Oral Glucose Tolerance Test Glucose Peak Time Is Most Predictive of Prediabetes and Hepatic Steatosis in Obese Girls

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Obese adolescent girls are at increased risk for type 2 diabetes, characterized by defects in insulin secretion and action. We sought to determine if later glucose peak timing (>30 minutes), 1-hour glucose >155 mg/dl, or monophasic pattern of glucose excursion during an oral glucose tolerance test (OGTT) reflect a worse cardiometabolic risk profile. Post-pubertal overweight/obese adolescent girls without diabetes were studied (N = 88; age, 15.2 ± 0.2 years; body mass index percentile, 97.7 ± 0.5). All participants completed an OGTT and body composition measures. Thirty-two girls had a four-phase hyperinsulinemic euglycemic clamp with isotope tracers, vascular imaging, and muscle mitochondrial assessments. Participants were categorized by glucose peak timing (≤30 min = early; >30 min = late), 1-hour glucose concentration (±155 mg/dL) and glucose pattern (monophasic, biphasic). Girls with a late (N = 54) vs earlier peak (n = 34) timing had higher peak glucose ($P < 0.001$) and insulin ($P = 0.023$), HbA1c ($P = 0.021$); prevalence of hepatic steatosis (62% vs 26%; $P = 0.003$) and lower oral disposition index ($P < 0.001$) and glucagon-like peptide-1 response ($P = 0.037$). When classified by 1-hour glucose, group differences were similar to peak timing, but minimal when classified by glucose pattern. In the >155 mg/dL group only, peripheral insulin sensitivity and fasting free fatty acids were worse. A later glucose peak or >155 mg/dL 1-hour glucose predicts metabolic disease risk in obese adolescent girls. This may defect incretin effects and first phase insulin response, and muscle and adipose insulin resistance.

Type 2 diabetes (T2D) prevalence is on the rise, with the obesity epidemic playing a major role, and youth are increasingly at risk for prediabetes and T2D. The burden from a public health standpoint also continues to increase, with the economic cost in 2015 alone estimated to be ~1.3 trillion dollars [1]. T2D is associated with an increased risk for comorbid conditions, including cardiovascular disease, nonalcoholic fatty liver disease, obstructive sleep apnea, depression, and cancer [2]. T2D represents the end of a spectrum of disease, which includes

Abbreviations: AUC, Area under the curve; BMI, body mass index; cIMT, carotid intimal medial thickness; FFA, free fatty acid; GIR, glucose infusion rate; IC 50, 50% inhibitory concentration; oDI, oral disposition index; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; IR, insulin resistance; ISI, insulin sensitivity index; oAIRg, oral acute insulin response to glucose; OGTT, oral glucose tolerance test; PCr, Phosphocreatine; Ra, rate of appearance; ROC, receiver operating characteristic; T2D, type 2 diabetes.

insulin resistance (IR) and β-cell failure with resulting hyperglycemia [2]. The degree of β-cell failure and hyperglycemia varies by patient population type [3]. Understanding this early pathophysiology is required for developing appropriate preventive strategies in youth, because once T2D develops in this population, the progression and onset of comorbidities appears faster than in adults [4].

Previous research has attempted to identify whether the glucose response to an oral glucose tolerance test (OGTT) can be predictive of cardiometabolic disease and risk for T2D. Beyond the definitions of impaired glucose tolerance (IGT) at the 2-hour time point, additional work has focused on describing the pattern of the glucose excursion, the timing of the glucose peak, and intermediate time points, including glucose concentrations 1 hour postglucose ingestion. Nolfe *et al.* [5] found that in obese youth, morphologies of the glucose curve seemed to reflect different phenotypes of insulin secretion and action. Kim *et al.* [6] compared OGTT to gold-standard measures of insulin sensitivity and secretion in obese youth. They found that in youth of all stages of puberty, a monophasic pattern (*i.e.*, one glucose peak) was associated with higher glucose and insulin excursions, a decreased early phase insulin response, and hepatic and peripheral IR [6]. However, recent data from adults at risk for T2D indicated that the timing of the glucose peak, rather than the pattern of the glucose curve, was more predictive of prediabetes status [7]. A 1-hour glucose peak >155 mg/dL has also been associated with worse β-cell function and potential increased risk for developing T2D in overweight/obese adolescents [8]. To our knowledge, comparisons of glucose excursion patterns compared with peak time or 1-hour glucose have not been performed in youth.

The pathogenesis of T2D in youth varies by sex, and hormonal changes in puberty contribute to a differential disease evolution compared with adults, a phenomenon first documented by Amiel *et al.* [9]. This finding of IR peaking midpuberty has been replicated many times [10]. Whereas adults with T2D can often control glucose levels with lifestyle changes for many years, ~50% of youth in the Treatment Options for Type 2 Diabetes in Adolescents and Youth study failed to respond to an intervention of lifestyle combined with medication and required escalation to insulin therapy by 2 years postdiagnosis [4]. Sex-dependent changes in body composition during puberty are also thought to play a role in the insulin sensitivity changes observed, with girls having the worst IR [11]. Further, in the Treatment Options for Type 2 Diabetes in Adolescents and Youth study, there was a female predominance of T2D and females with T2D also had a higher rate of failing single-agent metformin therapy, indicating that adolescent girls have an increased risk for both the development and progression of T2D, underscoring the need for more data in obese girls [4].

Based on the limited literature describing the distinct OGTT pattern/timing and potential underlying mechanisms in obese girls, who are at high risk for cardiometabolic disease and T2D, we sought to determine whether OGTT glucose concentration timing or glucose pattern was more informative for predicting disease and identifying underlying abnormal physiology.

1. Methods

A. Study Population

Eighty-eight participants ages 12 to 19 years were enrolled from the AIRS (Role of Androgens and Obesity in Insulin Resistance and Cardiovascular Disease in Polycystic Ovarian Disease, prior to NCT) and APPLE (Assessment of Hepatic Glucose and Fat Regulation in Overweight Adolescent Girls; NCT02157974) cohorts. Inclusion criteria were female sex, overweight/ obese status [body mass index (BMI) ≥ 85th percentile for age and sex], postpubertal status (Tanner stage 5), and sedentary status (<3 hours of regular exercise/week; validated with both a 3-day activity recall and 7-day accelerometer use). Exclusion criteria were as follows: diabetes, pregnancy, medications affecting insulin sensitivity (including metformin, antipsychotics, and systemic steroids), and hormonal contraception. The study was approved by the Colorado Multiple Institutional Review Board and Children's Hospital Colorado Scientific Advisory and Review Committee. Informed consent was obtained from all participants

18 to 20 years old, and parental consent and participant assent from all participants <18 years old.

B. Procedures

OGTTs were performed in the morning after a 12-hour fast. Fasting laboratories were drawn prior to the start of the OGTT and included markers of inflammation, lipid panel, adiponectin, and leptin. Samples were drawn at 0, 10, 20, 30, 60, 90, and 120 minutes for glucose and insulin, and 0, 10, 20, and 30 minutes for C-peptide. Samples for total GLP-1 were drawn at 0, 10, 20, 30, 60, and 90 minutes.

B-1. Body composition and mitochondrial function

Total body composition by dual-energy x-ray absorptiometry was performed to determine fat free mass. Visceral fat and hepatic fat fraction were determined by MRI with quantitation of fat performed using the Dixon method, as previously described [12]. Hepatic steatosis was defined as a hepatic fat fraction >5.5%. All MRI and ^{31}phosphorus magnetic resonance spectroscopy equipment and procedures have been previously described [13–15]. In summary, imaging and magnetic resonance spectroscopy were performed on a General Electric (Waukesha, WI) 3T with HDx MRI running version 15M4 software and a Siemens 3T with a Skyra platform (Seimens, Munich, Germany). The scanners are also equipped with multi-nuclear spectroscopy hardware and research software upgrades and used a custom built ^{1}H/^{31}P leg coil (Clinical MR Solutions, Brookfield, WI). The ^{31}phosphorus magnetic resonance spectroscopy exercise protocol consisted of measurements during rest for 60 seconds, isometric plantar flexion for 90 seconds at 70% maximal volitional contraction, and recovery for 8 minutes postexercise. Analysis of the spectroscopy was done as previously reported [13, 15–17]. Phosphate peaks are fit with time domain fitting using jMRUi and ADP calculated [18]. Calculations used data from the immediate recovery period. The rate of oxidative phosphorylation is calculated as ΔPhosphocreatine (PCr)/time from the first 10 seconds following cessation of exercise, and time constants for ADP and PCr were calculated via regression analyses with Sigmaplot (Systat Software, Inc., San Jose, CA).

B-2. Physical activity

A 3-day pediatric activity recall questionnaire recalling the physical activity of the three previous days was completed with study staff assistance [19]. Participants also wore an Actigraph GT3x accelerometer (Actigraph Corp., Pensacola, FL) for 7 days. All data collected were corrected for wear time and categorized into the following age-appropriate activity levels: sedentary, light, lifestyle, moderate, vigorous, and very vigorous [20].

B-3. Dietary intake

Customary macronutrient pattern was ascertained via diet interview by study staff using the SEARCH for Diabetes in Youth food frequency questionnaire, modified to incorporate common food choices among ethnically and regionally diverse youth aged 10 to 19 [19].

B-4. Hyperinsulinemic euglycemic clamp procedure

Briefly, the study day was preceded by 3 days of restricted physical activity and a fixed-macronutrient, weight-maintenance diet (55% carbohydrates, 30% fat, 15% protein). Following an inpatient 12-hour overnight monitored fast, a four-phase hyperinsulinemic euglycemic clamp was performed to determine adipose, hepatic and muscle insulin sensitivity, as previously described [21]. Following a 2-hour basal equilibration phase, consecutive

insulin doses for each 1.5-hour phase were 10, 16, and 80 mU/m^2/min, based on our [12, 22] and others' [23] previous experience with the higher insulin requirements in pubertal youth. Twenty percent dextrose (spiked with 6,6-^{2}H$_2$ glucose) was infused to maintain blood glucose at 95 mg/dL, with blood samples drawn every 5 minutes and run on a bedside Yellow Springs Instrument glucose analyzer. Glucose infusion rate (GIR) [mg.kglean.min] or M-value was determined based on steady-state measurements from the final 30 minutes of the final, high-dose phase of the clamp, as was GIR/steady-state insulin concentration.

B-5. Tracer infusion protocol

At 6 AM, baseline blood samples to measure background enrichment of glucose and glycerol, and concentrations of glucose, insulin, glycerol, and free fatty acid (FFA) were obtained. Then, a bolus of 4.5 mg/kg [6,6-^{2}H$_2$] glucose (Isotec, Miamiville, IA), followed by a continuous infusion at 0.03 mg/kg/min [6,6-^{2}H$_2$] glucose was paired with a primed (1.6 μmol/kg), then constant (0.11 μmol/kg/min) infusion of ^{2}H$_5$glycerol [24] (Isotec, Miamiville, IA). During the last 30 minutes of each of the four clamp phases, four samples, each 10 minutes apart, were drawn for glucose, glycerol, FFA, and insulin concentrations, and glucose and glycerol isotope tracer enrichments [25].

B-6. Vascular measures

Carotid intimal medial thickness (cIMT), carotid artery β-stiffness index and compliance, and distension were assessed with ultrasound as previously described [26]. Briefly, far wall cIMT was measured from a longitudinal two-dimensional B-mode image obtained with a Vivid 7 (General Electric, Fairfield, CT) ultrasound and analyzed using Vascular Analysis Tools software version 5.0 (MIA, Coralville, IA). All cIMT measurements were made at end diastole. The average of four separate measurements per subject were used for analysis. Blood pressure measures were performed with a manual sphygmomanometer in triplicate after 15 minutes of supine rest.

B-7. Sample analysis

Laboratory assays were performed by the research center core laboratory or Children's Hospital Colorado clinical laboratory. Serum insulin, leptin, and adiponectin were analyzed with radioimmunoassay (Millipore, Billerica, MA); plasma glycerol (R-Biopharm, Marshall, MI), FFA (Wako Chemicals, Inc., Richmond, VA), and GLP-1 (Alpco Diagnostics, Salem, NH) were determined enzymatically. HbA1c was measured by Diabetes Control and Complications Trial-calibrated ion-exchange high-performance liquid chromatography (Bio-Rad Laboratories, Hercules, CA). Total cholesterol, high density lipoprotein cholesterol, and triglyceride assays were performed enzymatically on a Hitachi 917 autoanalyzer (Boehringer Mannheim Diagnostics, Indianapolis, IN). Low density lipoprotein cholesterol levels were calculated by the Friedewald Equation (11). High sensitivity C-reactive protein was measured via immunoturbidimetric assay (Beckman Coulter, Brea, CA), C-peptide via chemiluminescent immunoassay (DiaSorin, Stillwater, MN) and estradiol and progesterone via chemiluminescent immunoassay (Beckman Coulter). Aspartate aminotransferase and alanine aminotransferase were measured via chemical reaction on a Vitros® 5600 chemistry system (Ortho Clinical Diagnostics, Rochester, NY). Dehydroepiandrosterone-sulfate, total testosterone, and SHBG were performed via liquid chromatography–mass spectrometry, and antimullerien hormone was measured via chemiluminescent immunoassay, all by Esoterix (Calabasas Hills, CA). Free androgen index was calculated from total testosterone and SHBG.

Analyses of ^{2}H$_5$glycerol and 6,6-^{2}H$_2$ glucose were done using a modification of the negative ion chemical ionization gas chromatography mass spectrometry as previously described [25, 27, 28].

C. Calculations

Monophasic and biphasic classifications were determined based on the definition described by Tschritter *et al.* [29]. A monophasic pattern consisted of a gradual increase in glucose concentrations followed by a decrease. A biphasic pattern consisted of a second rise in glucose concentrations after the decrease. A change of ≥4.5 mg/dL constituted an "increase" or "decrease." Area under the curve (AUC) calculations were done using the trapezoidal method. Zero-, 30-, 60-, 90-, and 120-minute time points were used in calculating the AUC for glucose and insulin. Zero-, 15-, and 30-minute time points were used to calculate AUC for c-peptide. Zero-, 10-, 20-, 30-, 60-, and 90-minute time points were used to calculate AUC for GLP-1. Insulin sensitivity index (ISI) was modeled using the Matsuda Equation (10,000/square root (fasting plasma glucose*fasting plasma insulin*mean G*mean I)). The oral acute insulin response to glucose (oAIRg) was calculated as area under the insulin curve, from zero, over the first 15 minutes of the OGTT. Relative β-cell function was modeled by the oral disposition index (oDI), calculated as ISI*oAIRg.

C-1. Tracer calculations

All isotopic enrichments were corrected for background enrichments. The glucose and glycerol rate of appearance (Ra), rate of disappearance, and metabolic clearance rate over the last 30 minutes of each phase of the clamp were calculated using the Steele non-steady-state equation, accounting for "spiked" glucose in the 20% dextrose infusion [27]. To describe the interaction between Ra across the different insulin concentrations of each phase, the 50% inhibitory concentration (IC 50) was calculated. The Ra and log insulin for each phase were plotted, and the slope of the curve of all four points was used to calculate the insulin concentration at the location on the curve equal to 50% suppression of the basal Ra, as described previously [27]. The inverse of this relationship, or predicted Ra at the average insulin concentration for the group was also calculated, and called the "predicted Ra."

D. Statistics

The distributions of all variables were examined and results presented as mean ± SD, median (minimum to maximum), or proportions, as appropriate. Using the OGTT glucose concentrations, participants were categorized in three ways: based on the (1) timing of their glucose peak, early = peak ≤ 30 minutes or late = peak > 30 minutes, (2) value of their 1-hour glucose (<155 mg/dL or >155 mg/dL), and (3) pattern of the glucose curve, either biphasic or monophasic. Those with "unclassified" OGTT glucose patterns were excluded from further comparisons by pattern only.

Group comparisons were made using χ^2 or Fisher's exact test for proportions and the t test or Kruskal-Wallis test for continuous variables. Impaired fasting glucose (IFG) (fasting glucose > 100 mg/dL) and IGT (2-hour post-OGTT glucose > 140 mg/dL) were used to define prediabetes status [30]. Receiver operating characteristic (ROC) curve analysis was used to evaluate the respective abilities of a later (>30 minutes) peak time, higher 1-hour glucose, and a monophasic pattern to predict worse outcomes in cardiometabolic risk measures and relative β-cell function. χ^2 analysis was used to determine the relationship between timing or pattern to defined components of the metabolic syndrome including a waist-to-hip ratio >0.85, hepatic fat fraction >5.5%, systolic blood pressure >130 mm Hg and triglycerides >150 mg/dL. Significance tests were two-tailed. P values < 0.05 were considered significant. All statistical analyses were performed with Sigma Plot Version 13.0 (Systat Software, San Jose, CA).

2. Results

A total of 88 overweight and obese nondiabetic adolescent girls ages 12 to 19 years were enrolled. Thirty-four of the participants had regular menses, and 54 had polycystic ovarian

Table 1. Demographics and Glucose Parameters by OGTT Descriptor

	30-Min Peak, N = 34	>30-Min Peak, N = 54	*P* Value	1 H < 155 mg/dL, N = 51
Physical characteristics				
Age, y	15.4 ± 1.6	15.0 ± 1.9	0.262	15 (14–16)
Race (White/Hispanic/ black/biracial)	(18/11/5/0)	(15/31/7/1)	0.074	(19/24/7/1)
Weight, kg	92.5 ± 15.6	92.9 ± 16.5	0.921	96.0 (80.4–101.0)
BMI, kg/m^2	34.4 (29.5–36.9)	34.3 (31.0–38.0)	0.606	34.5 (30.1–37.8)
BMI percentile	98.5 (96.6–98.9)	98.4 (96.9–99.3)	0.257	98.5 (96.8–99.1)
Glucose/insulin characteristics				
Prediabetes	**21%**	**52%**	**0.007**	**14%**
HbA1C, %	**5.2 ± 0.3**	**5.4 ± 0.3**	**0.021**	**5.2 (5.1–5.4)**
Fasting glucose, mg/dL	87 ± 6	89 ± 8	0.277	**85 (82–91)**
Fasting insulin, μU/mL	18 (16–24)	22 (16–31)	0.219	**18 (15–27)**
Fasting C-peptide, ng/mL	**2.3 (1.9–3.1)**	**2.9 (2.3–3.9)**	**0.023**	2.6 (2.2–3.1)
Peak glucose, mg/dL	**144 ± 18**	**163 ± 23**	**<0.0001**	**144 (131–150)**
Peak insulin, μU/mL	**193 (138–408)**	**316 (192–483)**	**0.023**	**226 (144–319)**
Peak C-peptide, ng/mL	10.9 ± 4.4	9.9 ± 3.4	0.267	9.8 ± 3.9
ISI	**1.86 (1.46–2.85)**	**1.54 (0.88–2.01)**	**0.004**	**1.85(1.49–2.81)**
Oral AIRg Min*(μU/mL)	508 (381–1249)	465 (311–705)	0.109	493 (316–769)
oDI	**1085 (727–1956)**	**592 (395–974)**	**<0.001**	**973 (587–1410)**

syndrome, but analyses showed that polycystic ovarian syndrome status was unrelated to each of the three OGTT categories. Thirty-four girls had an early glucose peak vs 54 with a late glucose peak; 51 had a 1-hour glucose <155 mg/dL vs 38 with a 1-hour >155 mg/dL; and 33 had a biphasic curve vs 51 with a monophasic curve. Four participants were excluded from pattern categorization due to an "unclassified" pattern to their OGTT glucose curve.

Participant demographics and OGTT details are shown in Table 1, divided by each measure. Age, race, and BMI were similar whether dividing by time of peak glucose, 1-hour glucose, or pattern (Table 1). All OGTT groups were equally sedentary and had a similar dietary intake of both micro and macronutrients including total calories, total fat, carbohydrate, protein, saturated fat, and fructose per dietary recall questionnaire (data not shown).

Peak timing and 1-hour glucose categorizations identified similar populations, with 88% of early peak subjects having a 1-hour glucose <155 mg/dL, and 60% of late peak participants having a 1-hour glucose >155 mg/dL ($P < 0.001$). There was a weaker association between timing and pattern, with 58% of those with biphasic patterns peaking early vs 29% of those with monophasic patterns ($P = 0.02$). Pattern and 1-hour glucose had a similarly weak association: 79% of those with biphasic patterns had 1-hour glucose <155 mg/dL vs 50% of those with monophasic patterns ($P = 0.01$).

Those with an early glucose peak were more likely to have prediabetes (IFG or IGT) on the OGTT ($P = 0.007$) as were those with a 1-hour >155 mg/dL ($P < 0.0001$), whereas no association was found between having a monophasic glucose pattern and prediabetes ($P = 0.75$). Additionally, a later >30-minute peak time and 1-hour glucose > 155 mg/dL were associated with higher HbA1c % ($P = 0.02$ for both), glucose AUC ($P < 0.0001$ for both), peak glucose ($P < 0.0001$ for both), and insulin ($P = 0.023$, $P < 0.001$, respectively). Fasting c-peptide concentrations were higher in the late vs early peak group ($P = 0.023$). Between the 1-hour <155 vs >155 mg/dL groups, fasting glucose ($P = 0.005$) and insulin ($P = 0.04$) were also different. In comparisons by pattern, a monophasic pattern was only associated with higher peak glucose ($P = 0.005$). Those who peaked early, or had a 1-hour <155 mg/dL had higher insulin sensitivity calculated by ISI ($P = 0.004$, $P < 0.001$, respectively). Whereas absolute insulin secretion modeled by the oAIRg was not different between groups, the relative first-phase insulin secretion, reflected by the oDI, was better in those with an early glucose peak and lower 1-hour glucose ($P < 0.001$, $P = 0.003$, respectively).

Table 1. Demographics and Glucose Parameters by OGTT Descriptor (Continued)

1 H > 155 mg/dL, N = 37	*P* Value	Biphasic, N = 33	Monophasic, N = 51	*P* Value
15 (13–17)	0.857	15.11 ± 1.6	15.4 ± 1.9	0.435
(12/20/6)	0.648	(16/14/3/0)	(17/25/8/1)	0.445
95.9 (77.2–100.1)	0.172	92.8 ± 18.5	92.9 ± 15.1	0.997
32.9 (29.4–37.0)	0.246	34.3 (29.7–37.2)	34.1 (30.0–37.6)	0.996
98 (96.8–99.1)	0.367	98.4 (96.7–99.0)	98.2 (96.9–99.2)	0.993
72%	**<0.001**	33%	39%	0.753
5.4 (5.2–5.6)	**0.022**	5.4 ± 0.3	5.3 ± 0.3	0.294
91 (86–95)	**0.005**	88 ± 7	88 ± 8	0.842
23 (16–37)	**0.044**	22 (15–30)	19 (16–29)	0.469
3.2 (2.2–4.2)	0.076	2.7 (2.1–3.4)	2.6 (2.2–3.6)	0.961
172 (164–187)	**<0.001**	145 (129–161)	**161 (145–171)**	**0.005**
374 (205–578)	**<0.001**	242 (145–369)	288 (169–440)	0.472
10.8 ± 3.7	0.266	10.4 ± 4.4	10.0 ± 3.3	0.632
1.25 (0.79–1.78)	**<0.001**	1.73 (1.26–2.62)	1.62 (1.09–2.18)	0.539
465 (324–729)	0.955	529 (335–848)	474 (312–704)	0.520
577 (349–837)	**0.003**	975 (538–1521)	689 (434–1255)	0.120

Data are shown as mean ± SD of the mean or median (25th percentile–75th percentile). Values with *P* < 0.05 in **bold.**
Prediabetes is either IFG or IGT status.
Abbreviation: AIR, acute insulin response.

Figure 1 shows the OGTT mean glucose and insulin concentration curves by peak time, peak pattern, and 1-hour value. Those with a >30-minute peak had a mean ($P < 0.001$) and AUC ($P < 0.001$) glucose concentration (Fig. 2A), with insulin excursions that parallel the glucose excursions (Fig. 1B; mean, $P < 0.004$; AUC, $P = 0.009$). Within the group having an earlier glucose peak, insulin levels also peaked earlier. Those with a later (>30 minutes) glucose peak are characterized by a lack of an early insulin peak, and instead had late and continuously rising insulin secretion. The glucose excursions were different between the biphasic and monophasic groups (Fig. 1C; AUC, $P = 0.002$). The insulin curves followed biphasic and monophasic patterns similar to those observed in glucose, but there were no differences in insulin curves between groups (Fig. 1D; AUC, $P = 0.193$). Participants with a 1-hour > 155 mg/dL had a greater mean and AUC glucose (Fig. 1E) and insulin (Fig. 1F) ($P < 0.001$ for all).

Figure 2 shows the GLP-1 response to OGTT by peak time, pattern of glucose curve, and 1-hour value. Whereas the GLP-1 response (Fig. 2A) was blunted in the >30-minute group (AUC, $P = 0.037$), there were no differences when groups were classified by pattern (Fig. 2B; AUC = 0.752) or 1-hour glucose value (Fig. 2C; $P = 0.986$).

A. Cardiometabolic Risk Markers

Rates of central obesity were not different based on OGTT classification (Table 2). When dividing by peak time, 62% of those with a late peak met the criteria for hepatic steatosis (>5.5% hepatic fat) compared with only 26% of the early peak group ($P = 0.003$). Between the 1-hour groups, 81% of those with >155 1-hour glucose had hepatic steatosis, compared with only 30% of those with a <155 1-hour glucose (<0.001). Hepatic steatosis was not distinguished by pattern classification. No other parameters of the metabolic syndrome, such as hypertension or hypertriglyceridemia, were distinguished by any of the methods.

However, in a subset of youth, hyperinsulinemic euglycemic clamp measurements of insulin sensitivity (Table 3) including GIR, GIR/steady-state insulin concentration, glucose IC 50, glycerol IC 50, fasting FFA, and steady-state FFA were not different between the groups, except that the 1-hour peak was related to GIR ($P = 0.019$) and fasting FFA
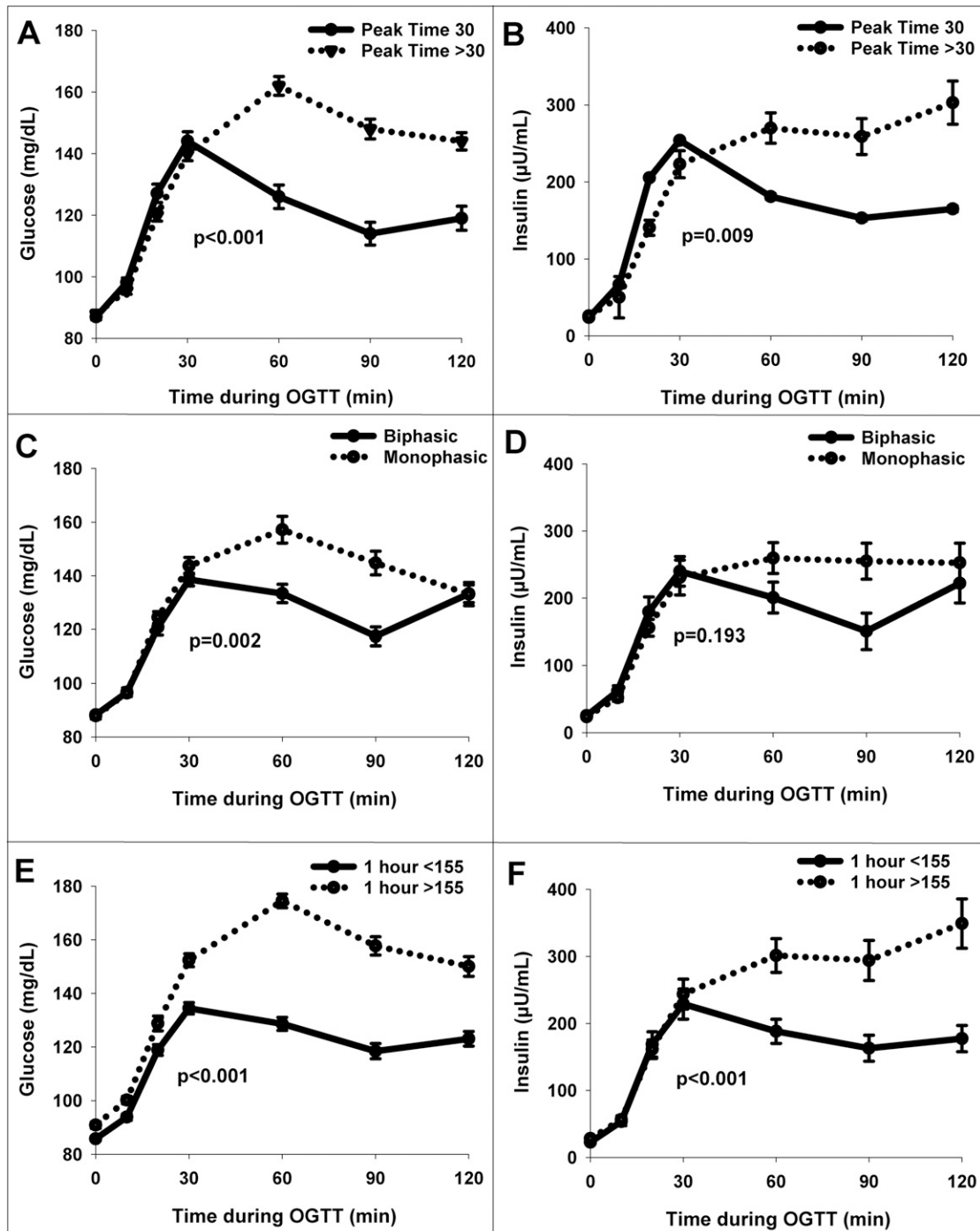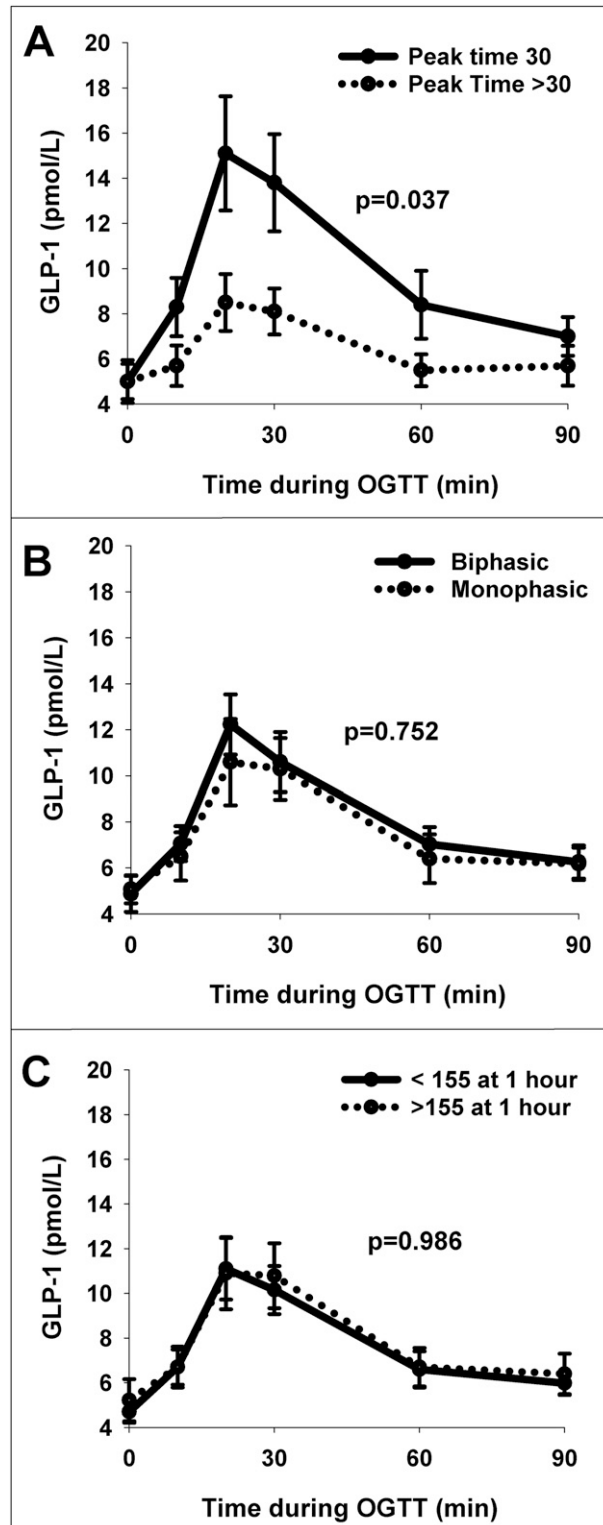
Figure 1. Glucose and insulin concentrations during OGTT per model. Data are shown as mean ± SEM. *P* values are for the comparison of the AUC for each group.

concentrations (*P* = 0.027). Measures of cardiovascular function and postexercise mitochondrial function were also not different.

A glucose peak >30 minutes predicted prediabetes (ROC AUC, 0.79; *P* < 0.0001) with a sensitivity and specificity of 85% and 57%, respectively, as did the 1-hour glucose (ROC AUC, 0.84; *P* < 0.0001, sensitivity 76%, specificity 88%). The monophasic pattern did not predict prediabetes status (ROC AUC, 0.50; *P* = 0.953). A glucose peak >30 minutes predicted having a waist-to-hip ratio >0.85 (ROC AUC, 0.64; *P* = 0.034) with a sensitivity and

Figure 2. GLP-1 response during OGTT per model. Data are shown as mean ± SEM. *P* values are for the comparison of the AUC for each group.

specificity of 38% and 80%, respectively. The 1-hour glucose did not predict a waist-to-hip ratio >0.85 (ROC AUC, 0.44; $P = 0.376$), nor did the monophasic pattern (ROC AUC, 0.48; $P = 0.352$). A glucose peak >30 minutes predicted hepatic steatosis (ROC AUC, 0.66; $P = 0.016$)

Table 2.   Metabolic Syndrome Components by OGTT Descriptor

	30-Min Peak, N = 34	>30-Min Peak, N = 54	*P* Value	1 H < 155 mg/dL, N = 51	1 H > 155 mg/dL, N = 38	*P* Value	Biphasic, N = 33	Monophasic, N = 51	*P* Value
Waist-to-hip ratio > 0.85, %	62	82	0.090	74	76	1.0	69	80	0.424
Hepatic steatosis (cutoff = 5.5%), %	**26**	**62**	**0.003**	**30**	**81**	**<0.001**	38	52	0.244
SBP > 130 mm Hg, %	23	11	0.212	18	10	0.351	16	16	1.000
TG >150 mg/dL, %	27	24	0.937	22	34	0.308	22	29	0.616

Data are shown as mean ± SD of the mean or median (25th percentile–75th percentile). Values with *P* < 0.05 in **bold**. Abbreviations: SBP, systolic blood pressure; TG , triglyceride.

with a sensitivity and specificity of 72% and 58%, respectively, as did the 1-hour glucose (ROC AUC, 0.79; $P < 0.0001$, sensitivity 77%, specificity 72%). The monophasic pattern did not predict hepatic steatosis (ROC AUC, 0.46; $P = 0.593$). None of the categories predicted an elevated blood pressure or triglycerides. Peak timing and 1-hour glucose also predicted oDI (AUC, 0.772; $P < 0.001$ timing; AUC, 0.681; $P = 0.004$ 1-hour glucose), whereas glucose pattern did not (AUC, 0.604; $P = 0.118$).

3. Discussion

Adolescent T2D has similarities and differences in comparison with T2D in adults, and some aspects may vary by sex [4]. We thus evaluated several different OGTT-based measures of glucose and insulin dynamics in obese adolescent girls, a population at high risk for cardiometabolic disease. We found that among adolescent overweight/obese girls, a later timing to glucose peak during an OGTT or a higher 1-hour glucose value during the OGTT were best able to distinguish glucose and insulin dynamics, as well as inflammation and hepatic steatosis. In contrast, these associations were not found when the groups were classified by OGTT curve pattern. ROC analysis supported that glucose peak timing >30 minutes or higher 1-hour glucose was a better parameter for predicting prediabetes and other risk factors of cardiometabolic disease compared with the monophasic pattern parameter. Based on the strong relationships between peak timing and oDI, we speculate that the timing of the glucose peak is related to the degree of early-phase insulin response, and may serve as a useful additional parameter for evaluating initial defects in β-cell function. This is also partially reflected in the 1-hour glucose measure, although GLP-1 was not different with this classification. Thus, the timing of glucose peak offers more insight into both insulin secretion and insulin sensitivity than the pattern of the glucose curve.

We also found that peak timing and 1-hour glucose were more predictive of β-cell function than pattern of the glucose curve. Our findings are very similar to those just published from an adult cohort with increased risk for T2D [7], suggesting that this pattern of pathology may be comparable in obese youth and adults. In adults with newly diagnosed T2D, time to glucose peak during an OGTT was assessed before and after 4 weeks of intensive insulin therapy, and improved β-cell function was associated with shift to an earlier time of peak [31]. Dynamic longitudinal models have also demonstrated that timing of the glucose peak shifts right as diabetes worsens [7]. Further, in a longitudinal study in which women completed OGTTs at 3 and 12 months postpartum, a delayed peak at the second test was associated with worsening β-cell function [32]. Our results and these previous studies suggest that timing of the glucose

peak could be influenced by β-cell function and timing of insulin response, with defects in relative β-cell function reflected by a later time to peak.

Several studies have previously linked the monophasic pattern to worse glycemia and lower insulin sensitivity among youth and adults [6, 29, 33–35]. OGTT glucose pattern was first noted to have significance in predicting glycemic status in an early Japanese study conducted by Fuchigami *et al.* [36]. They classified OGTT glucose curves into biphasic, domed, and upward morphologies, and found a greater prevalence of the biphasic morphology among patients with normal glucose concentrations and a greater prevalence of upward and domed morphology among patients with T2D [36]. Further study by Tschritter *et al.* [29] attempted to examine contributors to the pattern of glucose during an OGTT, and found that among those with a biphasic pattern, the ratio of normal glucose tolerance to IGT was slightly higher than in the monophasic group, though not significant ($P = 0.08$), but pattern did predict calculated insulin sensitivity [29]. These findings were validated by other large cohort studies, including clamp studies in obese youth of both sexes and all pubertal stages, that found a monophasic OGTT glucose curve pattern to be associated with significantly lower insulin sensitivity and impaired relative β-cell function [6].

The relationship between OGTT glucose pattern and timing of the first glucose peak may account for previous findings regarding pattern. The OGTT glucose pattern observed within a 2-hour window is influenced by the timing of the first peak: those who peak early are more likely to have a biphasic pattern, though a subgroup of those who peak early (healthier profile) could still have a monophasic pattern. In examining our data set both ways, we found a moderate concordance between early peak and biphasic pattern. Chung *et al.* [7] also found that 78% of those with a monophasic pattern had a glucose peak >30 minutes. A higher 1-hour glucose is often reflective of a later peak, and 60% of participants were concordant with these measures. However, only 50% of those with a 1-hour value >155 had a monophasic pattern, indicating that 1-hour glucose and pattern of the glucose curve do not appear to identify similar individuals.

We found several limitations to using pattern as a parameter for assessing metabolic health, particularly in a population of obese adolescent girls. The discrepancy between OGTT glucose pattern and peak glucose timing may relate to the poor reproducibility of OGTT-based parameters. When pattern, time to insulin peak, time to glucose peak, and 1-hour postchallenge glucose were examined, the only reproducible characteristic in healthy individuals was time to glucose peak, suggesting that the other parameters involve considerable intraindividual variability [31]. Further, even though the biphasic pattern has been associated with better glycemic outcomes, individuals with a monophasic pattern tend to present with heterogeneity in outcomes [37], suggesting that the pattern parameter is less sensitive at differentiating those at risk for prediabetes. Pattern is also less clearly defined, leaving room for ambiguity and variation depending on the definitions used. Because most studies of pattern morphology only look at time points within a 2-hour window, for instance, it is difficult to say whether a pattern classified as "monophasic" might in fact be classified as "biphasic" for certain individuals if the window was extended past 2 hours or the sampling interval decreased. These limitations of the pattern parameter further highlight the advantages of the timing parameter. If our findings on glucose peak timing can be validated by larger-cohort studies, it may serve as a better, simpler means of assessing first-phase insulin response and β-cell function.

Similar to findings in adults [7], our study also found that glucose peak timing and 1-hour glucose peak related to differences in the oDI. The oDI, which captures the early insulin response to glucose relative to insulin sensitivity, was significantly lower in individuals with >30-minut peak time. Further, those with >30-minute peak time were lacking the early insulin response, and instead had insulin levels that continued to rise into the 2-hour time point (Fig. 1B). Defects in the early insulin response have been demonstrated to precede T2D onset, an observation also documented in obese adolescents [38, 39]. Additionally, a study in nondiabetic Japanese Americans found that those with an insulin peak occurring at 120 minutes had the greatest risk of T2D development [40].

Table 3. Other Metabolic Measures in a Subset of Youth by OGTT Descriptor

	30-Min Peak, N = 11	>30-Min Peak, N = 21	P Value	1 H < 155 mg/dL, N = 18
GIR lean, mg.kglean.min	13.5 ± 3.9	11.3 ± 3.3	0.154	**12.8 ± 3.4**
GIR/insulin	0.034 (0.026–0.050)	0.022 (0.019–0.034)	0.133	0.061 ± 0.031
Glucose IC 50, μU/mL	57.1 ± 24.8	76.8 ± 34.9	0.134	60 ± 6.4
Glycerol Ra IC 50, μU/mL	64 (44–91)	73 (49–147)	0.474	73 ± 14
Fasting FFA, μmol/L	611 ± 128	600 ± 182	0.874	**413 (339–658)**
FFA end of clamp, μmol/L	35 (25–65)	41 (32–76)	0.510	37 (24–56)
PCr time constant, s	33.3 (30.2–34.6)	28.0 (25.2–34.6)	0.182	31 (26–35)
ADP time constant, s	21.0 (20.0–27.3)	21.4 (18.7–25.0)	0.897	23 ± 1.65
Oxidative phosphorylation, s	12 (9–21)	14 (8–19)	0.763	13 (8–20)
cIMT, cm	0.47 ± 0.02	0.46 ± 0.02	0.752	0.45 ± 0.03

The GLP-1 response was also significantly blunted in individuals whose glucose peak occurred >30 minutes, suggesting an impaired incretin response that could be modulating the decreased initial insulin response. Incretins such as GLP-1 enhance insulin response to oral glucose [41], and decreased GLP-1 is often associated with obesity [42] as well as altered glucose metabolism among obese youth [43]. In women, GLP-1 response was found to be reduced significantly for those with T2D and prediabetes compared with normal controls [44], independent of obesity and age. In obese adolescents, those with IGT also were found to have lower GLP-1 response during an OGTT than those with normal glucose tolerance [42]. Reduction in GLP-1 was shown to precede T2D onset, and GLP-1 concentrations during an OGTT were found to be negatively correlated with the degree of IR in obese subjects [45]. We speculate that an inappropriate GLP-1 response might contribute to a later glucose peak, with glucose concentrations increasing well beyond 30 minutes. This could contribute to greater rates of prediabetes and more overall hyperglycemia, as a late glucose peak also is associated with a much higher 2-hour glucose where IGT status is assessed.

Finally, we found that within an overweight/obese cohort, a >30-minute peak or a 1-hour value >155 mg/dL was related to increased fat partitioning to the liver, whereas prior literature has only linked later glucose timing with higher BMI [7]. This region-specific adiposity may be significant, as it is associated with greater risk of IR, metabolic syndrome, and cardiovascular disease [46]. Additionally, the greater hepatic fat fraction among participants with >30-minute glucose peak may reflect reduced insulin clearance by the liver, one of the compensatory mechanisms of early IR [47]. It is also of note that increasing severity of fatty liver is associated with more glucose dysregulation and greater β-cell defects among obese adolescents at risk for T2D [48]. Between the early and late peaking groups in our study, there were clearly differences in these risk factors, with the later-peaking group more likely to have T2D and metabolic syndrome characteristics. This could imply that for patients in which a later time to peak is observed, screening for these additional risk factors would be justified, and may facilitate detection and appropriate management strategies early on in disease.

In a subset of the cohort, we also performed multiple in-depth measures of tissue-specific insulin sensitivity with clamps, tracers, and magnetic resonance spectroscopy. With the exception of the 1-hour peak, we did not find any relationship between these measures and OGTT pattern, timing, or 1-hr glucose value. This underscores the fact that OGTT dynamics reflect many factors including insulin secretion, hepatic insulin clearance, and hepatic and muscle sensitivity. Furthermore, this highlights the need for more physiologic assessments, like OGTTs, for identification of risk factors.

There were several strengths and weaknesses to our study. Whereas prior studies in adolescents have included boys and girls of various pubertal stages, our cohort consisted entirely of late pubertal girls. Thus, we were able to control for sex and pubertal stage-dependent pubertal changes that affect IR. However, the specificity of the population we studied also limits the generalizability of our results. Our study also used a combination of experimental measures to establish a detailed characterization of both physiology and

Table 3. Other Metabolic Measures in a Subset of Youth by OGTT Descriptor (Continued)

1 H > 155 mg/dL, N = 13	P Value	Biphasic, N = 10	Monophasic, N = 19	P Value
8.8 ± 1.4	**0.019**	12.9 ± 2.9	12.1 ± 3.5	0.559
0.045 ± 0.022	0.185	0.065 ± 0.036	0.052 ± 0.022	0.285
68 ± 12	0.596	55.2 ± 29.9	71 ± 25.7	0.188
71 ± 13	0.914	60 (40–93)	67 (45–109)	0.800
616 (566–745)	**0.027**	531 ± 133	578 ± 130	0.416
38 (28–78)	0.921	34 (24–46)	45 (31–84)	0.244
28 (23–34)	0.364	31.3 ± 5.9	30.6 ± 5.8	0.779
18.1 ± 2.3	0.125	22.9 ± 7.0	22.2 ± 3.9	0.723
13 (8–19)	0.961	13 (5–19)	12 (8–19)	1.00
0.47 ± 0.04	0.245	0.46 ± 0.06	0.47 ± 0.07	0.796

Data are shown as mean ± SD of the mean or median (25th percentile–75th percentile). Values with $P < 0.05$ in **bold.**
Abbreviations: glucose Ra IC 50, hepatic insulin sensitivity as insulin concentration at 50% suppression of basal rate of appearance; glycerol Ra IC 50, adipose insulin sensitivity, insulin concentration at 50% suppression of basal rate of appearance.

metabolic function in our cohort. We used the gold standard MRI to measure hepatic fat fraction. We measured GLP-1 and sampled at extra time points in the first part of the OGTT to better understand the early response to the glucose. In addition, on a subset of patients, we performed the gold standard measurement for insulin sensitivity with a four-phase hyperinsulinemic clamp. We used glucose and glycerol tracers to directly measure adipose and hepatic IR, as well as measures of cardiovascular disease, which had not previously been performed simultaneously.

Our analyses support the conclusion that time to glucose peak may be a more reliable measure of β-cell function and risk for prediabetes when compared with the pattern of the glucose curve in populations at high risk for T2D. In the population we studied, assessing OGTT pattern did not yield appreciable differences predictive of risk. Rather, timing of the glucose peak or a higher 1-hour glucose offered more insight into insulin secretion and action in this population. Furthermore, glucose peak timing as it relates to cardiometabolic risk is worthy of further study, even in populations in which a monophasic/biphasic pattern does yield differences. In a clinical setting, a later time to peak or 1-hour glucose >155 mg/dL could also prompt screening for additional metabolic abnormalities, such as hepatic steatosis. Further work is needed to investigate these variables as they relate to cardiometabolic disease risk in other populations, as a shorter, more predictive OGTT could have significant economic and clinical implications for obesity care.

Acknowledgments

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