Women With Gestational Diabetes Mellitus Randomized to a Higher–Complex Carbohydrate/Low-Fat Diet Manifest Lower Adipose Tissue Insulin Resistance, Inflammation, Glucose, and Free Fatty Acids: A Pilot Study

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OBJECTIVE

Diet therapy in gestational diabetes mellitus (GDM) has focused on carbohydrate restriction but is poorly substantiated. In this pilot randomized clinical trial, we challenged the conventional low-carbohydrate/higher-fat (LC/CONV) diet, hypothesizing that a higher–complex carbohydrate/lower-fat (CHOICE) diet would improve maternal insulin resistance (IR), adipose tissue (AT) lipolysis, and infant adiposity.

RESEARCH DESIGN AND METHODS

At 31 weeks, 12 diet-controlled overweight/obese women with GDM were randomized to an isocaloric LC/CONV (40% carbohydrate/45% fat/15% protein; $n = 6$) or CHOICE (60%/25%/15%; $n = 6$) diet. All meals were provided. AT was biopsied at 37 weeks.

RESULTS

After ~7 weeks, fasting glucose ($P = 0.03$) and free fatty acids ($P = 0.06$) decreased on CHOICE, whereas fasting glucose increased on LC/CONV ($P = 0.03$). Insulin suppression of AT lipolysis was improved on CHOICE versus LC/CONV (56 vs. 31%, $P = 0.005$), consistent with improved IR. AT expression of multiple proinflammatory genes was lower on CHOICE ($P < 0.01$). Infant adiposity trended lower with CHOICE (10.1 ± 1.4 vs. 12.6 ± 2%, respectively).

CONCLUSIONS

A CHOICE diet may improve maternal IR and infant adiposity, challenging recommendations for a LC/CONV diet.

Diet therapy is the first line of treatment in women with gestational diabetes mellitus (GDM). Yet, the optimal diet in GDM is debated. The American College of Obstetricians and Gynecologists (1) and the Endocrine Society (2) support a low-carbohydrate diet, whereas the American Diabetes Association has withdrawn a specific diet recommendation. A diet lower in simple carbohydrates blunts postprandial glucose, which is associated with large-for-gestational-age infants (3).

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However, this approach often necessitates an increase in dietary fat given that protein intake is relatively constant (3). Higher dietary fat in humans and animals has been shown to promote insulin resistance (IR) (3). There is concern that increasing maternal IR could shunt more nutrients to the fetus and increase fetal fat accretion (3). Animal and nonhuman primate models support an intrauterine influence of dietary fat in promoting offspring adiposity, hepatic steatosis, and metabolic syndrome (4). In humans, maternal triglycerides (TGs) and free fatty acids (FFAs) are also strong predictors of excess fetal fat accretion (5,6). Thus, a low-carbohydrate, higher-fat (LC/CONV) diet in obese women with GDM with preexisting IR may have unintended consequences on infant health.

We set out to elucidate the effects of prepared diets on glucose and lipid profiles, adipose tissue (AT) IR, and infant adiposity in GDM. This highly controlled pilot began with a short-term crossover design. Women were randomized to either a conventional LC/CONV diet or a higher–complex carbohydrate, lower-fat (CHOICE, choosing healthy options in carbohydrate energy) diet at the time of GDM diagnosis. They remained on the second diet through delivery. We have previously published that in the randomized crossover trial, both diets resulted in controlled glycemia (7). We hypothesized in this pilot study that maternal IR (HOMA-IR, AT) and offspring adiposity would be improved after continuing the CHOICE diet versus LC/CONV for 6–7 weeks until delivery.

RESEARCH DESIGN AND METHODS

Subjects

This study was approved by the Colorado Multiple and the Kaiser Permanente Colorado institutional review boards. As previously described (7), GDM was diagnosed at 24–28 weeks using Carpenter and Coustan criteria (1). Women were aged 20–36 years and had BMI 26–39 kg/m^2, had fasting blood glucose (FBG) $<$110 mg/dL without comorbidities, and were treated with diet alone.

Study Protocol

Twelve women started diet intervention (all meals provided) in gestational weeks 30–31 and continued until delivery (7). A fasting (10-h) blood sample was collected at baseline (31 weeks), blood and AT were collected after 6–7 weeks of diet (37 weeks), and newborns were studied 2 weeks after birth. Women self-monitored fasting and 2-h postprandial glucose (targets $<$95 and $<$120 mg/dL, respectively) throughout pregnancy.

Techniques

Diet Protocol

Thorough information describing the diet protocol has been previously published (7). Diets were eucaloric and macronutrient distributions were as follows: LC/CONV, 40% carbohydrate/45% fat/15% protein; CHOICE, 60% carbohydrate/25% fat/15% protein, as previously described (7). Both diets were matched for fat (35% saturated fatty acids/45% monounsaturated fatty acids/20% polyunsaturated fatty acids) and simple sugars ($\leq$18% of kilocalories), and daily kilocalories were partitioned as breakfast 25%/lunch 25%/dinner 30%/snacks 20%. Both diets contained mainly low– to moderate–glycemic index foods. As previously described (7), we define "complex carbohydrate" as "polysaccharides and starches primarily derived from grains, vegetables, and fruits that tend to attenuate a sharp postprandial rise in plasma glucose." Fiber content was similar (LC/CONV $\sim$23.5 g/day; CHOICE $\sim$29.3 g/day). Menus were tailored to food preferences, and all meals were prepared by the Clinical Translational Research Center (CTRC) Nutrition Services. Women met with investigators when they picked up their meals (every 72 h).

AT Biopsy

Subcutaneous AT (10-h fasted) was biopsied (n = 11; one subject declined) from the upper gluteal/flank region using our established protocol (8,9). Isoproterenol-stimulated AT lipolysis $\pm$ insulin was measured as described by Engfeldt et al. (10). Gene expression (mRNA) for markers of inflammation and lipogenesis associated with IR in AT was measured by quantitative RT-PCR (11). In brief, RNA was isolated from AT samples using RNeasy (Qiagen, Valencia, CA), reverse transcribed (Bio-Rad, Hercules, CA), and run on an iQ5 instrument (Bio-Rad). mRNA was normalized to the mean of reference genes RPL13a, UBC, and TFRC.

Blood Measures

Lipids, ApoB, ApoA1, and Lp(a) were measured using VAP cholesterol testing (Atherotech, Birmingham, AL) and OxLDL by ELISA (Mercodia, Uppsala, Sweden) as previously reported (7).

Infant Body Composition

Infant weight, length, and adiposity (% fat by PEAPOD; Cosmed Inc., Concord, CA) were measured by the same investigators 2 weeks after birth.

Sample Size Determination and Statistical Methods

This pilot and feasibility study was an extension of the published randomized crossover study (7) performed to estimate power for a larger randomized clinical trial. Reported outcomes were prespecified, but not powered, end points. Equality of variance was confirmed using the Levene test. Paired Student t tests assessed within-group changes; independent Student t tests assessed between-group differences and change from baseline. Correlation analyses were Pearson R (SPSS V22.0; IBM, Armonk, NY).

RESULTS

Subject Characteristics

Subject characteristics did not differ statistically between maternal groups (Table 1). However, in the CHOICE group, four women were Caucasian and two were Asian. In LC/CONV, two were Caucasian, three were Hispanic, and one was Asian. Physical activity was not different between groups (7). Women attended all clinic visits and picked up all prepared meals for the duration of the protocol.

Blood Lipid, Glucose, and Insulin

There were no effects of either diet on cholesterol or lipoproteins (Table 1). Fasting TGs increased within both groups ($P <$ 0.01, LC/CONV; $P <$ 0.05, CHOICE). Both FBG ($P <$ 0.05) and FFAs (P = 0.06) decreased on the CHOICE diet, whereas FBG increased on LC/CONV ($P <$ 0.05) (Table 1).

AT Lipolysis and Markers of Inflammation

Insulin suppression of isoproterenol-stimulated lipolysis was higher on CHOICE versus LC/CONV (56 vs. 31%, respectively, $P <$ 0.01) (Table 1). AT gene expression of proinflammatory biomarkers interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), toll-like receptor-4 (TLR-4), cluster of differentiation molecule 11B (CD11B), CD11C, CD1D, and glycoprotein receptor 43 (GPR43) was lower in AT from CHOICE versus LC/CONV subjects (all $P <$ 0.01) (Table 1).

Table 1—Characteristics of diet-controlled mothers with GDM and infant outcomes, including within- and between-group fasting blood measure comparisons at 31 and 37 weeks' gestation (mean ± SEM; α <0.05 was significant)

	31 weeks' gestation		37 weeks' gestation		Delivery/2 weeks postnatal	
	CHOICE	LC/CONV	CHOICE	LC/CONV	CHOICE	LC/CONV
Subjects						
n	6	6				
Age (years)	30 ± 1	28 ± 2				
Study baseline, gestational age (weeks)	31.7 ± 1	31.2 ± 0.4				
BMI (kg/m^2), study entry	34.3 ± 1.6	33.4 ± 1.4				
Weight (kg), study entry	91.2 ± 5.8	86.5 ± 5.1				
Gravida/para	3/1	2/1				
Maternal at delivery						
Gestational age, delivery (weeks)					40.5 ± 0.5	39.2 ± 0.4
BMI (kg/m^2), delivery					35.4 ± 1.7	34.2 ± 1.4
Weight (kg), delivery					94.0 ± 6.1	88.3 ± 4.7
Weight gain while in study (kg)					2.3 ± 1.2	1.7 ± 1.6
Number of cesarean deliveries					0	2
Infant outcomes, delivery						
Sex (male/female)					3/3	2/4
Weight (g)					3,273.0 ± 104.0	3,421.0 ± 186.3
Length (cm)					50.93 ± 0.65	50.38 ± 1.16
Infant measures, 2 weeks postnatal						
Weight (g)					3,452 ± 113	3,683 ± 292
Adiposity (g), PEAPOD					392 ± 43	510 ± 124
Fat-free mass (g), PEAPOD					3,148 ± 96	3,188 ± 253
% body fat, PEAPOD					10.1 ± 1.4	12.6 ± 2.0
Blood measures						
Total cholesterol (mg/dL)	224 ± 11	235 ± 13	241 ± 12	246 ± 11		
HDL cholesterol (mg/dL)	52 ± 4	59 ± 4	54 ± 5	62 ± 3		
Non-HDL cholesterol (mg/dL)	172 ± 10	175 ± 11	186 ± 9	185 ± 10		
LDL cholesterol (mg/dL)	130 ± 13	142 ± 12	136 ± 15	151 ± 11		
Oxidized LDL (mg/dL)	62.2 ± 6.5	70.2 ± 7.0	69.8 ± 7.6	66.2 ± 5.0		
IDL (mg/dL)	29 ± 4	27 ± 4	31 ± 3	29 ± 3		
VLDL (mg/dL)	42 ± 9	33 ± 3	50 ± 10	34 ± 3		
Apolipoprotein B (mg/dL)	114 ± 7	122 ± 7	122 ± 7	125 ± 7		
Apolipoprotein A1 (mg/dL)	156 ± 4	163 ± 7	163 ± 4	169 ± 5		
Lp(a)	4.5 ± 1.1	7.3 ± 1.6	5.7 ± 0.9	7.0 ± 1.5		
Glucose (mg/dL)	79.3 ± 2.4*	82 ± 3.5*	75 ± 2†	86 ± 3†		
Insulin (μU/mL)	19.0 ± 3.3	26.0 ± 3.3	21 ± 4	29 ± 5		
HOMA-IR, calculated‡	3.7 ± 0.7	5.2 ± 0.8	4.0 ± 0.8	6.1 ± 1.0		
TGs (mg/dL)	220 ± 29*	201 ± 26*	280 ± 44	235 ± 29		
FFAs (μEq/L)	591 ± 82§	513 ± 69	394 ± 11	413 ± 56		
AT						
Lipolysis, glycerol release (% suppression)			55.8 ± 3.0‖	31.1 ± 10		
IL1-β			0.11 ± 0.04‖	0.87 ± 0.30		
TNF-α			0.39 ± 0.07‖	0.98 ± 0.20		
TLR-4			0.58 ± 0.08‖	1.15 ± 0.17		
CD11B			0.50 ± 0.12‖	1.19 ± 0.12		
CD11C			0.28 ± 0.09‖	1.01 ± 0.19		
CD1D			0.36 ± 0.08‖	1.01 ± 0.17		
CD34			0.65 ± 0.11	0.75 ± 0.16		
ADRA2A			0.76 ± 0.13	0.72 ± 0.15		
ADRAB2			0.56 ± 0.11	0.60 ± 0.13		
mTOR			0.62 ± 0.12	0.83 ± 0.09		
GPR41			0.63 ± 0.19	0.53 ± 0.16		
GPR43			0.13 ± 0.03‖	0.90 ± 0.28		
MCP1			0.72 ± 0.19	0.42 ± 0.06		
IL-6			1.12 ± 0.12	0.76 ± 0.12		
IL-10			0.90 ± 0.17	0.53 ± 0.13		
HSL			0.59 ± 0.13	0.75 ± 0.07		
Adiponectin			0.61 ± 0.15	0.55 ± 0.10		

ADRA2A, α-2A adrenergic receptor; ADRAB2, α-2B adrenergic receptor; HSL, hormone-sensitive lipase; MCP1, monocyte chemoattractant protein 1; mTOR, mammalian target of raptamycin. *$P < 0.05$ by paired Student *t* test, 31 vs. 37 weeks. †$P < 0.05$ by Student *t* test for independent group, change from 31 to 37 weeks, CHOICE vs. LC/CONV. ‡HOMA-IR was calculated as (fasting insulin [μU/mL]) * (fasting glucose [mg/dL] * 0.05551)/22.5. §$P = 0.06$ by Student *t* test for independent group, change from 31 to 37 weeks, CHOICE vs. LC/CONV. ‖$P < 0.05$ by Student *t* test for independent group, CHOICE vs. LC/CONV at 37 weeks.

Associations Between Maternal Blood and Infant Adiposity

Maternal fasting insulin ($r = 0.697$, $P = 0.01$) and HOMA-IR ($r = 0.731$, $P < 0.01$) at 37 weeks' gestation were correlated with infant adiposity.

CONCLUSIONS

This highly controlled pilot intervention demonstrated that women with GDM randomized to CHOICE compared with LC/CONV diet have greater AT insulin sensitivity (i.e., better suppression of isoproterenol-stimulated lipolysis) and strikingly lower proinflammatory gene expression. FBG was also reduced after CHOICE compared with an increase after LC/CONV, both of which limited simple carbohydrates. Fasting FFAs decreased on both eucaloric diets, but with a greater decrement on the CHOICE diet (33% compared with 19%). Among all women with GDM, fasting insulin and HOMA-IR measured at 37 weeks' gestation were strong correlates of increased infant adiposity. Taken together, our data suggest that the CHOICE diet, which is characterized by including more nutritious complex carbohydrates, may improve AT IR, resulting in a lower FBG and FFA exposure to the fetus. Therefore, AT insulin action may be an important target for diet therapy in GDM.

We previously demonstrated that glycemia was well controlled in these women with GDM when they consumed both diets for 3 days each in a randomized crossover trial. Importantly, postprandial FFAs were lower on CHOICE compared with the LC/CONV diet (7). Fasting glycemia was the strongest predictor for large-for-gestational-age infants in the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) trial (12), and in this pilot, prolonged exposure to the CHOICE diet decreased FBG. Mounting evidence supports that maternal TGs and FFAs also contribute to excess fetal fat accretion (5,6). In the second and third trimester, systemic IR increases to ensure adequate nutrient flux to the fetus, but insulin clamp studies demonstrate that women with GDM already enter pregnancy with chronic IR (13) and/or insufficient β-cell reserve. We have shown worsened skeletal muscle IR in GDM (14), and these data are the first to support an effect of diet on AT IR. Lowering dietary fat may improve insulin signaling through suppressing inflammation, resulting in less circulating FFAs, which we observed in subjects on the CHOICE diet (3). The reduced pattern of AT inflammatory and cytokine gene expression with CHOICE further supports this assertion. A reduction in the short-chain fatty acid receptor GPR43 suggests a potential favorable influence on the microbiota on the CHOICE diet (15).

Limitations to our study include the following: *1*) the small sample size, *2*) lack of a baseline (31 weeks) AT biopsy, and *3*) lack of a systemic measure of IR (beyond HOMA-IR estimates). It is a further limitation that three of six women in the LC/CONV group were Hispanic, as this could have influenced the outcomes. However, the well-matched women, highly controlled randomized design in which all meals were provided, and exclusion of the confounding effects of glucose-lowering medications make these data provocative (3). Based on these pilot data, we are conducting a larger randomized clinical trial powered to detect a between-group difference in infant adiposity following these two diets.

Our pilot data raise questions about current low-carbohydrate dietary recommendations for women with GDM. If maternal IR is a key regulator of nutrient exposure to the fetal-placental unit, and it may be attenuated by reducing dietary fat, then potential long-term programming effects and excessive fetal growth could be modifiable by diet therapy in GDM.

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