statin-naïve individuals (52). Oral DEA was well tolerated, while metformin causes severe gastrointestinal side effects in 1 of 4 users and 5% patients cannot tolerate metformin at all (53).

The proposed uses of metformin go far beyond the first line intervention in T2D. Metformin is touted as a promising treatment for obesity by inducing weight loss (54), and as a drug for cardiovascular disease (55), cancer (56), and life extension (57, 58). Our ongoing laboratory experiments suggest the potential application of DEA for these indications.

Table II. *Comparison of diethylazelate and metformin effects on glucose, insulin and lipid markers.*

Variable	DEA	Metformin	Study duration	Reference
Fasting plasma glucose	*5.9% decrease (apparent responders)	4.5% decrease	8 weeks (T2D risk)	48
Hypoglycemia	No effect	Infrequent event	Multiple studies	51
Fasting insulin	*38% decrease (apparent responders)	14.4% decrease	8 weeks (T2D risk)	48
HDL	8.7% increase	5% increase	1 year (T2D)	52
Cholesterol/HDL	*5.4% decrease (all), *8% (high A1c)	9.2% decrease	4 weeks (T2D)	47
LDL	4.3% decrease (all), 2% (high A1c)	5.6% decrease	1 year (T2D)	52
LDL/HDL	*6.5% decrease (all),*9.8% (high A1c)	11.7% decrease	1 year (T2D)	52
Side effects	Mild transient diarrhea (1/17 subjects)	Severe gastrointestinal effects	Multiple studies	53

*Significant effect in this study.

Interestingly, unlike the glucose and insulin effects of DEA in subjects with higher IR, we observed significant improvement in the diagnostic lipid ratios of cholesterol/HDL, LDL/HDL (59) and non-cholesterol HDL/HDL (60) in the entire cohort. These subjects were either overweight or obese and were thus at risk for comorbidities of metabolic syndrome including metaflammation (61), NAFLD and non-alcoholic steatohepatitis (NASH) (62), T2D, cardiovascular diseases, and cancer.

At present there are no approved drugs to treat NAFLD or NASH, and the lipid complications of metabolic syndrome are currently treated with statins (63). We found no overlap in statistically significant endpoints for DEA and statins except for decreased LDL/HDL ratios for DEA (9.8%; our 3-week study) *versus* statins [26.7%; 18-24-month study (64)]. However, statins may increase hyperglycemia and risk for T2D (65) especially on a high carbohydrate diet (66) and their adverse effects include severe muscle condition; rhabdomyolysis, further exacerbated by metabolic syndrome (67). Thus, the population that cannot tolerate statins may benefit from DEA treatment that may lower the risk of progressive diseases initiated and driven by dyslipidemia.

Future clinical studies with DEA will address optimization of both the dose and duration of the treatment for prediabetes/T2D and the efficacy of DEA in combination with other drugs and in the frank T2D population. The durability of the response to DEA and its effects on concomitant diseases-such as subjects with T2D and heart disease or cancer are as yet unknown and need to be examined.

The present study sheds more light on the mechanism of action of DEA. The correlation of the antihyperglycemic and lipid modulating effects of DEA with the degree of IR pathology can be rationalized in terms of the effects of DEA on plasma membrane fluidity, based on our *in vitro* laboratory experiments (data not shown). An increasing body of evidence suggests that even minor changes of membrane structure and composition affect host immune functions, inflammatory signaling and innate immune responses (68-70). Plasma membrane structure can be altered by various diseases (71, 72) but also by the diet. It has been proposed that dietary fats and sugars induce alterations in plasma membranes that result in pathological insulin signaling and diminished tissue glucose uptake associated with T2D (73). A small lipophilic molecule such as DEA may diffuse through the plasma membrane (74, 75), increase membrane fluidity and trigger metabolic changes that translate into health benefits.

Conclusion

DEA induced beneficial changes in metabolic markers of IR that are correlated with disease severity. We propose that these effects are achieved *via* modulation of plasma membrane fluidity using membrane-soluble molecules. Our observations support the development of drugs intentionally designed to modulate membrane physicochemical characteristics as a viable approach for the treatment of diverse human diseases. Given the very long history of oral use of azelates due to their natural occurrence, DEA holds promise as a safe long-term strategy for management of IR and related pathologies.

Conflicts of Interest

EI and RS are the owners and officers of New Frontier Labs L.L.C., the sponsor of the human study described in the manuscript.

Authors' Contributions

EI and RS conceived the project, supervised the clinical study and wrote the manuscript. CL performed statistical analysis of the results and reviewed the manuscript.

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