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Isocaloric Fructose Restriction Reduces Serum D-Lactate

ISOCALORIC FRUCTOSE RESTRICTION REDUCES SERUM D-LACTATE CONCENTRATION IN CHILDREN WITH OBESITY AND METABOLIC SYNDROME

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Objective: To investigate the link between dietary sugar consumption and two separate pathogenetic mechanisms associated with metabolic syndrome: *de novo* lipogenesis (DNL) and non-enzymatic glycation.

Design and participants: We assessed changes in serum D-lactate (the detoxification end-product of methylglyoxal) concentration in response to nine days of isocaloric fructose restriction in 20 children with obesity and metabolic syndrome, and examined correlations with changes in DNL, liver fat, insulin sensitivity and other metrics of hepatic metabolism.

Interventions: Nine days of dietary sugar restriction, with substitution of equal amounts of refined starch.

Main Outcome Measures: On day 0 and 10, children had lab evaluation of D-lactate levels and other analytes, and underwent oral glucose tolerance testing, magnetic resonance spectroscopy to quantify fat depots, and ¹³C-acetate incorporation into triglyceride to measure DNL.

Results: D-lactate was associated with baseline liver fat fraction ($p < 0.001$) and visceral adipose tissue ($p < 0.001$), but not with subcutaneous adipose tissue. At baseline, D-lactate was positively correlated with DNL-AUC ($p = 0.003$), liver fat fraction ($p = 0.02$), triglyceride ($p = 0.004$) and triglyceride to HDL ratio ($p = 0.002$). After nine days of isocaloric fructose restriction, serum D-lactate levels reduced by 50% ($p < 0.0001$), and changes in D-lactate correlated with both changes in DNL-AUC, and measures of insulin sensitivity.

Conclusion: Baseline correlation of D-lactate with DNL and measures of insulin sensitivity; and reduction in D-lactate following nine days of isocaloric fructose restriction suggest that DNL and non-enzymatic glycation are functionally linked via intermediary glycolysis in the pathogenesis

of metabolic syndrome, and points to fructose as a key dietary substrate that drives both pathways.

Nine days of isocaloric fructose restriction in children with obesity and metabolic syndrome reduced D-lactate levels, de novo lipogenesis and liver fat content; while improving insulin sensitivity.

INTRODUCTION

Two separate yet key pathways, mechanistically implicated in the current epidemic of metabolic syndrome, may be linked at the level of an insofar unexplored metabolic nidus: *de novo* lipogenesis leading to hepatic fat accumulation; and glycation leading to carbonyl stress. Intrahepatic fat accumulation uses the common pathway of glycerogenesis to esterify fatty acids that may come from different sources. Increased glycerogenesis implies triose generation from either glycolysis or fructolysis. The same trioses generate methylglyoxal (MG), the most toxic glycating agent known (1), which when increased recapitulates the phenotype of metabolic syndrome in animal models, even in the absence of hyperglycemia. We wondered whether hepatic fat and MG are parallel phenomena and/or are linked to metabolic flux dysfunction in the liver.

Non-alcoholic fatty liver disease (NAFLD) (2) currently exhibits a global prevalence of 24% (3), and is linked with other metabolic comorbidities such as type 2 diabetes, dyslipidemia, and cardiovascular disease. Although NAFLD is commonly associated with obesity, it also occurs in normal weight individuals (4-6), even in children (7).

Intrahepatic fat is in equilibrium between liver fat production and clearance. NAFLD can occur either due to increased liver triglyceride (TG) production from circulating free fatty acids (FFA) (8) or increased hepatic FFA synthesis by *de novo* lipogenesis (DNL) from carbohydrate sources (8-10); or from decreased liver fat clearance (i.e. decreased fatty acid oxidation (9-11) or decreased lipid export in the form of very low-density lipoproteins (VLDL) (9)).

The dietary monosaccharide fructose is a particularly lipogenic substrate through its stimulation of DNL (8). Dietary intake of fructose (sucrose, high-fructose corn syrup) has gradually increased in the past four decades around the world, concomitant with NAFLD prevalence and severity. Fructose not only differs structurally from its isomer glucose, but exhibits preferential hepatic metabolism as well, as hepatic extraction of a fructose bolus (12) is near complete (as compared to 20% for glucose). After conversion to fructose-1-phosphate by the enzyme fructokinase, the hepatic enzyme aldolase B splits the hexose into the trioses glyceraldehyde and dihydroxyacetone-phosphate (DHAP), to be utilized either in glycolysis or triglyceride production (13, 14). The process bypasses the 2 key regulatory steps in glycolysis and is prone to overload the system with the trioses glyceraldehyde-3-phosphate (G3P) and DHAP. DHAP is then converted to the highly reactive α -dicarbonyl compound methylglyoxal (MG) (15, 16). MG is a key precursor of the Maillard reaction (non-enzymatic glycation), which forms various advanced glycation end products (AGEs) and reactive oxygen species (ROS's). Fructose drives this reaction seven times faster than glucose (15, 17); yet MG drives the reaction even faster — 250 times faster than glucose (18).

MG is detoxified by enzymes glyoxalase 1 and 2 (Glo1, Glo2) to D-lactate (in contrast to L-lactate, the end product of glycolysis), which can be measured in peripheral blood, and serves as a surrogate marker of whole-body MG production (19). Previously, we demonstrated that nine days of an isocaloric fructose-restricted diet reduced available substrate for DNL and resultant liver fat in children with obesity and metabolic syndrome who identified as high habitual sugar consumers (20-22). Given the preferential hepatic metabolism of fructose, and despite the

replacement of dietary starch (and therefore glucose) for sugar in the aforementioned study, we hypothesized in the current analysis that isocaloric dietary fructose restriction would decrease MG formation and resultant D-lactate levels in these children and that the change in D-lactate would correlate with the change in DNL and that the D-lactate levels would be associated with other metrics of hepatic metabolism.

MATERIALS and METHODS

Study Design and Population

We recruited African-American and Latino children with obesity and metabolic syndrome who identified as high habitual sugar consumers (>15% sugar, >5% fructose) based on a food frequency questionnaire and an interview by a dietitian (21). Eligibility criteria included age 8-18 years, body mass index z-score >1.8, and at least 1 of the following: systolic blood pressure >95th percentile for age and sex, fasting triglycerides >150 mg/dL, alanine aminotransferase >40 U/L, fasting glucose 100-125 mg/dL, fasting insulin >15 mU/L, homeostatic model assessment of insulin resistance (HOMA-IR) >4.3 (23), or severe acanthosis nigricans as described elsewhere (21). Twenty out of 52 participants recruited to the main study had paired samples available to analyze for this paper.

The study protocol was approved by the Institutional Review Boards of the University of California, San Francisco (approval 10-03473) and Touro University-California (approval M-0609) and is registered with ClinicalTrials.gov (NCT01200043). We obtained informed consent / assent prior to a screening visit. Comprehensive metabolic assessments were performed at baseline (day 0) and following the 9-day dietary intervention (day 10).

Metabolic Assessments

Participating families were instructed to continue their usual daily routine (diet and exercise) prior to baseline study evaluation on day 0. On both day 0 and day 10, participants underwent metabolic studies at the University of California, San Francisco Pediatric Clinical Research Center (PCRC) following a fasting period of at least 8 hours. Weight and vital signs were measured and urine pregnancy testing was performed in female participants at the time of admission to PCRC. Body composition was measured by whole-body dual energy x-ray absorptiometry (GE/Lunar Prodigy, Madison WI). A 2-hour 75-g oral glucose tolerance test (OGTT) was performed, with glucose, insulin, and C-peptide measurements at 0, 30, 60, 90, and 120 minutes. Fasting glucose and insulin and their respective areas under the curve (AUC) were reported elsewhere (21).

Upon completion of the OGTT, postprandial DNL was measured through an 8-hour stable isotope feeding study providing liquid meals containing sodium [1-¹³C]-acetate (Cambridge Isotope Laboratories, Cambridge, MA) every 30 minutes. The liquid meals provided 67% of estimated daily energy requirement with total of 5-7 g acetate tracer, as described elsewhere (22). Glucose polymer was utilized to provide the remainder of carbohydrate. Blood samples were drawn in K₂EDTA tubes before the first test meal and every hour thereafter.

Magnetic Resonance Imaging and Spectroscopy

Participants underwent a magnetic resonance imaging (MRI) exam on a 3-Tesla scanner (GE Healthcare, Waukesha, WI) and liver fat fraction, visceral adipose tissue, and subcutaneous adipose tissue volumes were measured, as described in a previous report (22).

Outpatient Dietary Intervention

Upon their discharge from the PCRC on study day 0, participants were provided 3 days of food with instructions to return at 3-day intervals to pick up fresh food for a total duration of 9 days. On day 10, all day 0 assessments were repeated. As described previously (21), the University of California, San Francisco Clinical Research Service Bionutrition Core designed and provided individualized menus for each child after restricting sugar and fructose intake to 10% and 4% of total energy intake, respectively, by substituting an equal number of calories from refined starch to match overall proportional carbohydrate consumption in each participants' self-reported usual diet (21). Total energy content was estimated using Institute of Medicine formulas for weight maintenance in overweight boys and girls (24) and adjusted if weight changed >2% during outpatient feeding.

De Novo Lipogenesis, Insulin Sensitivity, and D-Lactate

Samples collected on both study days underwent ultracentrifugation to isolate lipid fractions and then fractional DNL measured as described elsewhere (22) to calculate integrated DNL-AUC during both study days. We used insulin and glucose data from the OGTT to compute the composite insulin sensitivity index (CISI) (25).

D-lactate was measured by kinetic spectrophotometric assay, in which D-lactate is specifically oxidized by bacterial D-lactate dehydrogenase, using the D-lactate Colorimetric Assay kit MAK058 from Sigma (St. Louis, MO, USA). Serum samples were ultra-filtered to eliminate interference by the reaction of serum LDH with L-lactate and then incubated at 37 °C, and the reaction was followed kinetically to achieve maximal sensitivity and linearity (26). The <10-kDa fractions were separated by ultrafiltration through 0.5 mL Amicon ultra centrifugal filters spun at 14,000g for 30 min in a refrigerated centrifuge at 4 °C; the ultrafiltrate was employed to measure D-lactate (27). The limit of detection was 1 μmol/L, and the reaction was linear up to 15 μmol/L. To further ensure specificity, we ran the reaction with and without 1 mmol/L L-lactate (upper limit of reference range in serum) and found <5% interference ($p < 0.05$), in agreement with data on the literature (26, 28). The intraassay coefficient of variation was 5% at 2 μmol/L and 3% at 10 μmol/L.

Statistical Analyses

Normal distribution was tested by box-plot, q-norm plot, and Shapiro-Wilk tests. Descriptive statistics are reported as mean \pm SD for normally distributed values and as median (first quartile, third quartile) for non-normally distributed data. Clinical, anthropometric, and biochemical characteristics of participants with and without paired baseline fasting serum sample, and outcome variables on day 0 and day 10, were compared by paired t-test if distributed normally, or by Mann-Whitney U-test for non-normally distributed data. Generalized estimating equation (GEE) models were created to assess the association between baseline measurements (liver fat fraction, visceral adipose tissue, subcutaneous adipose tissue, DNL-AUC, triglyceride, triglyceride to HDL ratio, CISI and HOMA-IR) and D-lactate on day 0 controlling for gender, ethnicity and weight at baseline. Time interactions were included with the GEE model to analyze whether baseline predictors were associated with change over time. Analyses were performed using STATA software, version 15 (StataCorp, College Station, TX). P-values are based on 2-tailed tests. A p-value of 0.1 was used for statistical significance for interaction terms, otherwise 0.05 was used for statistical significance. Investigators remained blinded to key study outcomes until data collection and analysis were completed. All authors had access to the study data, and reviewed and approved the final manuscript.

RESULTS

As reported previously (21), out of 52 Latino and African-American children that were recruited, 41 completed both study days. Of those 41 participants, 20 had adequate paired fasting serum samples to perform D-lactate analysis; their clinical, anthropometric, and biochemical characteristics at baseline were not significantly different from those who did not have adequate paired serum samples for D-lactate analysis (**Table 1**). The subgroup for D-lactate analysis consisted of 15 female and 5 male; 8 African-American and 12 Latino participants. The median age was 14.5 years (range 12.6 - 15.8 years), median body mass index z-score was 2.4 (range 2.2 - 2.6) and mean total body fat was $46.7\% \pm 5.0\%$. They were all pubertal except for one Latina female participant. Fifteen of the twenty participants lost weight (1.1 ± 1.0 kg) during the dietary intervention. Clinical, anthropometric and biochemical characteristics of participants with serum samples for D-lactate analysis are summarized in **Table 2**.

Changes in serum D-lactate level are shown in **Figure 1**. Serum D-lactate level on day 10 (3.1 ± 1.1 μM) was significantly lower than serum D-lactate levels on day 0 (6.2 ± 2.1 μM) ($p < 0.0001$). D-lactate levels decreased in 19 of 20 participants. D-lactate levels reduced by 50% from Day 0 to Day 10.

On day 0, D-lactate was positively correlated with DNL-AUC (Pearson r : 0.62; $p = 0.003$), liver fat fraction (Spearman Rho : 0.52; $p = 0.02$), triglyceride (Spearman Rho : 0.65; $p = 0.004$) and triglyceride to HDL ratio (Spearman Rho : 0.64; $p = 0.002$) (**Figure 2a-d**). Percent change in D-lactate level (Day 10 - Day 0 / Day 0) was positively correlated with percent change in DNL-AUC (Spearman Rho : 0.59; $p = 0.007$) and change triglyceride level (Pearson r : 0.48; $p = 0.04$) (**Figure 2e-f**).

In the GEE model after controlling for weight at baseline, gender and ethnicity, serum D-lactate level at baseline (day 0) was associated with baseline liver fat fraction (β : 1.2; $p < 0.001$; 95% CI 0.64, 1.77) and visceral adipose tissue (β : -0.003; $p < 0.001$ 95% CI -0.004, -0.001), but not with subcutaneous adipose tissue (β : -0.001; $p = 0.14$; 95% CI -0.001, 0.002). DNL-AUC, CISI and HOMA-IR were also significantly associated with serum D-lactate levels at baseline in GEE models controlling for weight (data not shown).

DISCUSSION

Unlike L-lactate (the end-product of anaerobic glycolysis), D-lactate is the end-product of the detoxification of MG, an obligate glycolytic intermediate that may have its own detrimental effects on metabolic pathways (15). We hypothesized that excessive dietary fructose consumption, through insulin-independent and unregulated hepatic glycolysis, would drive excess production of the trioses G3P and DHAP, which would both provide excess substrate for DNL and of necessity drive MG production through the law of mass action. Therefore, we were interested in whether isocaloric fructose restriction would reduce D-lactate production and consequently correlate with a reduction in DNL, and whether these reductions would correlate with markers of hepatic metabolism.

In this paper, we attempt to address the mechanisms by which dietary fructose and its restriction alters hepatic metabolism and expression of surrogate markers for these diseases, exclusive of calories or obesity. As previously reported, isocaloric fructose restriction resulted in improvements in many aspects of metabolic health in these children including insulin sensitivity/secretion, dyslipidemia, diastolic blood pressure as well as decreased DNL and reduction in hepatic fat (20-22). We now demonstrate that serum D-lactate levels reduced by 50%, despite the fact that these children received more polymerized glucose (as refined starch) in their diets during the 9-day study. As depicted in **Figure 3**, as the liver possesses GLUT2 which

mediates hepatic glucose and fructose transport (29), this finding would suggest a reduction of hepatic fructose burden. Consistent with this premise, we found correlations between baseline serum D-lactate, DNL, liver fat fraction, and serum triglycerides. It would appear that isocaloric fructose restriction reduced triose availability, thereby reducing MG production (**Fig 3**, blue), glycerogenesis to esterify fatty acids, and possibly acetyl-CoA for DNL (**Fig 3**, red). Consistent with this, we found that the reduction in serum D-lactate correlated with reduction in DNL and reduction in serum triglycerides over the 9 days of isocaloric fructose restriction.

The role of MG in the pathogenesis of metabolic syndrome has been recently postulated (26). MG is a particularly reactive intermediate because of its α -dicarbonyl moiety. The reactivity of MG in the Maillard reaction is 250 times greater than that of glucose, and 35 times greater than of fructose; thus rendering it the primary substrate for advanced glycation end-product formation (19). Indeed, MG reacts with amino-groups in lysine and arginine. LCMS/MS proteomics shows that methylglyoxal hydroimidazolone-1 (MGH-1) is the major intracellular Maillard adduct (19), which may also possess its own toxic properties (15). MG is normally detoxified by the enzymes Glyoxalase1 and 2 (Glo1, Glo2) into D-lactate using glutathione as a reducing agent (15). This phenomenon has been demonstrated in primary hepatocyte culture, in which fructose becomes toxic in the absence of glutathione(30); and in the methionine-choline deficient rat (31). Furthermore, a knockout model of Glo1 in *Drosophila* resulted in elevated levels of MG, which recapitulated the metabolic disturbances of type 2 diabetes; i.e. insulin resistance and hyperinsulinemia, with eventual obesity and hyperglycemia (1). This raises the question of whether elevated MG formation might be involved in the pathogenesis of type 2 diabetes in humans.

Masania et al. reported higher fasting serum levels of MG and D-lactate in subjects with obesity compared to non-obese adults following an isocaloric diet for 2 weeks (26). These findings are in line with the current literature suggestive of plasma D-lactate level as a surrogate marker for MG flux, which is increased in obesity (26, 32-34). Furthermore, in a cross-sectional study, we previously demonstrated that serum D-lactate levels were higher in obese Latino adolescents and correlated with serum triglycerides and small dense LDL levels (27). Therefore, the notion that MG might be a toxic intermediate that precipitates metabolic disease in humans needed to be explored in an intervention study.

Most studies of the Maillard reaction have addressed the role of glucose as a glyating agent and substrate for MG generation. However, as dietary sugar is now a component of virtually all processed foods (32), we wished to evaluate whether fructose, because of its increased lipogenic potential and as a more potent stimulus for MG production, could generate more metabolic perturbation than does glucose. In the current study, we restricted dietary sugar consumption from 28% to 10% of total calories, while substituting equal amounts of refined starch in its place, for 9 days (21). Not only did we observe a reduction in hepatic DNL and serum TG levels, we also observed a reduction in liver fat and improvement in insulin kinetics (22). If glucose were an equivalent substrate to fructose for either DNL or MG production, we would not have expected either DNL or serum D-lactate levels to have changed significantly. Our data, as summarized in **Figure 3**, suggest that fructose is a unique hepatic precursor which drives triose flux (in green) for both DNL (in red) and MG synthesis (in blue), both of which are linked to the pathogenesis of metabolic syndrome as described earlier.

Therefore, we postulate that fructose plays a key role in obesity and metabolic syndrome by increasing hepatic MG production (and thus increasing its detoxified metabolite D-lactate) independent of its caloric equivalence. One mechanism by which increased hepatic MG flux

may contribute to fructose-induced oxidative stress stems from its own catabolism. The detoxification enzyme Glo1 requires the antioxidant glutathione in its reduced state to provide the sulfhydryl group necessary for the hydration of MG to form D-lactate (**Figure 3**).

Unfortunately, the pool of this antioxidant is limited and made worse by a processed food diet high in sucrose (31). Small molecule inducers of Glo1 expression have been developed as possible treatments for metabolic syndrome. More research will be needed to ascertain and substantiate these cellular mechanisms. If confirmed, perhaps Glo1 inducers will serve as adjuncts to fructose restriction in treating the current NAFLD and metabolic syndrome epidemics.

We acknowledge some limitations in this study. D-lactate is a marker of whole-body MG metabolism. Because fructose metabolism is 90% hepatic, these changes in D-lactate mirror other changes in liver metabolism. However, D-lactate also may be produced from intestinal microbiota, and fructose may change the flora (35). Thus, changes in D-lactate levels after fructose restriction may not solely reflect liver metabolism; however, it would be difficult to explain the correlations with DNL, liver fat, and triglycerides if serum D-lactate was coming from the gut. We did not measure MG and MG-H1 levels in plasma, because they are poor reflections of liver MG and MG-H1, respectively (26). In addition, Masania et al. showed no difference in serum MG-H1 levels in obese and normal weight subjects (26). We were not able to analyze the impact of ethnicity in our participants given the small number of participants. Lastly, as discussed previously (21), despite attempts to maintain each individual's weight throughout the 10-day study interval, an average decline of 1.1 kg was noted. One potential concern is that the weight loss was a manifestation of unintended caloric deficit, and that weight loss alone resulted in metabolic improvement. However, the temporal pattern of weight change in the first 4 days with subsequent weight stability argues against persistent caloric deficit (21), and it is unlikely such a small reduction (especially with increased dietary glucose consumption) would improve D-lactate levels, or account for the correlation between D-lactate, DNL, and insulin sensitivity.

In conclusion, this study demonstrates that serum D-lactate concentrations correlate with DNL, liver fat, and measures of insulin sensitivity in obese adolescents, suggesting a link between MG flux and hepatic perturbations associated with metabolic syndrome. Secondly, isocaloric fructose restriction reduced D-lactate levels in spite of a concomitant increase in glucose consumption during the intervention, pointing to fructose as a “neglected” generator of hepatic MG. Lastly, the changes in D-lactate produced by the intervention strongly correlated with reduction of DNL (proximally) and of serum TG (distally), suggesting a mechanistic link between the two processes. These findings support the importance of both public health and pharmacologic interventions to reduce fructose consumption in order to curb the metabolic syndrome epidemic.

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Authors have nothing to disclose.

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Figure 1: Changes in individual serum D-lactate levels in obese children before and after nine days of isocaloric fructose restriction with average changes (mean \pm SEM) (n=20). * p < 0.0001.

Figure 2: Correlations between serum D-lactate level and DNL–AUC (a), liver fat fraction (b), triglyceride (c), and triglyceride/HDL ratio (d) at baseline on day 0, percent change in serum D-lactate level and change in DNL – AUC (e) and change in serum triglyceride (f) from day 0 to day 10 (n=20).

Figure 3: Hepatic fructose metabolism and its effects on DNL (**red**) and methylglyoxal production (**blue**). Trioses are a metabolic crossroad that link both pathways (**green**). After absorption from the gut and transport to the liver via the portal vein, fructose is quickly phosphorylated by fructokinase-C (1), bypassing regulatory steps in glycolysis and increasing the flux of both trioses (2) and fatty acids (FA) (3). These are turned into fat through *de novo* lipogenesis (4). The process impairs FA oxidation by the mitochondria, as malonyl-CoA inhibits carnitine palmitoyl transferase-1 (CPT-1) and FA transport into mitochondria for β -oxidation. Some of the trioses decompose into the toxic metabolite methylglyoxal (MG) (5), which can damage either proteins or DNA (6), or be detoxified to D-lactate (7, 8), by the enzyme glyoxalase 1 (Glo1), which is critical for this process and dependent on hepatic supplies of glutathione (GSH) (9).

Table 1: Comparison of clinical, anthropometric and biochemical characteristics of participants with and without D-Lactate data (n=41)

	Group with D-Lactate data (n=20)	Group without D-Lactate data (n=21)	p-value
Sex			ns
Female	15	11	
Male	5	10	
Ethnicity			ns
African-American	8	7	
Caucasian	12	14	
Puberty Status			ns
Pre-pubertal	1	4	
Pubertal	19	17	
Age (year)	14.5 (12.6 - 15.8)	13.4 (11.8 - 14.4)	ns
Weight (kg)	94.5 \pm 25.4	91.7 \pm 20.4	ns
Height (cm)	160.3 \pm 8.7	161.7 \pm 7.9	ns
BMI (kg/m²)	35.8 (31.1 - 39.6)	34.3 (31.2 - 35.7)	ns
Systolic blood pressure (mmHg)	123.8 \pm 10.8	121.9 \pm 10.6	ns
Diastolic blood pressure (mmHg)	69.2 \pm 9.7	69.3 \pm 8.4	ns
Total body fat percentage (%)	46.9 \pm 5.0	46.7 \pm 4.9	ns
Liver fat fraction (%)	7.8 (2.9 - 25.8)	8.7 (2.5 - 13.3)	ns
Glucose (mg/dl)	99.6 \pm 9.9	96.2 \pm 6.5	ns
Insulin (mU/L)	30.8 (17.4 - 42.9)	30.2 (20.1 - 39.8)	ns
L-lactate (mmol/L)	1.2 \pm 0.5	1.2 \pm 0.4	ns
Triglyceride (mg/dl)	112.5 (64 - 155.5)	104 (73 - 117)	ns
Total Cholesterol (mg/dl)	157.7 \pm 34.6	155.1 \pm 25.5	ns
LDL-Cholesterol (mg/dl)	91.3 \pm 26.5	88.6 \pm 22.8	ns
HDL-Cholesterol (mg/dl)	43.8 \pm 10.9	44.4 \pm 8.2	ns

Data are shown as mean \pm SD for normal distribution; median and interquartile range for non-normally distributed variables.

Difference between groups were calculated by t-tests for normally distributed variables or Mann-Whitney U-test for non-normally distributed variables.

ns: Not significant

Table 2: Clinical, anthropometric and biochemical characteristics of participants (n=20)

	Day 0	Day 10	p-value
Age (year)	14.5 (12.6 - 15.8)		
Ethnicity			
Latino	12		

African-American	8		
Puberty Status			
Pre-pubertal	1		
Pubertal	19		
Height (cm)	160.3 ± 8.7		
BMI (kg/m²)	35.8 (31.1 – 39.6)		
Weight (kg)	94.5 ± 25.4	93.4 ± 25.5	< 0.01
Systolic blood pressure (mmHg)	123.8 ± 10.8	121.2 ± 11.1	0.28
Diastolic blood pressure (mmHg)	69.2 ± 9.7	61.7 ± 7.9	< 0.01
Total body fat percentage (%)	46.9 ± 5.0	46.6 ± 5.5	0.32
Liver fat fraction (%)	7.8 (2.9 – 25.8)	4.9 (2.2 – 21.4)	<0.01
Glucose (mg/dl)	99.6 ± 9.9	94.2 ± 8.7	< 0.01
Insulin (mU/L)	30.8 (17.4 – 42.9)	20.9 (14.1 – 30.3)	<0.01
L-Lactate (mmol/L)	1.2 ± 0.5	0.8 ± 0.2	< 0.01
Triglyceride (mg/dl)	112.5 (64 – 155.5)	74 (52.5 - 113)	< 0.01
Total Cholesterol (mg/dl)	157.7 ± 34.6	139.8 ± 34.8	< 0.01
LDL - Cholesterol (mg/dl)	91.3 ± 26.5	83.3 ± 27.6	0.04
HDL - Cholesterol (mg/dl)	43.8 ± 10.9	40.3 ± 9.3	< 0.01
DNL - AUC	64.8 (45.5 – 78.9)	26.7 (19.2 – 35.5)	< 0.01

Data are shown as mean ± SD for normal distribution; median and interquartile range for not-normally distributed variables.

Difference between groups were calculated by t-tests for normally distributed variables or Mann-Whitney U-test for non-normally distributed variables.

ns: Not significant





