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Fasting, post-OGTT challenge, and nocturnal free fatty acids in prediabetic vs. normal glucose tolerant overweight and obese Latino adolescents

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Abstract

Background and Objective—Type 2 diabetes risk and its relationship to free fatty acid (FFA) exposure and visceral fat by prediabetes status in minority adolescents has yet to be explored. Therefore, the objective of this study was to examine the association of circulating FFA under varying conditions with prediabetes in Latino adolescents and to determine the relative relationships of FFA and visceral adiposity to insulin sensitivity, secretion, and β-cell function.

Subjects and Outcome Measures—Overweight or obese, but otherwise healthy Latino adolescent males and females (n=164, 14.2±2.5 years) were recruited for assessment of prediabetes, abdominal fat, and FFA levels taken at a fasting state (FFA_F), during an OGTT (FFA_{OGTT}), and overnight ($FFA_{NOCTURNAL}$).

Results—Prediabetic adolescents had a higher FFA_F than those with normal glucose tolerance when controlling for age, sex, pubertal status, total percent body fat, and visceral fat. FFA_{OGTT} and $FFA_{NOCTURNAL}$ did not differ between participants with prediabetes and those with normal glucose tolerance after adjusting for covariates. Visceral fat was independently related to insulin sensitivity and secretion in pubertal adolescents, however in post-pubertal adolescents, FFA_F and visceral fat were both independent and negatively related to β-cell function.

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Statement of Human and Animal Rights

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

Statement of Informed Consent

Informed consent was obtained from all patients for being included in the study.

Conflicts of interest: Claudia Toledo-Corral, Tanya Alderete, Joyce Richey, Paola Sequeira, Michael Goran, and Marc Weigensberg have nothing to disclose.

Conclusion—These results support a plausible progression of the lipotoxicity theory of diabetes development during the pubertal transition.

Keywords

Prediabetes; Latino; FFA; visceral fat

INTRODUCTION

Studies in prediabetic and diabetic adults suggest that elevated circulating levels of fasting free fatty acids (FFA_F) may play a role in the development of type 2 diabetes (T2DM) [1, 2]. Elevated FFA_F have been linked to the development of insulin resistance, and defects in insulin secretion [3, 4], and have been shown to be an independent risk factor for progression to T2DM [5]. In addition to imparting diabetes risk, increases in FFA_F may be linked to the development of non-alcoholic fatty liver disease [6], metabolic syndrome, [7] and other obesity-related metabolic disorders. While some studies in children have reported relationships between FFA_F and T2DM factors [8, 9], others have failed to observe similar relationships [10, 11].

Like adults, T2DM in children and adolescents moves through a pathophysiologic sequence initially characterized by a prediabetic state, which by current definitions includes impaired fasting glucose, impaired glucose tolerance, and/or elevated A1C [12]. We have previously shown that overweight Latino children and adolescents are at high risk for the development of T2DM, demonstrating declining insulin sensitivity and increased visceral fat over time in those with prediabetes compared to those with normal glucose tolerance (NGT) [13–15]. Although studies have shown that insulin sensitivity declines during puberty and recovers post-puberty [16], there is sparse literature on the role of FFA_F metabolism and adiposity as it relates to the progression from NGT to prediabetes during the pubertal transition.

Emerging evidence from animal studies suggest that elevated overnight FFA ($FFA_{NOCTURNAL}$) may be relevant in the pathogenesis of diabetes. For example, data from an insulin-resistant canine model demonstrated that after 6 weeks of high fat feeding, these “pre-diabetic” dogs had elevated $FFA_{NOCTURNAL}$ levels [17]. Data has shown that FFA is elevated between 4–8 AM in T2DM patients, which may be due to an increased rate of lipolysis and FFA flux [18]. The fluctuations of $FFA_{NOCTURNAL}$ in children with prediabetes have not been described; however, one study found that obese children had higher $FFA_{NOCTURNAL}$ when compared to their lean counterparts [19]. Together, these studies suggest that obesity and metabolic dysfunction may be associated with elevated $FFA_{NOCTURNAL}$.

Our primary objective was to examine fasting FFA_F, FFA across an OGTT (FFA_{OGTT}) and $FFA_{NOCTURNAL}$ in prediabetic and NGT overweight Latino adolescents. Our secondary objective was to determine relationships between FFA and visceral adiposity with β-cell function. The specific hypotheses were: (1) FFA would be higher and would remain higher following an oral glucose challenge in those with prediabetes vs. those with NGT; 2) plasma levels of $FFA_{NOCTURNAL}$ would be higher in those with prediabetes vs. those with NGT; 3)

FFA exposure would be inversely related to insulin sensitivity and β-cell function and; 4) FFA, in addition to visceral fat, would independently contribute to T2DM risk factors.

METHODS

Participants

One hundred sixty-four overweight or obese, but otherwise healthy Latino adolescent males and females were recruited from greater Los Angeles (LA) County through community health clinics, health fairs, and word of mouth for participation in either the DREAM (Diabetes Risk and Ectopic Adiposity in Minority youth) or the SOLAR-2 (Study of Overweight Latino Adolescents at Risk-2) study. Participants met the following inclusion criteria: 1) Latino ethnicity (all four grandparents of Latin-American descent); 2) age 8–17 years; 3) age and sex-adjusted BMI ≥85th percentile. SOLAR-2 criteria additionally required that participants be in Tanner stage 2, 3 or 4, and have a family history of T2DM (at least one immediate family member was diagnosed with T2DM). In DREAM, a family history of T2DM was not an inclusion criterion; however, 54.1% (n=55) of participants reported such family history. Exclusions included previous major illness, medications or conditions known to influence body composition, insulin action, or insulin secretion. Participants and their parents provided written informed consent. Studies were approved by the Institutional Review Board of University of Southern California (USC) at the Health Sciences Campus, LA.

Procedures

All participants attended two visits at the USC General Clinical Research Center at the LA County General Hospital or, after 2008, at the Clinical Trials Unit at the USC University Hospital. On the first visit, participants received a comprehensive physical examination by a licensed health care provider. Pubertal stage was determined as breast stage for girls and pubic hair stage for boys [20, 21]. Blood was drawn at the fasting state and then at 30, 60 and 120min post oral glucose challenge (1.75 g oral glucose solution/kg body weight, maximum 75g).

Within approximately 2 months following the first visit, participants were admitted for their inpatient visit and served dinner and a snack before 2000hr, after which only water was permitted. The following morning, subjects underwent a 13-sample insulin-modified frequently sampled intravenous glucose tolerance test (FSIVGTT) beginning shortly after 0700hr as previously described [14, 15, 22]. FSIVGTT glucose and insulin were entered into MINMOD software (version 6.02; RN Bergman, Los Angeles, CA) for calculation of whole body insulin sensitivity, the acute insulin response (AIR), and the disposition index (DI) [23]. All subjects underwent abdominal MRI scanning to determine volume of visceral fat on a commercial 3-Tesla MRI system (Excite HD, GE Healthcare, Waukesha, WI) as previously described [22].

Participants from the SOLAR-2 study underwent nocturnal blood sampling (n=65). An intravenous (IV) catheter was placed on the antecubital fossae of the either the right or left arm. Immediately after, participants were served 1 of 2 standardized dinner options and were

allowed 30min to complete their meal. Thereafter, only water was permitted. Blood samples were drawn hourly overnight from 0000hr to 0600hr. At approximately 0530hr, participants were awakened to have a second IV catheter placed on the antecubital fossae of the non-catheterized arm and underwent the FSIVGTT procedure as described above.

Assays

Hemoglobin A1c was measured by HPLC (Tosoh 11c 2.2 HLC-723, Tokyo, Japan), an assay approved by the International Federation of Clinical Chemistry Working Group (IFCC-WG) on A1C standardization (14). Glucose was assayed using a Yellow Springs Instruments analyzer (YSI INC., Yellow Springs, OH) that uses a membrane bound glucose oxidase technique. Insulin was assayed using an automated enzyme immunoassay (Tosoh AIA 600 II analyzer, Tosoh Bioscience, Inc., South San Francisco, CA). An in vitro enzymatic colormetric method assay was used for the quantitative determination of FFA (Wako NEFA-HR (2) series; Wako Diagnostics, USA).

Statistical Analysis

Prediabetes (n=57) was defined as: fasting glucose ≥100mg/dL and/or 2hr glucose 140–199mg/dL and/or A1C 6.0–6.4% (42–46mmol.mol)] and NGT (n=107) as: fasting glucose <100mg/dL, 2hr glucose <140mg/L, and A1C <6.0% (42mmol/mol)]. The A1C criteria is based on the recommendations of the International Expert Committee [12], and our prior work demonstrating compromised β-cell function in overweight Latino adolescents with A1C between 6.0–6.4% (42–46 mmol/mol) [14]. Insulin and FFA data from the OGTT were used to calculate insulin and FFA area under the curve (insulin AUC_{OGTT}, and FFA AUC_{OGTT}) using the trapezoidal rule. The nocturnal blood sampling data from 0000h to 0600h was used to calculate insulin $AUC_{NOCTURNAL}$ and FFA $AUC_{NOCTURNAL}$.

For descriptive purposes, independent t-tests and chi-square tests were used to determine mean physical and metabolic characteristics by prediabetes status. To test our first aim, a repeated measure ANCOVA was used to test for differences in FFA during the OGTT in those with and without prediabetes while adjusting *a priori* covariates of age, sex, pubertal status [puberty (Tanner 2–4) vs. post-puberty (Tanner 5)] [16], total percent body fat, and visceral fat. To assess potential differences by study, we adjusted for family history of T2DM; however, results remained the same so these variables were not included in the final analyses. Differences in FFA $AUC_{NOCTURNAL}$ and insulin $AUC_{NOCTURNAL}$ by prediabetes status were tested with an ANCOVA.

For our second aim, we used linear regression analysis to determine the contribution and relationship of FFA_F and visceral fat to each metabolic outcome (insulin sensitivity, AIR_G, and DI). Standardized betas and change in variance are shown in Table 2 by pubertal status. Data were analyzed using SPSS18.0 (IBM Inc., Chicago, IL), with an *a priori* significance level of $p<0.05$.

RESULTS

Physical and metabolic characteristics in subjects by prediabetes status are shown in Table 1. BMI and BMI z-score was higher in those with prediabetes than NGT. Total fat mass,

total lean tissue, total percent fat, visceral fat and subcutaneous abdominal adipose tissue did not differ by prediabetes status ($p>0.05$). By definition, those with prediabetes had higher fasting & 2-hour glucoses, and A1C ($p<0.01$). Those with prediabetes had higher insulin AUC_{OGTT}, insulin $AUC_{NOCTURNAL}$, FFA_F, FFA AUC_{OGTT}, and lower insulin sensitivity and DI than those with NGT ($p<0.05$).

As shown in Figure 1, participants with prediabetes had higher FFA_F levels compared to those with NGT (0.725 vs 0.677 mmol/L, $p=0.001$). FFA AUC_{OGTT} was higher in those with prediabetes than NGT (0.720 vs 0.627 mmol/L × 120min, $p=0.001$), but after controlling for FFA_F, this relationship disappeared ($p=0.86$). FFA levels were significantly higher in those with prediabetes than NGT at 30min ($p<0.05$) but not 60min or 120min ($p>0.05$). Although fasting insulin levels did not differ by prediabetes status ($p>0.05$), insulin at 30min, 60min, and 2-hours post OGTT were higher in those with prediabetes than NGT ($p=0.02–0.05$). Lastly, overall exposure of insulin during the OGTT challenge (INS AUC_{OGTT}) was higher in those with prediabetes than NGT participants (255.1 vs 169.1 uU/mL, $p=0.01$).

$FFA_{NOCTURNAL}$ and $insulin_{NOCTURNAL}$ patterns over a 6-hour period are shown in Figure 2. There were no differences in the $FFA_{NOCTURNAL}$ patterns or FFA $AUC_{Noc\ 6HR}$ by prediabetes status ($p_{between}>0.05$), even after adjusting for age, sex, pubertal status, and total percent body fat. Additionally, $insulin_{NOCTURNAL}$ patterns and INS $AUC_{NOCTURNAL}$ did not differ between those with prediabetes and NGT ($p=0.10–0.14$). Insulin levels from 0300hrs to 0500hrs were significantly higher in participants with prediabetes than NGT ($p=0.03- <0.05$).

Given the importance of the pubertal transition and visceral fat in affecting insulin resistance and risk for T2DM, we explored the interaction between pubertal status and visceral fat. Overall, we found that a more advanced pubertal status interacted with visceral fat to predict a lower insulin sensitivity and DI ($p<0.05$). Therefore, predictors of insulin sensitivity, AIR_G, and DI by pubertal status are shown in Table 2. Visceral fat was more strongly related to a lower insulin sensitivity in post-pubertal ($\beta_{standardized\ post\text{-}pubertal}= -0.69$, $p<0.001$) than pubertal adolescents ($\beta_{standardized\ pubertal} = -0.44$, $p<0.001$). Additionally, visceral fat accounted for 27% of the variance explained in insulin sensitivity in post-pubertal adolescents but only 13% in pubertal adolescents. FFA_F showed a trend for an inverse association with insulin sensitivity ($\beta_{standardized}= -0.16$, $p=0.08$) in post-pubertal but not pubertal adolescents ($p=0.20$). In post-pubertal adolescents, only visceral fat was related to AIR_G ($\beta_{standardized}= 0.39$, $p=0.01$) while age and visceral fat were related to AIR_G ($\beta_{standardized}= -0.27$, $p=0.02$ and $\beta_{standardized}= 0.38$, $p=0.004$, respectively).

Both visceral fat and FFA_F were related to a lower DI in pubertal adolescents ($\beta_{standardized}= -0.44$, $p=0.003$ and $\beta_{standardized}= -0.29$, $p=0.02$, respectively), each accounting for 10% and 7% of the variance. Conversely, only age was related to a lower DI in pubertal adolescent ($\beta_{standardized}= -0.27$, $p=0.02$), accounting for 1.7% of the variance. FFA AUC_{OGTT} and FFA $AUC_{NOCTURNAL}$ were not related to insulin sensitivity, AIR_G, DI (data not shown).

DISCUSSION

In overweight and obese Latino adolescents, we found that FFA_F, but not FFA_{OGTT} nor $FFA_{NOCTURNAL}$, was independently associated with prediabetes. Visceral fat was negatively related to insulin sensitivity and secretion in all adolescents, while in post-pubertal adolescents, FFA_F and visceral fat were both independently and negatively related to β-cell function. Together, these results suggest that although visceral fat may contribute to insulin sensitivity and secretion across the pubertal transition, FFA_F may play an additional role in prediabetes and β-cell function after puberty is complete.

Despite data in adults showing an association between elevated FFA and T2DM risk [24, 25], there is little research in children and adolescents. In studies of European Caucasian children, no association was observed between FFA_F and insulin resistance using HOMA-IR [10, 11]. In an elegant study among Caucasian and African-American (AA) adolescents, overall plasma FFA and various long and short-chain acylcarnitine species did not differ in those who were NGT, obese, or had T2DM [26]. In contrast, other studies have shown evidence of alterations in FFA and T2DM risk. Cali et al. reported increased fasting plasma FFA in children with prediabetes vs. those with NGT in a group of Caucasian and African-American children [8]. In a group of AA women and girls, Goree et al. showed that increased FFA flux was related to insulin secretion and action [9]. In our study, we found elevated levels of FFA_F in prediabetic vs. NGT adolescents. A plausible explanation involves insulin resistance of adipose tissue, leading to increased lipolysis and increased release of FFA into the bloodstream, which results in elevated FFA_F. Over time, this process may stress β-cells and impair adequate secretion of insulin post-prandially and therefore results in decreased ability to suppress FFA and increased risk for T2DM. Future longitudinal data are needed to examine this hypothesis.

Elevation in FFA during the night may also be relevant in the pathogenesis of T2DM. FFA have been shown to be elevated between 4–8 AM in those with T2DM [18] and in a canine model for insulin resistant metabolic disease, dogs fed a high fat diet for 6 weeks developed visceral fat and insulin resistance [27]. Studies from the canine model demonstrated that after 6 weeks of high fat feeding, these “pre-diabetic” dogs had elevated $FFA_{NOCTURNAL}$ levels when compared to controls and daytime levels between the two groups did not differ [17]. Our current analysis provided a unique opportunity to examine whether $FFA_{NOCTURNAL}$ levels might be elevated in Latino adolescents with prediabetes compared to those who were NGT. Contrary to our hypothesis, we found that overall exposure to $FFA_{NOCTURNAL}$ did not differ significantly between participants with prediabetes and those with NGT in pubertal adolescents.

Insulin resistance has been also closely associated with an elevation of plasma FFA. The “lipotoxicity theory” of T2DM pathogenesis states that in the presence of insulin resistance, increased lipolysis, and increased fatty acid flux constitute a major pathway of progression to diabetes [28, 29]. Visceral fat has been shown to contribute to elevated FFA in obesity and to be a strong predictor of T2DM [27]. The increased mobilization of FFA contributes to adipose tissue insulin resistance, exacerbating the inability of insulin to suppress lipolysis, and possibly leading to a vicious cycle of increasing levels of plasma FFA. The increase in

FFA flux is proposed to lead to β-cell dysfunction and a gradual decrease in insulin secretion, likely due to increased β-cell apoptosis [30]. In prospective studies in Pima Indians, elevated FFA_F concentrations were an independent risk factor for the development of T2DM, possibly by reducing insulin secretion [31]. Moreover, Salgin et al [32] reported data from a large longitudinal study where in both children and adults, higher FFA_F was associated with lower insulin secretion following a 30min oral glucose challenge in children with NGT. Finally, in a study using an overnight intravenous lipid infusion, Hughan et al. [33] showed declines in insulin sensitivity and β-cell function in AA and Caucasian overweight and obese children, indicating β-cell lipotoxicity. Collectively, these studies show that elevated FFA may lead to alterations in insulin secretion and β-cell function, either independently or in conjunction with obesity. However, none of the aforementioned studies assessed differences in FFA by pubertal status. Given the natural progression of worsening insulin sensitivity during the pubertal transition [16], assessing the role of puberty is key in examining the progression of T2DM, particularly in Latino children who have been shown to have high visceral adiposity and insulin resistance [34] and in whom DI progressively deteriorates across puberty [16]. We showed that in pubertal adolescents, age was the independent predictor of DI whereas in post-pubertal adolescents, FFA_F and visceral fat were the independent predictors of DI. Interestingly, visceral fat, but not FFA_F, was the primary predictor of insulin sensitivity and AIR_G in all adolescents. Our results suggest that whole body insulin sensitivity and insulin secretion may be more strongly related to visceral fat metabolism while β-cell dysfunction may a related to both increased visceral fat and elevated FFA.

To our knowledge, this is the first study to describe $FFA_{NOCTURNAL}$ and to examine differences in FFA_F and FFA_{OGTT} in an exclusively Latino youth population. Our use of recently recommended A1C cutoffs [34] for defining prediabetes makes this the first study to do so in the examination of FFA levels and T2DM risk. By using the A1C criteria for T2DM risk, we were able to identify more youth with heightened diabetes risk (i.e. prediabetes). Secondly, by using rigorous direct measures of insulin action and secretion were we able to better determine relationships between FFA and insulin sensitivity/secretion dynamics than would not have been possible using coarser measures, such as fasting insulin or HOMA-IR. Despite these strengths, the FSIVGTT method with Minimal Modeling is designed only to provide whole body (ie, predominantly muscle) insulin sensitivity, but is unable to isolate hepatic insulin sensitivity, nor to determine hepatic insulin clearance that can be achieved by C-peptide modeling. Since we did not measure C-peptide during the IVGTT, we cannot determine the degree of contribution to AIR related to hepatic insulin clearance, which is a limitation particularly given the role that FFA released from visceral fat may play on hepatic insulin catabolism [35, 36]. Next, our FFA assay only supplied total concentrations of FFA and it did not provide information on specific FFA (i.e., saturated and unsaturated fatty acids). This may be particularly important since saturated and unsaturated FFA may have differential effects on muscle insulin signaling, as has been shown in vitro [37, 38], and in biopsies from children [39]. Another limitation of this study is that $FFA_{NOCTURNAL}$ data was limited to pubertal adolescents, which inhibited our ability to compare differences in pubertal and post-puberty with regard to $FFA_{NOCTURNAL}$ and $insulin_{NOCTURNAL}$ exposure. Finally, our studies did not include non-Latino ethnicities,

lean, or pre-pubertal children, so our findings cannot be generalized beyond overweight and obese, pubertal and post-pubertal Latino adolescents.

In conclusion, in a group of overweight and obese Latino adolescents, FFA_F, but not FFA_{OGTT} or $FFA_{NOCTURNAL}$, was independently associated with prediabetes. These results suggest that visceral fat independently contributed to insulin sensitivity and secretion across the pubertal transition, but elevated FFA_F along with increased visceral fat may play a concomitant role in βcell dysfunction during post-puberty in overweight Latino adolescents. Our findings are supported by the natural progression of worsening insulin sensitivity during the pubertal transition, which may have an impact on the role of circulating FFA and development of T2DM.

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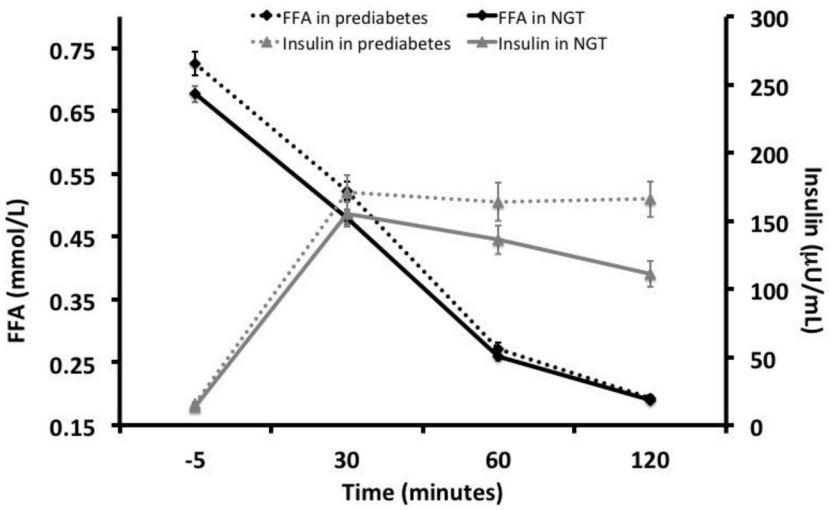

Figure 1. FFA and insulin concentration during the OGTT in those with prediabetes vs. NGT

Repeated measures ANCOVA; covariates: age, sex, pubertal stage, total percent body fat and visceral fat.

$P<0.05$

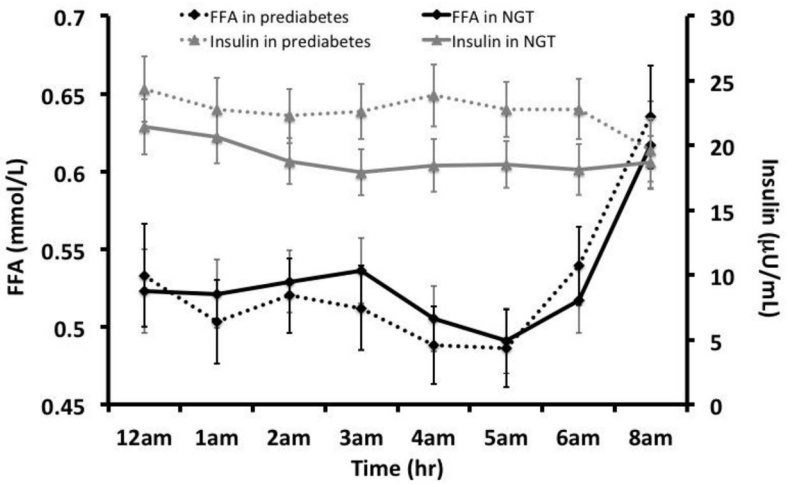

Figure 2.

Nocturnal plasma FFA and insulin 8-hour profiles by prediabetes status in pubertal adolescents (n=55)

Table 1

Anthropometric and metabolic parameters of Latino adolescents (n=164) 2-way ANOVA; #sample size =55

	NGT (n=107)	Pre-diabetes (n=57)	P-value
Age (years)	14.3 ± 2.4	14.0 ± 2.6	0.532
Sex (Male/Female)	62/45	33/24	
Tanner Stage			
2	27	20	
3	12	9	
4	20	8	
5	48	20	
Weight (kg)	78.3 ± 20.0	83.0 ± 23.8	0.186
Height (cm)	160.5 ± 11.6	159.4 ± 12.4	0.563
BMI (kg/m^2)	30.1 ± 5.8	32.3 ± 6.8	**0.030**
BMI z-score	1.9 ± 0.5	2.1 ± 0.5	**0.017**
Total Fat mass (kg)	29.2 ± 1.0	30.6 ± 1.1	0.401
Total Lean mass (kg)	45.7 ± 1.2	47.6 ± 1.3	0.340
Total % Fat	37.5 ± 6.7	38.0 ± 7.2	0.672
Visceral Adipose Tissue (L)	1.6 ± 1.0	1.9 ± 1.2	0.137
Subcutaneous Abdominal Adipose Tissue (L)	6.5 ± 3.3	7.2 ± 3.9	0.193
Fasting Glucose (mg/dL)	86.7 ± 5.6	90.2 ± 7.7	**0.003**
2-hr Glucose (mg/dL)	115.2 ± 15.1	138.6 ± 23.3	**<0.001**
HbA1c (%)	5.5 ± 0.3	5.9 ± 0.3	**<0.001**
HbA1c (mmol/mol)			**<0.001**
Fasting Insulin (μU/mL)	13.1 ± 8.9	16.0 ± 11.3	0.091
Insulin AUC$_{OGTT}$ (μU/mL x min)	175.1 ± 110.0	229.1 ± 163.8	**0.029**
Insulin AUC$_{NOCTURNAL}$ (μU/mL x min)	52.4 ± 26.0	71.7 ± 39.0	**0.040**
Fasting FFA (mmol/L)	0.68 ± 0.14	0.73 ± 0.14	**0.032**
FFA AUC$_{OGTT}$ (mmol/L x min)	0.61 ± 0.13	0.66 ± 0.13	**0.045**
FFA AUC$_{NOCTURNAL}$ (mmol/L x min)	1.56 ± 0.36	1.51 ± 0.28	0.542
Insulin sensitivity [(x10^{-4} min^{-1})/(μU/mL)]	2.02 ± 1.19	1.45 ± 0.86	**0.001**
Acute Insulin Response (μU/mL x 10min)	1302 ± 853	1448 ± 987	0.351
Disposition index	2188 ± 1087	1735 ± 1035	**0.041**

Table 2

Predictors of SI, AIR_G, and DI in overweight and obese Latino adolescents

	Pubertal			Post-Pubertal		
	Standardized β	**p-value**	**Change in Variance**	**Standardized β**	**p-value**	**Change in Variance**
Model 1: SI						
Age	−0.092	0.405	3.5%	0.142	0.107	2.9%
Sex	−0.133	0.243	13.8%	−0.048	0.724	12.4%
Total % Fat	−0.124	0.239	0.2%	−0.030	0.811	11.4%
Visceral Fat	**−0.436**	**<0.001**	**13.0%**	**−0.685**	**<0.001**	**27.3%**
Fasting FFA	−0.127	0.201	1.5%	*−0.163*	*0.081*	*2.3%*
Model 2: AIR_g						
Age	**−0.270**	**0.024**	**1.7%**	0.005	0.964	0.1%
Sex	0.096	0.431	8.2%	0.086	0.639	6.6%
Total % Fat	−0.054	0.631	1.2%	0.175	0.307	0.4%
Visceral Fat	**0.378**	**0.004**	**9.8%**	**0.390**	**0.011**	**9.9%**
Fasting FFA	0.126	0.236	1.5%	−0.162	0.198	2.3%
Model 3: DI						
Age	**−0.382**	**0.003**	**10.4%**	0.12	0.278	1.5%
Sex	−0.026	0.839	0.3%	0.053	0.755	2.7%
Total % Fat	−0.179	0.133	2.7%	0.059	0.709	7.9%
Visceral Fat	−0.013	0.924	0%	**−0.435**	**0.003**	**10.2%**
Fasting FFA	0.013	0.906	0%	**−0.290**	**0.016**	**7.3%**

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