Online-Only Supplemental Material

Lisa T Jansen, Nianlan Yang, Julia MW Wong, Tapan S Mehta, David B Allison, David S Ludwig, Cara B Ebbeling. Prolonged Glycemic Adaptation Following Transition from a Low- to High-Carbohydrate Diet: a Randomized Controlled Feeding Trial

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Protocol Amendment History

Current Version

Protocol: 2020.04.10
Consent (Screening): 2019.11.27
Consent (Trial):   2019.11.27

Dates			Amendment Summary
IRB Amendment	**Protocol Version**	**Consent Versions**	Prepared: 2021.08.26
2020.04.10	2020.04.10	Screening: 2019.11.27 Trial: 2019.11.27	*Study Organization* • COVID-19 Mitigation Plan: staffing assessments at END, participant departure and travel
2020.03.18	2020.03.17	Screening: 2019.11.27 Trial: 2019.11.27	*Study Organization* • COVID-19 Mitigation Plan: Cohort 4 completion, Cohort 5 put on hold
2019.11.25	2019.11.27	Screening: 2019.11.27 Trial: 2019.11.27	*Screening* • Note that Background checks will be completed prior to Residential phase *Main Study Consent* • Specify kidney stone formation in relation to consuming a VLC diet as a possible risk
2019.09.27	2019.09.23	Screening: 2019.03.29 Trial: 2019.09.20	*Outcomes* • Add new component to optional postprandial energy expenditure measurement: CO2/O2 Breath and Respiration Analyzer (COBRA) - test as a portable alternative to a stationary ventilated hood to obtain estimates of energy expenditure and respiratory quotient. *Main Study Consent* • Add COBRA • Add potential for discomfort when wearing the COBRA during the voluntary assessment as a possible risk • Extend buccal cheek swab brush time from 15 to 30 seconds
2019.08.21	2019.08.14	Screening: 2019.03.29 Trial: 2019.08.13	*Outcomes* • Add outcomes to be assessed during the test-diet phase. Participant could opt-in to participate in assessment of postprandial energy expenditure and postprandial respiratory quotient *Main Study Consent* • Add voluntary postprandial energy expenditure assessment (calories burned after breakfast) • Add potential for boredom, inconvenience to sit still and claustrophobia while participating in the voluntary assessment

Dates			Amendment Summary
IRB Amendment	**Protocol Version**	**Consent Versions**	Prepared: 2021.08.26
2019.07.29	2019.07.18	Screening: 2019.03.29 Trial: 2019.07.11	*Outcomes* • Add tissue-level insulin signaling and immune status as secondary outcomes at START and END (composition of leukocytes, RNA expression profile of immune cells, and serum cytokines) • Add microRNA as secondary outcome at START and END *Screening* • Conduct criminal and sex offender background checks through Sterling prior to PRE visit *Main Study Consent* • Add buccal cell collection via cheek swab to measure new secondary outcomes • Add irregular menstrual cycles as potential risk
2019.05.17	2019.05.15	Screening: 2019.03.29 Trial: 2019.04.18	*Eligibility* • Eliminate upper limit for BMI from inclusion criteria
2019.04.08	2019.04.09	Screening: 2019.03.29 Trial: 2019.04.09	*Main Study Consent* • Revise section on potential risks: Add fainting to the existing risk factor of "feeling faint" associated with IV and blood draws, Add hair thinning/loss and keto rash while on the VLC diet • Clarify schedule: Physical activity and sleep monitoring schedule consecutive for START and END assessments and alternating weeks during the test diet period *Screening* • Add remote screening methods for participants unable to conveniently travel to Boston: LabCorp as remote screening lab option, Sterling to complete background checks, HIPPA-compliant AnswerNet for telephone screening, Pearson web-based survey (The Brief Symptom Inventory 18) to assess psychological health at the informational visit *Participant Support* • Offer access to a licensed mental health counselor • Implement Participant Contract to clarify expectations • Offer optional dietary support at end of study
2019.01.28	2019.02.01	Screening: 2018.11.20 Trial: 2019.02.01	*Outcomes* • Add possible future analyses of archived samples: Incretins (GLP-1, GIP), glucagon, oxytocin, oxyntomodulin • Change CGM device from iPro2 (Medtronic) to Freestyle Libre Pro (Abbott)
2019.01.07	2018.12.13	Screening: 2018.11.20 Trial: 2018.12.12	*Screening* • Include vaping and e-cigarette use in Telephone Screening Form *Main Study Consent* • Use telephone only for telehealth visits • Add daily questionnaire for improved participant support, including self-reported daily weight obtained at home using provided scales • Specify daily monitored exercise session during Residential phase, via Bluetooth heart rate monitor, with at least one supervised exercise session per week

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IRB Amendment	**Protocol Version**	**Consent Versions**	Prepared: 2021.08.26
2018.12.10	2018.11.27	Screening: 2018.11.20 Trial: 2018.11.20	*Eligibility* • Add willingness to discuss work options (e.g., remote work) with employer, and make appropriate arrangements prior to the Residential phase (if employed), as an inclusion criterion *Results Reporting* • Note that participants will receive results letter containing select body composition data for personal use shortly after completing participation in the study
2018.09.05	2018.08.22	Screening: 2018.07.19 Trial: 2018.07.19	*Randomization* (prior to any random assignment) • Add stratification factor: serum insulin concentration at 30 minutes into a standardized OGTT (insulin-30) measured at PRE • Stratification cut point: ≤100 µIU/mL vs. >100 µIU/mL
2018.07.17	2018.07.12	Screening: 2018.07.11 Trial: 2018.06.28	*Screening Consent* • Add Quest Diagnostics as lab option for obtaining and analyzing screening labs
2018.07.02	2018.06.28	Screening: 2018.06.05 Trial: 2018.06.28	*Dietary Intervention* • For participants reporting no hunger and lower than expected weight loss during the Run-in phase, specify minimum energy intake of 800 kcal/day
2018.06.21	2018.06.12	Screening: 2018.06.05 Trial: 2018.06.05	*Eligibility* • Modify exclusion criterion for weight change from 5% to 10% in the past 6 months, to account for highly fluctuating weights among populations with overweight and obesity, thereby avoiding unnecessary exclusion
2018.05.04	2018.05.03	Screening: 2018.04.04 Trial: 2018.05.08	*Outcomes* • Remove C-Peptide from the archived sample list • Add the 3D Body Scanner to body composition measurements *Remuneration* • Reimburse out-of-state participants for travel expenses up to $750 for the PRE visit *Main Study Consent* • Add 3D Body Scanner to body composition measurements • Specify individuals who may see participant information, including those employed by the 3D Body Scanner company (Fit3D) and companies which prepare and deliver foods and beverages • For out-of-state participants, reimburse for travel expenses for the PRE visit *Eligibility* • Require flu shot for participants in Winter/Spring cohort • Background checks: SORI (sex offender) checks will be completed prior to PRE visit; CORI (criminal offender) checks will be completed prior to the Residential phase
2018.04.04	2018.03.30	Screening: 2018.04.04 Trial: 2018.01.26	*Outcomes* • Change ghrelin from an archived specimen analysis to a secondary outcome • Add sex hormone-binding globulin (SHBG) to the list of possible analyses using archived specimens

Dates			Amendment Summary
IRB Amendment	**Protocol Version**	**Consent Versions**	Prepared: 2021.08.26
2018.03.12	2018.03.08	Screening: 2018.03.13 Trial: 2018.01.26	*Eligibility* • Add plasma uric acid as a screening lab to assess kidney function • No one will be enrolled if plasma uric acid is above the upper limit of the normal range
2018.01.26	2018.01.24	Screening: 2018.01.26 Trial: 2018.01.26	*Eligibility* • Change BMI upper limit from 45 to 40 kg/m^2 • Change weight upper limit from 425 to 350 pounds • Add inclusion criterion: willingness to obtain seasonal flu shot or provide documentation of flu shot for current flu season. • Add exclusion criterion: use of recreational drugs • Add exclusion criteria: current diagnosis or history of kidney stones, gout, or gall stones; or removal of gall bladder • Add exclusion criterion: exercise restrictions or at high risk for complications during exercise *Screening* • Change screen lab from fasting glucose to HbA1c *Outcomes* • Add uric acid (blood) as a secondary outcome at all assessment time points to assess risk for kidney stones • Collect 24-hour urine samples at START and END (aliquots for analysis of C-peptide will be archived at these time points only)

Table S1. Participant Eligibility Criteria

Inclusion Criteria	Exclusion Criteria
• Aged 18 to 50 years • BMI ≥ 27 kg/m^2 • Weight ≤ 350 lb • Medical clearance from a primary care provider • Willingness to follow a VLC weight-loss diet • Willingness to reside in a research unit for 3 months and eat/drink only provided study foods and beverages • If employed, willingness to discuss work options (e.g., remote work) with employer, and make appropriate arrangements prior to the Residential phase • No major food allergies or aversions • Willingness to obtain seasonal flu shot or provide documentation of flu shot for current flu season (winter/spring cohort only)	• Change in body weight ≥ 10% during prior 6 months • Specialized diets (e.g., for medical or religious reasons) • Chronic use of any medication or dietary supplement that could affect study outcomes (e.g., insulin, metformin, thyroxine) • Current smoking (1 cigarette in the last week) • Use of recreational drugs • Greater than moderate alcohol consumption (> 14 drinks/wk) or history of binge drinking (≥ 5 drinks in 1 day within past 6 months) • Current diagnosis or history of kidney stones, gout, or gall stones; or removal of gall bladder • Exercise restrictions or at high risk for complications during exercise • Physician diagnosis of a major medical illness or eating disorder • Laboratory tests: ALT > 2x upper limit; abnormal HbA1c; abnormal TSH; abnormal creatinine; plasma uric acid above the upper limit of normal (using the male upper limit for both sexes) • Failed criminal offender background check or sex offender background check
Additional Exclusion Criteria for Women	
• Menopausal • Any change in birth control medication during the 3 months prior to enrollment • Pregnancy or lactation during the 12 months prior to enrollment, or intent to become pregnant during study participation	

Table S2. Macronutrient Composition of Test Diets

Dietary Variable	VLC	HC-Starch	HC-Sugar
Carbohydrate (% of total energy)	5 *	57	57
Fat (% of total energy)	77	25	25
Protein (% of total energy)	18	18	18
Whole grain products (% total energy)	0	25	25
Refined grain products (% total energy)	0	20	$\leq$2
Added sugars (% of total energy)	0	$\leq$2	20
* Capped at 30g/d digestible carbohydrate, with the difference made up by fat			

Table S3. Example Breakfasts (calculated for a 2,000-kcal/d diet)

VLC			HC-Starch			HC-Sugar		
Scrambled eggs	90	Gram	Scrambled eggs	26	Gram	Scrambled eggs	26	Gram
			Scrambled egg whites	58	Gram	Scrambled egg whites	58	Gram
			Avocado oil	3	Gram	Avocado oil	3.8	Gram
MCT Oil (mixed into eggs)	11	Gram						
Collagen protein powder (mixed into eggs)	3.5	Gram						
Vegetarian sausage patty	22	Gram	Vegetarian sausage patty	22	Gram	Vegetarian sausage patty	22	Gram
Cheddar cheese (shredded)	20	Gram						
Fresh avocado slices	23	Gram						
Walnut halves	28	Gram						
			Whole wheat English muffin	50	Gram	Whole wheat English muffin	50	Gram
			Butter	6	Gram	Butter	6	Gram
			Milk, 1%	115	Gram	Milk, 1%	115	Gram
			Mini wheats, bite size	20	Gram	Mini wheats, bite size	20	Gram
			Raisins	18	Gram	Raisins	18	Gram
			Cream of rice, cooked	166	Gram			
						Kool-Aid (peach-mango, prepared with water)	347	Gram

Figure S1. Flow of Participants through the Trial

For analysis, CGM data were available for 64 participants (because a different device, yielding incomparable data, was used for 6 participants in the initial cohort), and OGTT data were available for 41 participants (due to elimination of the OGTT at END for 29 participants in the final cohort, as part of risk mitigation in response to COVID-19).

Assessed for Eligibility (n=22,638)	Excluded (n=17,648) ♦ Unable to contact for telephone screen (n=9,172) ♦ Declined telephone screen (n=1,089) ♦ Did not meet eligibility criteria (n=7,387)
Telephone Screening & Informational Visit (n=4,990)	Excluded (n=4,583) ♦ Did not meet eligibility criteria (n=2,338) ♦ Unable to reside in study housing for 3 months (n=274) ♦ Other reasons (n=1,971)
Screening & Communication with Primary Care Provider (n=407)	Excluded (n=241) ♦ Screening labs and/or PCP clearance unavailable (n=138) ♦ Did not meet eligibility criteria (n=67) ♦ Other reasons (n=36)
Signed Main Consent Form (n=166)	Excluded (n=2) ♦ Did not meet eligibility criteria (n=1) ♦ Other reasons (n=1)
Began Run-in Phase (n=164)	Excluded (n= 76) ♦ Did not achieve target weight loss (n=73) ♦ Other reasons (n=3)
Began Residential Phase (n=88)	Excluded (n= 11) ♦ Inability to comply (n=7) ♦ Medical reasons (n=4)

Enrollment

Allocation

Randomized (n=77)

VLC (n=26)	**HC-Starch** (n=26)	**HC-Sugar** (n=25)

Follow-Up

Completed Intervention (n=26)	**Completed Intervention** (n=24) ♦ Dismissed for non-compliance (n=2)	**Completed Intervention** (n=20) ♦ Dismissed for non-compliance (n=2) ♦ Dismissed for other reasons (n=3)

Analysis

| ♦ Included in CGM analysis (n=23) ♦ Included in OGTT analysis (n=16)	♦ Included in CGM analysis (n=22) ♦ Included in OGTT analysis (n=13)	♦ Included in CGM analysis (n=19) ♦ Included in OGTT analysis (n=12)

Figure S2. OGTT Curves for Glucose and Insulin at PRE and START
Data are depicted as mean (CI) for glucose (Panel A) and insulin (Panel B).
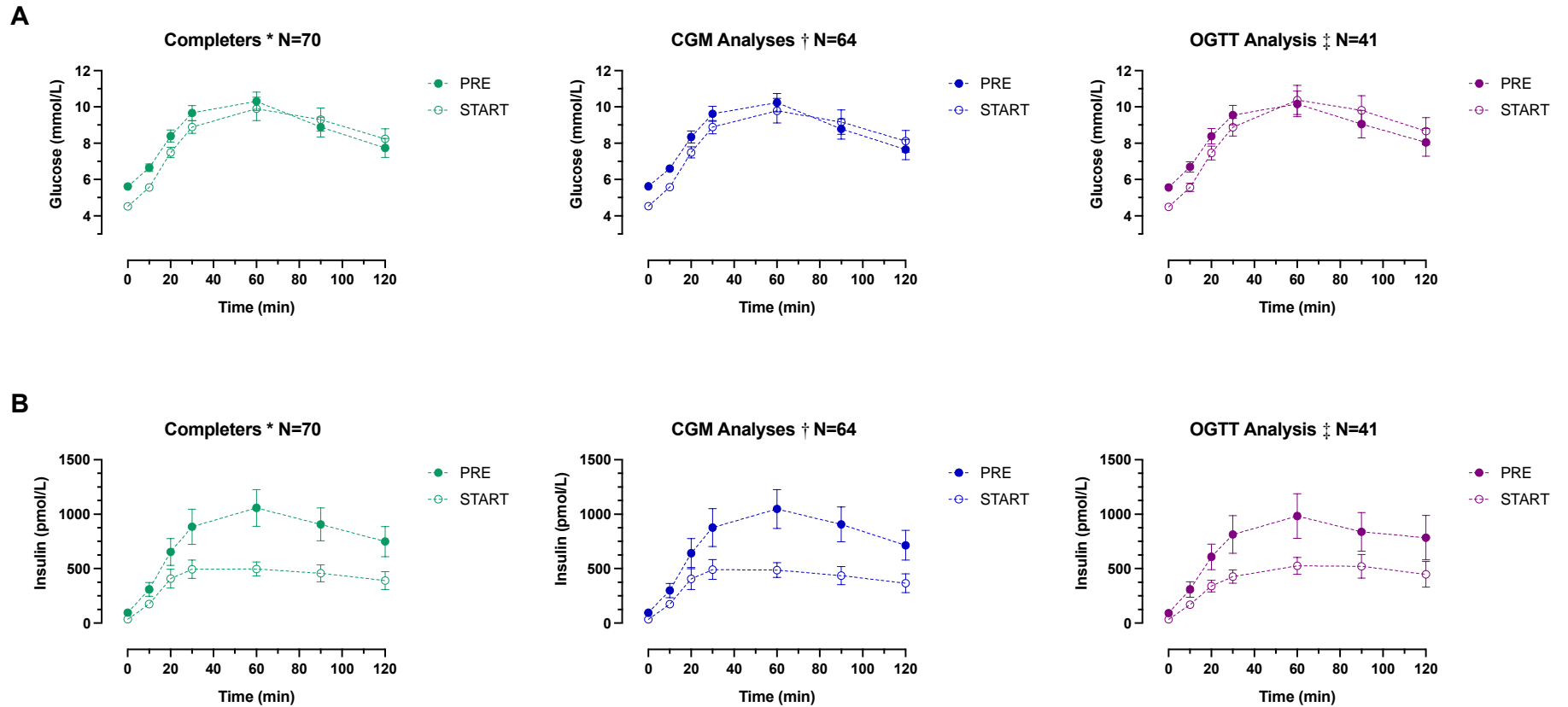
* Of the 77 randomized participants, 70 were retained at END (Completers).

† Data for CGM Analyses were available for 64 retained participants (because a different device, yielding incomparable data, was used for 6 participants in the initial cohort).

‡ Data for OGTT Analysis were available for 41 retained participants (due to elimination of the OGTT at END for 29 participants in the final cohort, as part of risk mitigation in response to COVID-19).

Figure S3. Linear Trends for CGM Metrics During the Test Diet Period by Diet Group
Data are depicted as mean (CI) for fasting (Panel A), 2-hr (Panel B) and peak
glucose (Panel C). Data points for week 0 (last week of Run-in diet) and week 1
(first week of Test diet) are depicted to illustrate the full-time course of changes but
were not included in the models.

A
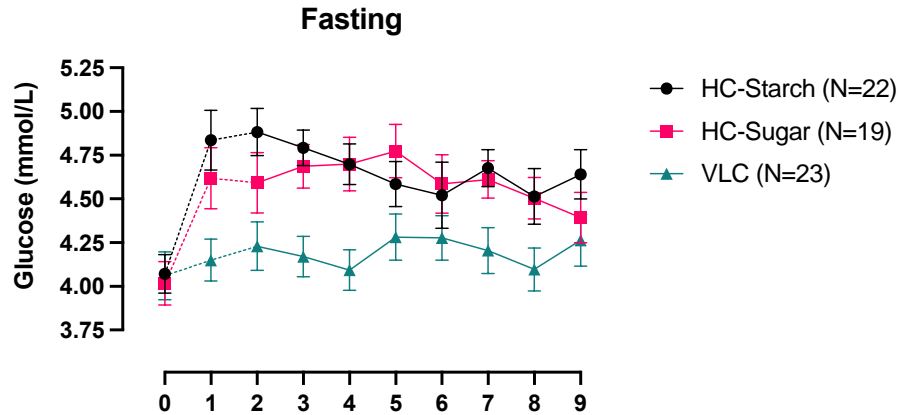
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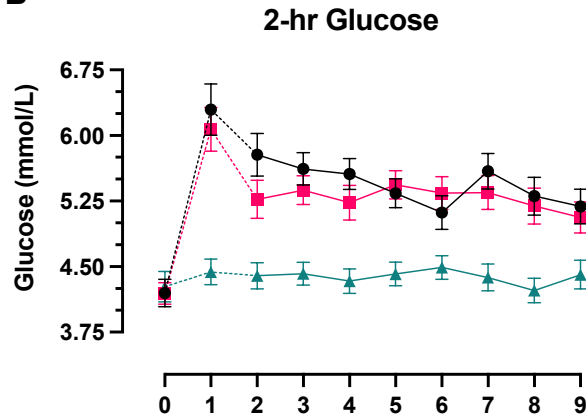
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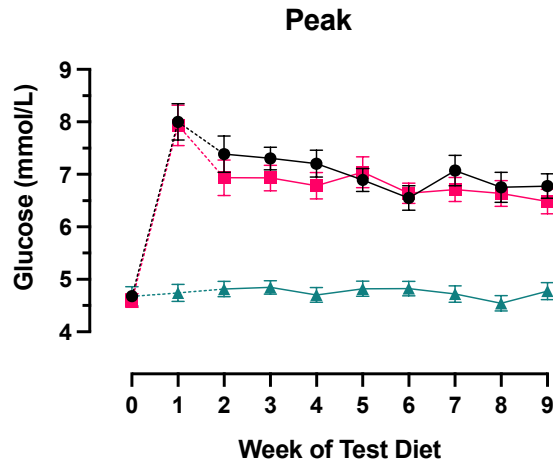
Table S4. Original and Bootstrap Results for Segmented Regression Modeling from Weeks 2 to 9 [*]

Outcome	HC-Starch (n=22)	HC-Sugar (n=19)
	Changepoint Estimate (95% CI) †	Changepoint Estimate (95% CI) †
Fasting Glucose		
Original	5.2 (4.1, 6.3)	4.6 (3.6, 5.6)
Bootstrap	5.3 (4.1, 6.2)	4.7 (3.4, 5.8)
2-hr Glucose		
Original	5.3 (3.2, 7.3)	5.7 (3.8, 7.7)
Bootstrap	5.4 (3.4, 6.9)	5.7 (3.9, 7.7)
[*] A random sample bootstrap procedure was conducted with 10,000 replications for each variable.		
† Estimates are means for original asymptotic estimates based on regression modeling assumptions and medians for bootstrap results.		

Table S5. Within-Group Comparisons of Slope Dynamics Before and After the Week 5 Time Index *

Outcome Interval	Piecewise Linear Mixed Model					
	VLC (n=23)		HC-Starch (n=22)		HC-Sugar (n=19)	
	Estimate (95% CI)	*P*	Estimate (95% CI)	*P*	Estimate (95% CI)	*P*
Fasting Glucose						
Before †						
mg/dL per week	-0.1 (-1.2, 1.0)	0.868	-1.9 (-3.0, -0.8)	0.001	0.7 (-0.5, 1.9)	0.234
mmol/L per week	-0.01 (-0.07, 0.06)		-0.10 (-0.17, -0.04)		0.04 (-0.03, 0.11)	
After †						
mg/dL per week	-0.2 (-0.9, 0.6)	0.675	-0.1 (-0.9, 0.7)	0.880	-1.6 (-2.5, -0.7)	0.001
mmol/L per week	-0.01 (-0.05, 0.03)		-0.00 (-0.05, 0.04)		-0.09 (-0.14, -0.04)	
Change ‡						
mg/dL per week	-0.1 (-1.7, 1.5)	0.928	1.8 (0.2, 3.5)	0.033	-2.3 (-4.1, -0.5)	0.012
mmol/L per week	-0.00 (-0.09, 0.08)		0.10 (0.01, 0.19)		-0.13 (-0.23, -0.03)	
2-hr Glucose						
Before						
mg/dL per week	-0.2 (-1.9, 1.4)	0.798	-1.8 (-3.5, -0.1)	0.035	0.6 (-1.2, 2.4)	0.487
mmol/L per week	-0.01 (-0.10, 0.08)		-0.10 (-0.19, -0.01)		0.04 (-0.07, 0.14)	
After						
mg/dL per week	-0.2 (-1.1, 0.8)	0.724	-1.2 (-2.2, -0.3)	0.014	-1.6 (-2.6, -0.6)	0.003
mmol/L per week	-0.01 (-0.06, 0.04)		-0.07 (-0.12, -0.01)		-0.09 (-0.15, -0.03)	
Change						
mg/dL per week	0.1 (-2.2, 2.3)	0.964	0.6 (-1.7, 2.9)	0.594	-2.2 (-4.7, 0.2)	0.074
mmol/L per week	0.00 (-0.12, 0.12)		0.03 (-0.09, 0.16)		-0.12 (-0.26, 0.01)	

* Time index of 5 weeks derived from segmented regression analysis.
† Within diet group estimates are slopes Before (2 to 5 weeks) and After (6 to 9 weeks) the 5-week time index derived from a piecewise linear mixed model.
‡ Change is difference in slope between the two intervals (After – Before).

Table S6. Pairwise Contrasts of Slope Dynamics Before and After the Week 5 Time Index *

Outcome Interval	Pairwise Contrasts †					
	HC-Starch – HC-Sugar		HC-Starch - VLC		HC-Sugar - VLC	
	Estimate (95% CI)	*P*	Estimate (95% CI)	*P*	Estimate (95% CI)	*P*
Fasting Glucose						
Before						
mg/dL per week	-2.6 (-4.2, -1.0)	0.003	-1.8 (-3.4, -0.2)	0.026	0.8 (-0.8, 2.4)	0.32
mmol/L per week	-0.14 (-0.24, -0.05)		-0.10 (-0.19, -0.01)		0.05 (-0.04, 0.14)	
After						
mg/dL per week	1.5 (0.3, 2.7)	0.012	0.1 (-1.0, 1.2)	0.854	-1.4 (-2.6, -0.3)	0.018
mmol/L per week	0.08 (0.02, 0.15)		0.01 (-0.06, 0.07)		-0.08 (-0.14, -0.01)	
2-hr Glucose						
Before						
mg/dL per week	-2.5 (-4.9, 0.0)	0.052	-1.6 (-4.0, 0.8)	0.178	0.8 (-1.6, 3.3)	0.492
mmol/L per week	-0.14 (-0.27, 0.00)		-0.09 (-0.22, 0.04)		0.05 (-0.09, 0.18)	
After						
mg/dL per week	0.4 (-1.0, 1.8)	0.580	-1.0 (-2.4, 0.3)	0.119	-1.4 (-2.8, -0.1)	0.042
mmol/L per week	0.02 (-0.06, 0.10)		-0.06 (-0.13, 0.02)		-0.08 (-0.16, -0.00)	

* Time index corresponding to week 5 was derived from segmented regression analysis.
† Pairwise contrasts assess differences in patterns of change between groups.

Table S7. HbA1c and OGTT Metrics at START by Diet Group *

Outcome		VLC (n=16)	HC-Starch (n=13)	HC-Sugar (n=12)
HbA1c	%	4.69 (0.33)	4.82 (0.35)	4.98 (0.31)
	mmol/mol	28 (3.6)	29 (3.8)	31 (3.4)
Fasting Glucose	mg/dL	79.4 (6.7)	82.1 (5.4)	82.0 (6.7)
	mmol/L	4.4 (0.4)	4.6 (0.3)	4.6 (0.4)
Fasting Insulin	μIU/mL	6.6 (3.8)	5.2 (3.3)	4.7 (2.3)
	pmol/L	39.4 (22.7)	31.5 (19.8)	28.5 (13.5)
2-hour Glucose	mg/dL	152.8 (37.1)	172.1 (50.4)	143 (37.5)
	mmol/L	8.5 (2.1)	9.6 (2.8)	7.9 (2.1)
* Data are mean (SD).				

Figure S4. OGTT Curves for Glucose and Insulin at START and END by Diet Group
Data are depicted as mean (CI) for glucose and insulin for VLC (Panel A), HC-Starch (Panel B), and HC-Sugar (Panel C).