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Lean adolescents with insulin resistance display higher angiopoietin like protein 3, ApoC-III and chylomicron remnant dyslipidemia

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ABSTRACT

Background: Triglyceride-rich lipoproteins (TRL: chylomicrons and VLDL) are a key component of diabetes dyslipoproteinemia and cardiovascular risk. We have shown that it is already prevalent in obese adolescents in association with lipoprotein lipase (LPL) dysregulation. Insulin resistance (IR) suffices to produce TRL dyslipoproteinemia and LPL dysfunction even in the absence of obesity.
Methods: This cross-sectional study included euglycemic adolescents between 15 and 19 y, classified in 4 groups according to BMI, HOMA-IR and fasting lipid as: metabolically healthy lean (MHL, n = 30), metabolically unhealthy lean (MUL, n = 25), metabolically healthy obese (MHO, = 30), and metabolically unhealthy obese (MUO, n = 42).
Results: As compared to MHL, MUL participants showed 73% higher concentrations of ApoB-48; 84% of ApoC-III; 24% ANGPTL-3; 200% of TG; 218% of VLDL-C and 238% of TG/HDL-C [c], No changes were found in LPL mass. Interestingly, the differences in these parameters between MUL and MHO were not significant.
Conclusion: Euglycemic lean adolescents with IR display TRL dyslipoproteinemia with increased inhibition of LPL as highlighted by higher concentrations of ANGPTL-3, ApoC-III and fasting chylomicron remnants (ApoB-48).

1. Introduction

Over the last few decades obesity has reached global epidemic proportions in children as well as adults [1]. Despite its drawbacks and limitations, one of today's most important population health parameters is high body mass index (BMI) [2]. Children or adolescents with obesity already display one or more cardiovascular risk factors such as dyslipidemia, impaired glucose tolerance, even type 2 diabetes, arterial hypertension, and others [3]. Elevated concentrations of triglyceride-rich lipoproteins (TRL), such as chylomicrons (CM) and very low density lipoprotein (VLDL), which are the main carriers of triglycerides (TG) in the blood, have been associated with an increased risk of cardiovascular disease (CVD) [4]. ApoB-48, the apolipoprotein in chylomicrons (carriers of exogenous TG), is a marker of chylomicron remnant lipoproteins [5], high concentrations in serum are thought to be one of the risk factors for atherosclerosis and CVD [6].

We have recently shown that increases in TRL are precociously prevalent in adolescents with obesity in association with lipoprotein lipase (LPL) dysregulation which suggest a role for delayed catabolism of TRL as a causative factor [7].

Capillary LPL is the key enzyme linked to plasma TG catabolism [8]. TG hydrolysis and uptake of the resulting fatty acids are largely dependent of this enzyme [4]. Angiopoietin-like peptide 3 (ANGPTL-3) and apolipoprotein C-III (ApoC-III) are the main inhibitors of LPL [9,10]. Unrestrained inhibition of LPL by either ApoC-III or ANGPTL-3 has been shown to be a major factor in CVD residual risk [7] and the issue is so important as to justify the need to study the different factors involved in the regulation of lipid metabolism that could provide potential therapeutic approaches for the treatment of dyslipidemia-related diseases.

Additionally, current evidence highlights the pivotal role of lipoprotein-associated phospholipase A2 (Lp-PLA2) and cytokines in mediating vascular inflammation, the earliest steps to atherosclerosis, making it a valuable marker of subclinical ongoing arterial lesion [11,12]. The hallmark of atherosclerosis is (ectopic) lipid accumulation and inflammation in vessel walls which underlie development of CVD [13].

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Most of the studies about risk of cardiovascular disease in adolescents have been conducted in populations with obesity, whether they are free of cardiovascular risk factors called "Metabolically Healthy Obese" (MHO) [14] or the ones that have alterations in the lipid and glucose profile called "Metabolically Unhealthy Obese" (MUO) [15]. Many studies compare these populations with "Metabolically Healthy Lean Adolescents" (MHL). However, there is a subgroup that has not been adequately studied: adolescents with normal BMI but with alteration in their lipid and glucose profile known as "Metabolically Unhealthy Lean Adolescents" (MUL) [16].

Indeed, in recent years and at least in adults, a subgroup of people, named TOFI (thin on the outside, fat on the inside) has been described [17]. They display metabolic abnormalities previously associated with obesity without increased BMI. The main reason is the presence of ectopic fat, generating insulin resistance. Thus, it becomes imperative to test whether even adolescents with normal weight show cardiometabolic dyslipidemia that can lead to the development of cardiovascular diseases, since early intervention is key to prevention [18]. Therefore, we hereby tested the hypothesis that insulin resistance (IR) suffices to produce TRL dyslipoproteinemia and LPL dysfunction even in the absence of obesity.

2. Material and methods

2.1. Participants

This comparative cross-sectional study was conducted between August 2018 and May 2019 in 5 different educational institutions. A total of 1473 Mexican adolescents were screened for eligibility, of which 317 met the inclusion criteria but only 127 (72 adolescents with obesity and 55 with normal weight) agreed to participate in the study. These adolescents were between 15 and 19 years old, without chronic, autoimmune, hormonal or infectious diseases. The participants were classified in 4 groups according to BMI and whether or not they had lipid alterations (TC > 170 mg/dl, TG > 85 mg/dl HDL-C < 35 mg/dl) [19] and IR which was defined as a HOMA-IR > 3.0 [20]. Adolescents were classified as metabolically unhealthy when they met the one or more conditions of either dislypidemia (TC > 170 mg/dl, TG > 85 mg/dl, HDL-C < 35 mg/dl) or HOMA-IR > 3.0. Metabolically healthy means to fulfill all of the following conditions, TC < 170 mg/dl, TG < 85 mg/dl, HDL-C > 35 mg/dl, and HOMA-IR < 3.0.

Thus, the 4 groups were as follows: metabolically healthy lean (MHL, n = 30), metabolically unhealthy lean (MUL, n = 25), metabolically healthy obese (MHO, = 30), and metabolically unhealthy obese (MUO, n = 42). This study was approved for the Institutional Committee of Bioethics of the University of Guanajuato (CIBIUG-P24-2018). Both adolescents and their parents or tutors signed an informed consent form.

2.2. Anthropometric measures and blood pressure

The height and weight of the subjects were measured using a SECA stadiometer and a SECA scales, respectively, following standardized methods, BMI was calculated as according to WHO child growth charts [21].

Blood pressure was measured with an Omron HEM-7320-LA electronic monitor (Omron Healthcare Co. Ltd.) in the non-dominant hand after 10 min of rest in the sitting position, and the average of 3 measurements was recorded.

2.3. Biochemical measurements

A venous blood sample was obtained after 8–12 h of fasting and was processed the same day to measure glucose, (GOD-PAP^{TM}) and lipids using enzymatic methods in an autoanalyzer (Spinreact-Spinlab). Serum aliquots were stored at –80° C until further analyses by ELISA kits to measure insulin (ALPCOTM), ANGPTL-3 (DuoSet), Human IL-6 by Quantikine ELISA Kit). Apolipoprotein B-48 (Fujifilm), APO C-III (Millipore Sigma) and LPL (MyBioSource Inc.) Homeostatic model assessment-insulin resistance (HOMA-IR) was calculated according to Matthews et al [22] and insulin resistance was defined as above 95% percentile, according to a previous report in Mexican adolescents [23].

To determine the alterations in the lipid profile we employed the recommendations for children and adolescents of the National Cholesterol Education Program, total cholesterol < 170 mg/dl, triglycerides < 85 mg/dl, HDL-C > 35 mg/dl and glucose < 90–130 mg/dl [19]. The triglycerides/HDL-C and total cholesterol/HDL-C indexes were calculated.

2.4. Statistical analysis.

The distribution of the data was evaluated with the Shapiro-Wilk test and the results are expressed as mean ± SD for continuous variables with normal distribution and the median and interquartile range for variables with a skewed distribution. The differences between groups were evaluated by ANOVA for variables with normal distribution and Kruskal Wallis test for nonparametric variables and the post hoc analysis by Tukey-HSD test. Spearman correlation analysis was used to determine the univariate correlation between the different variables in the study. All analyses were performed using Statistica 7 software (StatSoft Inc.). Significance was defined as a value of $p < 0.05$.

3. Results

The clinical and biochemical characteristics of the adolescents are summarized in Table 1. As shown in Table 2 by design, lipid profile in the adolescents, were significantly different between the groups. The adolescents in the group MUL have higher concentration of total cholesterol, LDL-C, VLDL-C and triglycerides than the MHL and MHO but similar concentrations as compared with the MUO group.

Fig. 1 shows the differences in ApoC-III and ANGPLTL-3 between the groups. ApoC-III is 84% higher in MUL (82.7 ± 44 μg/ml) and ANGPTL-3 is 24% higher in MUL (98.3 ± 29.9) compared with MHL (ApoC-III: 44.7 ± 27.05 μg/ml) and (ANGPTL-3: 79.6 ± 18.8 ng/ml) and shows no significantly differences compared with MUO (ApoC-III: 59.4 ± 51.55 μg/ml) and (ANGPTL-3: 95.7 ± 22.3 ng/ml).

To explore the presence of delayed catabolism of chylomicrons as a result of the changes in ANGPTL-3 and ApoC-III shown above, we measured fasting ApoB-48, which showed a trend to be higher in obese vs metabolically healthy lean adolescents in spite of the large inter individual variability. Of note ApoB-48 is up by 73% in MUL as compared with MHL and the highest concentration compared with the other three groups, suggesting an early delay in chylomicron catabolism when IR is present even in lean subjects (Table 3). To probe whether the changes found could be due in part to changes in LPL mass, we measured the circulating fraction of LPL. No changes were found among the groups. Given the key role of inflammation as a primer for IR and a marker of dysfunctional visceral fat, we measured one key cytokine, IL-6. IL-6 shows a gradient in the four groups: MUO > MHO > MUL > MHL, with statistical differences between MUO and the rest of the groups. In the light of the lipoprotein changes and to determine if there was inflammatory damage at the vascular level, we measured Lp-PLA2. Fortunately, no significant differences were found between the groups.

Table 4 shows the correlations found between HOMA-IR as an index of IR and the parameters of interest. As expected, HOMA-IR correlated with BMI, TG/HDL-C and TC/HDL-C. Of note, ApoB-48 also correlated with HOMA-IR (Rho = 0.186, p = 0.04) (Table 4) and so did IL-6 (Rho 0.43, p < 0.00001).

4. Discussion

In this exploratory and comparative cross-sectional study on adolescents with different BMI and metabolic conditions we show that lean

Table 1
Clinical and biochemical characteristics of adolescents.

Groups Variable	Lean adolescents MHL(n = 30)	 MUL (n = 25)	Adolescents with obesity MHO(n = 30)	 MUO(n = 42)	p value**ANOVA**	Paired comparisons p(MHLvsMHO, MUO)	 p(MULvsMHO, MUO)	 pMHLvsMUL /p (MHOvsMUO
Female/male	13/17	16/9	19/11	27/15				
Age (y)	16.1 ± 1.1	16.1 ± 1.1	16.3 ± 1.2	16.4 ± 1.0	0.48			
Weight (kg)	55.6 ± 8.1	57.3 ± 7.3	81.0 ± 9.8	91.3 ± 13.8	<0.00001	(<0.0001, <0.0001)	(<0.0001, <0.0001)	
Height (m)	1.6 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	0.92			
BMI (Kg/m^2)	20.4 ± 1.6	21.1 ± 2.0	30.2 ± 2.6	33.4 ± 4.1	<0.00001	(<0.0001, <0.0001)	(<0.0001, <0.0001)	
SBP (mm/Hg)	108.8 ± 12.4	106.7 ± 14.2	106.0 ± 9.6	116.5 ± 9.2	<0.002	(NS, p < 0.03)		(<0.04)
DBP (mm/Hg)	68.5 ± 6.1	68.0 ± 7.1	71.0 ± 6.5	76.3 ± 7.5	<0.00003	(NS, <0.001)	(NS, <0.009)	(<0.03)
Fasting glucose (mg/dl)	91.3 ± 9.4	89.8 ± 10.6	87.5 ± 9.1	95.8 ± 9.9	<0.004		(<0.009, NS)	
Insulin mlU/l*	5.2 (3.1–7.5)	11.3 (7.7–20.8)	9.1 (6.9–10.6)	24.2 (18.2–30.1)	<0.00001	(NS, <0.001)	(<0.0009), <0.0009)	(<0.0001/(<0.0001)
HOMA-IR*	1.2 (0.7–1.7)	2.5(1.8–4.2)	1.8(1.4–2.5)	5.8(4.3–7.5)	<0.00001	(NS, <0.0001)	(<0.0009), <0.0009)	(<0.0001)/(<0.0001)
Creatinine* (mg/dl)	0.8 (0.8–0.9)	0.8(0.7–1.0)	0.9(0.8–0.9)	0.8(0.7–0.9)	0.29			

Data are shown as mean ± SD except for *Non– parametric data, median (interquartile range). MHL (Metabolically healthy lean), MUL (Metabolically unhealthy lean), MHO (Metabolically healthy obese), MUO (Metabolically unhealthy obese). Weight and BM, SBP (systolic blood pressure), DBP (diastolic blood pressure), HOMA-IR (Homeostatic model assessment of insulin resistance)

Table 2
Lipid profile of adolescents.

Groups Variable	Lean adolescents MHL (n = 30)	 MUL (n = 25)	Adolescents with obesity MHO (n = 30)	 MUO (n = 42)	ANOVA p value	Paired comparisons p(MHLvsMHO, MUO)	 p(MULvsMHO, MUO)	 pMHLvsMUL /p (MHOvsMUO
Total Cholesterol (mg/dl)	149.4 ± 26.3	176.7 ± 34.6	155.7 ± 29.2	180.4 ± 35.8	<0.0001	(NS, <0.01)		(<0.02)/(<0.003)
LDL-C (mg/dl)	88.8 ± 27.0	106.5 ± 30.5	92.3 ± 28.6	112.4 ± 28.4	<0.002	(NS, <0.02)		
HDL-C (mg/dl)	49.2 ± 10.3	45.2 ± 10.7	43.2 ± 11.0	41.2 ± 7.6	<0.007	(NS, <0.02)		
Non– HDL-C (mg/dl)	100.2 ± 26.8	131.5 ± 30.0	112.6 ± 25.5	139.2 ± 35.0	<0.000001	(NS, <0.0001)		(<0.004)/(<0.003)
VLDL-C (mg/dl)	11.4 ± 3.1	24.5 ± 6.1	16.9 ± 6.5	27.4 ± 12.9	<0.00001	(NS, <0.0001)	(<0.007, NS),	(0.00001)/(<0.00007)
Triglycerides (mg/dl)	57.3 ± 15.2	122.8 ± 30.1	84.3 ± 32.0	133.8 ± 67.3	<0.00001	(NS, <0.0001)	(<0.008, <0.008)	(0.00001)/(<0.0003)
TG/HDL-C	1.2 ± 0.4	2.9 ± 1.0	2.1 ± 1.1	3.5 ± 2.0,	<0.00001	(NS, <0.00001)		(0.0001)/(<0.003)
TC/HDL-C	3.1 ± 0.7	4.0 ± 1.0	3.7 ± 0.7	4.5 ± 1.0	<0.00001	(NS, <0.004)		(<0.04)

Data are shown as mean ± SD. MHL (Metabolically healthy lean), MUL (Metabolically unhealthy lean), MHO (Metabolically healthy obese), MUO (Metabolically unhealthy obese).

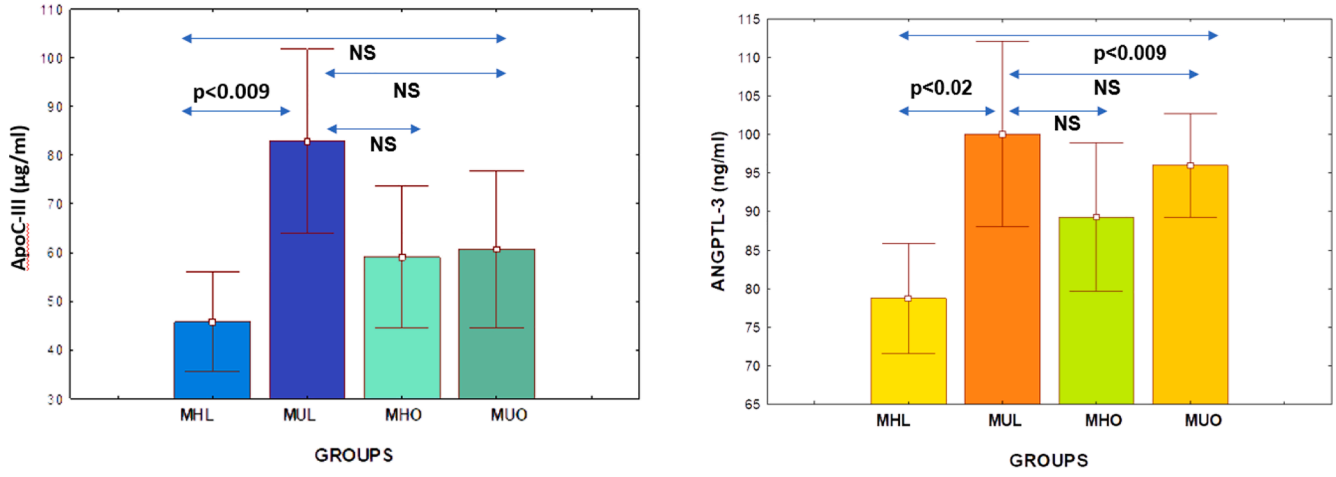

Fig. 1. Concentrations of ApoC-III and ANGPLTL-3 in the different groups of adolescents Data are shown as mean ± S D. Metabolically healthy lean (MHL); metabolically unhealthy lean (MUL); metabolically healthy obese (MHO); metabolically unhealthy obese (MUO).

Table 3
ApoB-48, LPL mass, IL-6 and Lp-PLA2 in adolescents.

Groups	Lean adolescents		Adolescents with obesity			Paired comparisons		
Variable	MHL (n = 30)	MUL (n = 25)	MHO (n = 30)	MUO (n = 42)	p value**ANOVA**	p(MHLvsMHO, MUO)	p(MULvsMHO, MUO)	P(MHLvsMUL)/p (MHOvsMUO
ApoB-48 (μg/ml)*	7.12(4.5–9.8) [œ]	1.2 (8.4–2.0) [œ]	9.2(5.6–1.3)	9.9(6.7–1.3)	<0.004			(0.002)/NS
LPL (ng/ml)*	11.0 (9.7–1.4)	10.7(9.9–11.7)	10.1 (8.7–12.2)	10.2(9.3–13.4)	NS			
IL-6 (pg/ml)*	1.10(0.63–1.6) [‡]	1.57(0.52–3.3)	1.94(1.10–3.1) [§]	3.49(2.07–4.5) [‡, §]	<0.00001	(0.02,<0.0003)	(NS, p < 0.02)	(<0.02)
Lp-PLA2 (ng/ml)(n)	451.5 ± 105.6 (13)	485.0 ± 100.1 (16)	554.1 ± 118.2 (16)	461.8 ± 121.4 (17)	NS			

Data are shown as mean ± SD except for *Non-parametric data, median (interquartile range). MHL (Metabolically healthy lean), MUL (Metabolically unhealthy lean), MHO (Metabolically healthy obese), MUO (Metabolically unhealthy obese. Apolipoprotein B-48 (ApoB-48), Lipoprotein lipase (LPL), Interleukin-6 (IL-6), Lipoprotein-associated phospholipase A2 (Lp-PLA2).

Table 4
Spearman correlation analysis in the total group.

Correlations in the whole cohort		
Variable	Rho	p value
HOMA-IR		
BMI	0.574	<0.00001
TG/HDL-C	0.534	<0.00001
TC/HDL-C	0.459	<0.00001
ANGPTL-3	0.156	NS
ApoB-48	0.180	0.04
ApoC-III	0.054	NS
IL-6	0.456	<0.00001

Spearman correlation coefficient. ApoC-III: apolipoprotein C-III; ApoB-48: apolipoprotein B48; ANGPTL-3: angiopoietin-like protein 3: IL-6: interleukin 6.

adolescents with IR have higher serum concentrations of ANGPTL-3, ApoC-III and ApoB-48 than lean adolescents without IR, and similar concentrations than the adolescents with obesity. MUL also present alterations in the lipid profile similar to those shown by MUO, such as total cholesterol and triglycerides. When the TG/HDL-C and TC/HDL-C indexes are compared, MUO showed similar scores as compared to MUL, which suggests that both groups are at risk for cardiometabolic dyslipidemia, and this risk is exacerbated by obesity. Another important finding is that MUL adolescents display a change in IL-6 consistent with altered adipose tissue signaling that favors inflammation and dyslipidemia. On the other hand, absence of changes in Lp-PLA2 suggest that, fortunately, there appears to be no sign of early vascular inflammation.

Our group had recently evaluated ANGPTL-3 in adolescents with normal weight and adolescents with obesity, finding higher concentrations in the group with obesity [7]. Our current study, with a different and larger cohort confirms the previous finding and adds the stratification of lean adolescents in two groups which allowed us to observe that MUL adolescents have significantly higher ANGPTL-3 (24%) than MHL and very similar to MUO.

ANGPTL-3 is secreted from the liver and functions as a potent circulating inhibitor of LPL and endothelial lipase (EL) [24], most notably in the fed state. Inhibition of LPL in selected tissues i.e. adipose vs muscle is an important regulatory control in the fast-fed cycle [25]. During fasting, hydrolysis of VLDL-TG is preferentially shifted to muscle capillaries where fatty acids are used for energy whereas during feeding it is shifted to adipose where TG are employed for re esterification and storage [26]. ANGPTL-3 (more active during fasting) inhibits LPL activity and EL activity, and retards clearance of triglyceride-rich lipoproteins upstream of LDL production in a tissue-specific manner that directs the TRL to adipose tissue preferentially. When its secretion is poorly regulated, the physiological action goes awry and excessive inhibition raises plasma concentrations of triglycerides and reduces high-density lipoprotein cholesterol (HDL-C) [27–29]. In addition to inhibiting LPL, ANGPTL-3 also inhibits the activity of EL, which hydrolyzes HDL phospholipids [30]. These actions explain the increase in circulating TG concentrations in MUL due to the inhibitory activity of higher concentrations of ANGPTL-3 on LPL activity. The importance of ANGPTL-3 in TRL dyslipoproteinemia is highlighted by the fact that phase I and II trials with either monoclonal antibodies (Evinacumab) or ASOs (IONIS-ANGPTL-3-LRX) and siRNA ARO-ANG3 are very promising therapeutic strategies in early development for the treatment of hypertriglyceridemia [7,31–33].

The other key LPL inhibitor that we measured in this study is ApoC-III. Studies have shown that impaired catabolism of TRL, is linked to increased concentrations of plasma ApoC-III. ApoC-III regulates TRL fluxes as it both inhibits LPL-catalyzed hydrolysis of TRLs and attenuates the uptake of TRL remnants by the liver [34,35]. We found increased ApoC-III concentrations in MUL (84%) vs MHL. A possible increase in the percentage of visceral fat could be the cause of the insulin resistance already present in MUL.

As occurs for ANGPTL-3, phase I and II trials with ASOs (AKCEA-pAPO-III-LRX) and siRNA ARO-APOC3 are new therapeutic strategies for the treatment of hypertriglyceridemia and residual risk for atherosclerosis [7,9,36,37].

These main inhibitors of LPL that are now drugs targets are increased not only in obese subjects as we showed before, but in lean subjects as well provided they have IR. This finding is important as it suggests that obesity is not necessary for adolescents to have cardiometabolic dyslipidemia, it aggravates it when present.

Another significant finding of this study is the higher concentration of ApoB-48 in MUL (73%) than MHL, and more importantly the fact that it is higher than MHO and MUO. Our results show that adolescents with normal weight but alterations in their lipid profile or insulin resistance can have fasting high concentrations of ApoB-48. This implies the likelihood that this is far worse in the postprandial state (which we did not study here and deserves further exploration). Chylomicron remnant dyslipoproteinemia can lead to the development of cardiovascular diseases in later stages of life. There are few published studies evaluating ApoB-48 only in adolescents. One of this studies suggest that fasting plasma ApoB-48 and remnant lipoproteins associate with early cardiovascular diseases in adolescents and youth [38]. Vine et al evaluated ApoB-48 lipoprotein metabolism in female adolescents with polycystic ovary, finding that the group with obesity have elevated fasting and postprandial plasma TG and ApoB-48 lipoprotein remnants compared with the normal weight group [39]. ApoB-lipoprotein remnants are derived from both the intestine (chylomicrons and chylomicron remnants containing ApoB-48) and the liver (very low-density lipoprotein, and low-density lipoprotein [LDL] remnants containing ApoB-100) [40]. Impaired ApoB-48 chylomicrons metabolism in the fasted and nonfasted state has been observed in normolipidemic conditions and in those with increased incidence of CVD, including obesity, MetS, and diabetes [41]. Since humans are in the postprandial most of the day, the continuous generation of remnants after each meal may be an important causal risk-

factor for the development of atherosclerosis [42]. These results provide evidence or the early development of subclinical cardiovascular risk in adolescents even in absence of obesity.

The findings of higher ApoC-III, ANGPTL-3 and ApoB48 in MUL as compared with obese adolescents are interesting and deserves further exploration. We posit that early IR in MUL induces rapid "acute" changes in lipoprotein metabolism. In time, when IR leads to obesity compensatory mechanisms may be at play to curb some of those changes. Longitudinal studies are needed to substantiate this putative mechanism.

IL-6 was higher in MUO compared to the other groups, but it is important to highlight that the MUL and the MHO group did not differ. IL-6 is upregulated in response to ROS and vascular injury and highly representative of vascular inflammation [43]. Previous studies show serum IL-6 is a significant predictor of cardiovascular mortality [11,44].

Lp-PLA2 concentrations did not show significant difference among the four groups, suggesting that fortunately our cohort, although showing lipoprotein alterations, does not show evidence of arterial lesions. There is still no established cutoff point for children and adolescents for this biomarker. However, interestingly, the mean of the four groups is higher than 200 ng/ml, which is the cutoff point in adults to consider it as a high CVD risk [45]. Lp-PLA2 is involved in the modification of lipids within atheromatous plaques. Lp-PLA2 was found to be predictive of thromboembolic episodes in adults [46].

The strengths of this work reside in the fact that there is a paucity of studies that evaluate adolescents in these four groups as well as the LPL and its main regulators. Despite having a small number of participants, the differences between the groups were significant. One of the limitations is that we did not study the postprandial state of adolescents, and changes may be even more important. Another limitation of this study was that, due to the small sample size, it was not possible to evaluate a possible gender effect on our results. Given the role of ethnicity in the prevalence of obesity our results may only apply to the Mexican population. Further studies are needed for generalization of the impact of these findings.

5. Conclusion

Euglycemic lean adolescents with IR display TRL dyslipoproteinemia with increased inhibition of LPL as shown by higher concentrations of ANGPTL-3 compounded with increased ApoC-III and associated with higher fasting chylomicron remnants (ApoB-48). The significance of these findings is double. First, they show that the dyslipidemia is indistinguishable from that found in MUO showing obesity is not necessary to produce it. Secondly, they suggest a mechanism for TRL dyslipidemia even in lean adolescents: increased LPL inhibition impairs VLDL and chylomicron catabolism leading to atherogenic remnants.

CRediT authorship contribution statement

Karla Paola Gutiérrez Castro: Conceptualization, Formal analysis, Investigation, Writing – original draft, Visualization. **Alma Patricia González:** Investigation. **Russell Caccavello:** Validation, Investigation. **Ma. Eugenia Garay-Sevilla:** Conceptualization, Methodology, review and editing, Supervision, Project administration, Funding aquisition. **Alejandro Gugliucci:** Conceptualization, Methodology, Resources, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] L. Pacifico, E. Bonci, G. Andreoli, S. Romaggioli, R. Di Miscio, C.V. Lombardo, C. Chiesa, Association of serum triglyceride-to-HDL cholesterol ratio with carotid artery intima-media thickness, insulin resistance and nonalcoholic fatty liver disease in children and adolescents, Nutr. Metab. Cardiovasc. Dis. 24 (7) (2014) 737–743, https://doi.org/10.1016/j.numecd.2014.01.010.
[2] L.M. Jaacks, S. Vandevijvere, A. Pan, C.J. McGowan, C. Wallace, F. Imamura, D. Mozaffarian, B. Swinburn, M. Ezzati, A. Expand, The obesity transition: stages of the global epidemic, Yearb. Paediatr. Endocrinol. 7 (2019) 231–240, https://doi.org/10.1530/ey.16.13.15.
[3] P. Weihe, S. Weihrauch-Blüher, Metabolic Syndrome in Children and Adolescents: Diagnostic Criteria, Therapeutic Options and Perspectives, Curr. Obes. Rep. 8 (2019) 472–479, https://doi.org/10.1007/s13679-019-00357-x.
[4] R. Zhang, The ANGPTL3-4-8 model, a molecular mechanism for triglyceride trafficking, Open Biol. 6 (2016), 150272, https://doi.org/10.1098/rsob.150272.
[5] K. Nakajima, T. Nagamine, M.Q. Fujita, M. Ai, A. Tanaka, E. Schaefer, Apolipoprotein B-48: A unique marker of chylomicron metabolism, Adv. Clin. Chem. 64 (2014) 117–177, https://doi.org/10.1016/B978-0-12-800263-6.00003-3.
[6] B. Staňková, J. Macášek, M. Zeman, M. Vecka, E. Tvrzická, M. Jáchymová, A. Slabý, A. Žák, Polymorphisms rs2167444 and rs508384 in the SCD1 gene are linked with high APOB-48 concentrations and adverse profile of cardiometabolic risk factors, Folia Biol. (Czech Republic) 65 (2019) 159–169.
[7] R. Rodríguez-Mortera, R. Caccavello, M.E. Garay-Sevilla, A. Gugliucci, Higher ANGPTL3, apoC-III, and apoB48 dyslipidemia, and lower lipoprotein lipase concentrations are associated with dysfunctional visceral fat in adolescents with obesity, Clin. Chim. Acta. 508 (2020) 61–68, https://doi.org/10.1016/j.cca.2020.05.014.
[8] M. Kockx, L. Kritharides, Triglyceride-Rich Lipoproteins, Cardiol. Clin. 36 (2) (2018) 265–275, https://doi.org/10.1016/j.ccl.2017.12.008.
[9] M.R. Taskinen, J. Borén, Why Is Apolipoprotein CIII Emerging as a Novel Therapeutic Target to Reduce the Burden of Cardiovascular Disease? Curr. Atheroscler. Rep. 18 (2016) 1–8, https://doi.org/10.1007/s11883-016-0614-1.
[10] A. Köster, Y.B. Chao, M. Mosior, A. Ford, P.A. Gonzalez-DeWhitt, J.E. Hale, D. Li, Y. Qiu, C.C. Fraser, D.D. Yang, J.G. Heuer, S.R. Jaskunas, P. Eacho, Transgenic angiopoietin-like (Angptl)4 overexpression and targeted disruption of Angptl4 and Angptl3: Regulation of triglyceride metabolism, Endocrinology 146 (2005) 4943–4950, https://doi.org/10.1210/en.2005-0476.
[11] X. Lu, X. Xu, Y. Zhang, Y. Zhang, C. Wang, X. Huo, Elevated inflammatory Lp-PLA2 and IL-6 link e-waste Pb toxicity to cardiovascular risk factors in preschool children, Environ. Pollut. 234 (2018) 601–609, https://doi.org/10.1016/j.envpol.2017.11.094.
[12] S.M. Ragab, M.A. Safan, O.M. Obeid, A.S. Sherief, Lipoprotein-associated phospholipase A2 (Lp-PLA2) and tumor necrosis factor-alpha (TNF-α) and their relation to premature atherosclerosis in β-thalassemia children, Hematology. 20 (4) (2015) 228–238, https://doi.org/10.1179/1607845414Y.0000000180.
[13] J. Li, L. Li, D.M. Guo, S.Y. Li, Y.X. Zeng, C.H. Liu, R. Fu, M.Q. Huang, W. Xie, Triglyceride metabolism and angiopoietin-like proteins in lipoprotein lipase regulation, Clin. Chim. Acta. 503 (2020) 19–34, https://doi.org/10.1016/j.cca.2019.12.029.
[14] A.D. Karelis, M. Faraj, J.-P. Bastard, D.H. St-Pierre, M. Brochu, D. Prud'homme, R. Rabasa-Lhoret, The metabolically healthy but obese individual presents a favorable inflammation profile, J. Clin. Endocrinol. Metab. 90 (7) (2005) 4145–4150, https://doi.org/10.1210/jc.2005-0482.
[15] C. Iacobini, G. Pugliese, C. Blasetti Fantauzzi, M. Federici, S. Menini, Metabolically healthy versus metabolically unhealthy obesity, Metabolism. 92 (2019) 51–60, https://doi.org/10.1016/j.metabol.2018.11.009.
[16] L. Basurto, L. Sánchez, A. Díaz, M. Valle, A. Robledo, C. Martínez-Murillo, Differences between metabolically healthy and unhealthy obesity in PAI-1 level: Fibrinolysis, body size phenotypes and metabolism, Thromb. Res. 180 (2019) 110–114, https://doi.org/10.1016/j.thromres.2019.06.013.
[17] E.L. Thomas, G. Frost, S.D. Taylor-Robinson, J.D. Bell, Excess body fat in obese and normal-weight subjects, Nutr. Res. Rev. 25 (1) (2012) 150–161, https://doi.org/10.1017/S0954422412000054.
[18] E.L. Thomas, J.R. Parkinson, G.S. Frost, A.P. Goldstone, C.J. Doré, J.P. McCarthy, A.L. Collins, J.A. Fitzpatrick, G. Durighel, S.D. Taylor-Robinson, J.D. Bell, The missing risk: MRI and MRS phenotyping of abdominal adiposity and ectopic fat, Obesity. 20 (2012) 76–87, https://doi.org/10.1038/oby.2011.142.
[19] Z. Gómez Cruz, E. Romero Velarde, A.H. Tinoco, H.V. Sánchez, R.M.F. Gómez, Y. L. Illan, L.G. Mejía, Estado nutricional y perfil de lípidos en adolescentes de un escuela rural, Rev. Mex. Pediatría. 80 (2013) 5–9.
[20] W Q Ding, Y K Yan, M X Zhang, H Cheng, X Y Zhao, D Q Hou, J Mi, Hypertension outcomes in metabolically unhealthy normal-weight and metabolically healthy obese children and adolescents, J. Hum. Hypertens. 29 (9) (2015) 548–554, https://doi.org/10.1038/jhh.2014.124.

[21] World Health Organization, BMI-for.age (5-19 years) charts, 2007. (n.d.). https://www.who.int/tools/growth-reference-data-for-5to19-years/indicators/bmi-for-age.
[22] D.R. Matthews, J.P. Hosker, A.S. Rudenski, B.A. Naylor, D.F. Treacher, R.C. Turner, Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man, Diabetologia 28 (1985) 412–419, https://doi.org/10.1007/BF00280883.
[23] C. Aradillas-García, M. Rodríguez-Morán, M.E. Garay-Sevilla, J.M. Malacara, R. A. Rascon-Pacheco, F. Guerrero-Romero, Distribution of the homeostasis model assessment of insulin resistance in Mexican children and adolescents, Eur. J. Endocrinol. 166 (2012) 301–306, https://doi.org/10.1530/EJE-11-0844.
[24] P.A. Nidhina Haridas, Jarkko Soronen, Sanja Sädevirta, Raghavendra Mysore, Fabiana Quagliarini, Arja Pasternack, Jari Metso, Julia Perttilä, Marja Leivonen, Cynthia M. Smas, Pamela Fischer-Posovszky, Martin Wabitsch, Christian Ehnholm, Olli Ritvos, Matti Jauhiainen, Vesa M. Olkkonen, Hannele Yki-Järvinen, Regulation of angiopoietin-like proteins (ANGPTLs) 3 and 8 by insulin, J. Clin. Endocrinol. Metab. 100 (10) (2015) E1299–E1307, https://doi.org/10.1210/jc.2015-1254.
[25] M.J. Ladu, H. Kapsas, W.K. Palmer, Regulation of lipoprotein lipase in adipose and muscle tissues during fasting, Am. J. Physiol. - Regul. Integr. Comp. Physiol. 260 (5) (1991) R953–R959.
[26] Maryam Ahmadian, Robin E Duncan, Kathy Jaworski, Eszter Sarkadi-Nagy, Hei Sook Sul, Triacylglycerol metabolism in adipose tissue, Future Lipidol. 2 (2) (2007) 229–237, https://doi.org/10.2217/17460875.2.2.229.
[27] Zahid Ahmad, Poulabi Banerjee, Sara Hamon, Kuo-Chen Chan, Aurelie Bouzelmat, William J. Sasiela, Robert Pordy, Scott Mellis, Hayes Dansky, Daniel A. Gipe, Richard L. Dunbar, Inhibition of Angiopoietin-Like Protein 3 with a Monoclonal Antibody Reduces Triglycerides in Hypertriglyceridemia, Circulation 140 (6) (2019) 470–486, https://doi.org/10.1161/CIRCULATIONAHA.118.039107.
[28] Viktoria Gusarova, Corey A. Alexa, Yan Wang, Ashique Rafique, Jee Hae Kim, David Buckler, Ivory J. Mintah, Lisa M. Shihanian, Jonathan C. Cohen, Helen H. Hobbs, Yurong Xin, David M. Valenzuela, Andrew J. Murphy, George D. Yancopoulos, Jesper Gromada, ANGPTL3 blockade with a human monoclonal antibody reduces plasma lipids in dyslipidemic mice and monkeys, J. Lipid Res. 56 (7) (2015) 1308–1317, https://doi.org/10.1194/jlr.M054890.
[29] E. Geladari, P. Tsamadia, N.G. Vallianou, ANGPTL3 Inhibitors: Their role in cardiovascular disease through regulation of lipid metabolism, Circ. J. 83 (2019) 267–273, https://doi.org/10.1253/circj.CJ-18-0442.
[30] Mitsuru Shimamura, Morihiro Matsuda, Hiroaki Yasumo, Mitsuyo Okazaki, Kazunori Fujimoto, Keita Kono, Tetsuya Shimizugawa, Yosuke Ando, Ryuta Koishi, Takafumi Kohama, Naohiko Sakai, Kazuaki Kotani, Ryutaro Komuro, Tatsuo Ishida, Kenichi Hirata, Shizuya Yamashita, Hidehiko Furukawa, Iichiro Shimomura, Angiopoietin-like protein3 regulates plasma HDL cholesterol through suppression of endothelial lipase, Arterioscler. Thromb. Vasc. Biol. 27 (2) (2007) 366–372, https://doi.org/10.1161/01.ATV.0000252827.51626.89.
[31] F.J. Raal, R.S. Rosenson, L.F. Reeskamp, G.K. Hovingh, J.J.P. Kastelein, P. Rubba, P.D. Shazia Ali, P. Banerjee, K. Chan, D.A. Gipe, N. Khilla, R. Pordy, D. M. Weinreich, G.D. Yancopoulos, Y. Zhang, D. Gaudet, Evinacumab for Homozygous Familial Hypercholesterolemia, N. Engl. J. Med. 383 (2020) 711–720, https://doi.org/10.1056/NEJMoa2004215.
[32] V.M. Olkkonen, J. Sinisalo, M. Jauhiainen, New medications targeting triglyceride-rich lipoproteins: Can inhibition of ANGPTL3 or apoC-III reduce the residual cardiovascular risk? Atherosclerosis. 272 (2018) 27–32, https://doi.org/10.1016/j.atherosclerosis.2018.03.019.
[33] Mark J. Graham, Richard G. Lee, Teresa A. Brandt, Li-Jung Tai, Wuxia Fu, Raechel Peralta, Rosie Yu, Eunju Hurh, Erika Paz, Bradley W. McEvoy, Brenda F. Baker, Nguyen C. Pham, Andres Digenio, Steven G. Hughes, Richard S. Geary, Joseph L. Witztum, Rosanne M. Crooke, Sotirios Tsimikas, Cardiovascular and Metabolic Effects of ANGPTL3 Antisense Oligonucleotides, N. Engl. J. Med. 377 (3) (2017) 222–232.
[34] M.R. Taskinen, C.J. Packard, J. Borén, Emerging Evidence that ApoC-III Inhibitors Provide Novel Options to Reduce the Residual CVD, Curr. Atheroscler. Rep. 21 (2019) 27, https://doi.org/10.1007/s11883-019-0791-9.
[35] Jan Borén, Gerald F. Watts, Martin Adiels, Sanni Söderlund, Dick C. Chan, Antti Hakkarainen, Jesper Lundbom, Nina Lundbom, Niina Matikainen, Juhani Kahri, Bruno Vergès, P. Hugh R. Barrett, Marja-Riitta Taskinen, Kinetic and related determinants of plasma triglyceride concentration in abdominal obesity: Multicenter tracer kinetic study, Arterioscler. Thromb. Vasc. Biol. 35 (10) (2015) 2218–2224, https://doi.org/10.1161/ATVBAHA.115.305614.
[36] J. Schmitz, I. Gouni-Berthold, APOC-III Antisense Oligonucleotides: A New Option for the Treatment of Hypertriglyceridemia, Curr. Med. Chem. 25 (2018) 1567–1576, https://doi.org/10.2174/0929867324666170609081612.
[37] V.J. Alexander, S. Xia, E. Hurh, S.G. Hughes, L. O'Dea, R.S. Geary, J.L. Witztum, S. Tsimikas, N-acetyl galactosamine-conjugated antisense drug to APOC3 mRNA, triglycerides and atherogenic lipoprotein concentrations, Eur. Heart J. 40 (2019) 2785–2796, https://doi.org/10.1093/eurheartj/ehz209.
[38] J.A. Krysa, D.F. Vine, L.J. Beilin, S. Burrows, R.C. Huang, T.A. Mori, S.D. Proctor, ApoB48-remnant lipoproteins are associated with increased cardiometabolic risk in adolescents, Atherosclerosis. 302 (2020) 20–26, https://doi.org/10.1016/j.atherosclerosis.2020.04.021.
[39] D.F. Vine, Y. Wang, M.M. Jetha, G.D. Ball, S.D. Proctor, Impaired apob-lipoprotein and triglyceride metabolism in obese adolescents with polycystic ovary syndrome, J. Clin. Endocrinol. Metab. 102 (2017) 970–982, https://doi.org/10.1210/jc.2016-2854.
[40] D.F. Vine, L.J. Beilin, S. Burrows, R.C. Huang, M. Hickey, R. Hart, S.D. Proctor, T. A. Mori, ApoB48-lipoproteins are associated with cardiometabolic risk in adolescents with and without polycystic ovary syndrome, J. Endocr. Soc. 4 (2020) 1–12, https://doi.org/10.1210/jendso/bvaa061.
[41] Anette Varbo, Marianne Benn, Anne Tybjærg-Hansen, Børge G. Nordestgaard, Elevated remnant cholesterol causes both low-grade inflammation and ischemic heart disease, whereas elevated low-density lipoprotein cholesterol causes ischemic heart disease without inflammation, Circulation 128 (12) (2013) 1298–1309, https://doi.org/10.1161/CIRCULATIONAHA.113.003008.
[42] E. Björnson, C.J. Packard, M. Adiels, L. Andersson, N. Matikainen, S. Söderlund, J. Kahri, A. Hakkarainen, N. Lundbom, J. Lundbom, C. Sihlbom, A. Thorsell, H. Zhou, M.-R. Taskinen, J. Borén, Apolipoprotein B48 metabolism in chylomicrons and very low-density lipoproteins and its role in triglyceride transport in normo- and hypertriglyceridemic human subjects, J. Intern. Med. 288 (4) (2020) 422–438, https://doi.org/10.1111/joim.13017.
[43] A.R. Brasier, The nuclear factor-B-interleukin-6 signalling pathway mediating vascular inflammation, Cardiovasc. Res. 86 (2010) 211–218, https://doi.org/10.1093/cvr/cvq076.
[44] Katalin Eder, Noemi Baffy, Andras Falus, Andras K. Fulop, The major inflammatory mediator interleukin-6 and obesity, Inflamm. Res. 58 (11) (2009) 727–736, https://doi.org/10.1007/s00011-009-0060-4.
[45] Michael H. Davidson, Marshall A. Corson, Mark J. Alberts, Jeffrey L. Anderson, Philip B. Gorelick, Peter H. Jones, Amir Lerman, Joseph P. McConnell, Howard S. Weintraub, Consensus Panel Recommendation for Incorporating Lipoprotein-Associated Phospholipase A2 Testing into Cardiovascular Disease Risk Assessment Guidelines, Am. J. Cardiol. 101 (12) (2008) S51–S57, https://doi.org/10.1016/j.amjcard.2008.04.019.
[46] S. Sakka, T. Siahanidou, C. Voyatzis, P. Pervanidou, C. Kaminioti, N. Lazopoulou, C. Kanaka-Gantenbein, G.P. Chrousos, I. Papassotiriou, Elevated circulating concentrations of lipoprotein-associated phospholipase A2 in obese children, Clin. Chem. Lab. Med. 53 (2015) 1119–1125, https://doi.org/10.1515/cclm-2014-1081.