Online-Only Supplemental Material

Carbohydrate tolerance threshold for unannounced snacks in children and adolescents with Type 1 Diabetes using an advanced hybrid closed-loop system

Supplement Appendix

RESEARCH DESIGN AND APPROACHES

Study system

The Institutional Review Board approved the protocol ("No bolus for breakfast" study - IRB-BURLO 07/2021 28.07.2021). Patients were enrolled at the Diabetes Unit of the Institute for Maternal and Child Health "Burlo Garofolo", Trieste, Italy. All participants and their parents (for participants aged <18 years) gave written informed consent/assent before enrolment. The study was proposed to eligible individuals prospectively enrolled during a follow-up visit. Participants with other types of diabetes than Type 1, with concomitant celiac disease or pregnancy were excluded. Data on sensor-specific measures of glycemic control in the two weeks before the study and the two hours after each snack were extracted with CareLink™ Personal Software. The "G2 clinico" platform (management system specialist activities) was employed to access patients' clinical data (age, sex, weight, age at Type 1 diabetes onset). Participants were considered as pre-pubertal when in Tanner stage B/G 1, pubertal in Tanner stage B/G 2-4, and post-pubertal in Tanner stage B/G 5.

Statistical analysis

Assuming $\alpha=0.05$ and $1-\beta=0.80$, supposing the mean paired difference between Pre-BG and Post-BG is equal to 50 mg/dL for each quantity of carbohydrate in the 12 days, with SD=60 mg/dL, the sample size required to conduct the study was 14 subjects (paired t-test - two tails - size=0.83).

Paired t-test was performed to check the differences of paired data in the continuous variables. A multivariate repeated measures model was applied to identify factors influencing glycemia level

changes between pre- and post-meal. Independent variables were blood glucose before the meal, amount of simple CHO, amount of complex CHO, and amount of fats, while age and sex were adjustment variables. Day in the breakfast scheme was considered the repeated variable within subjects. To evaluate the number of subjects with hyperglycemia, the mean paired difference between pre- and post-glycemia for each quantity and type of snack was categorized into two classes (hyperglycemia: yes/no). Frequency distribution was provided, and associations with variables of interest were studied using the Chi-square test or t-test for two independent groups, as appropriate.

RESULTS

Blood glucose before and after the snacks

Pre-BG was slightly lower in pre-pubertal children (99±16 mg/dL) compared to pubertal (112±15 mg/dL) or post-pubertal individuals (114±16 mg/dL), without statistical significance (p=0.48). No significant differences were either found in Post-BG (pre-pubertal 137±47 mg/dL, pubertal 140±32 mg/dL, post-pubertal 149±35 mg/dL, p=0.45) or ΔBG (pre-pubertal 37±48 mg/dL, pubertal 29±31 mg/dL, post-pubertal 35±36 mg/dL, p=0.47), although not finding statistical difference among different groups could also depend on the scarcity of the number of subjects evaluated.

ΔBG was not associated with age (p=0.90) or diabetes duration (p=0.84), while it was associated with TIR≥70% (p=0.01) and TAR<25% (p<0.01).

Hyperglycemia at 2 hours (ΔBG ≥50 mg/dL) after the unannounced snack was always avoided when eating ~10 gr of mostly complex or simple CHO, and most of the time (>75% of cases) when eating up to ~30 gr of simple CHO and ~10 gr of mostly complex or simple CHO with fats. The greatest ΔBG and the highest BG were reported when eating ~30 gr of mostly complex CHO (mean ΔBG 75 mg/dl, maximum ΔBG 156 mg/dl, maximum BG 217 mg/dl).

Post-BG was always <180 mg/dl when eating up to 10 gr of CHO (simple or complex, with or without fats) and most of the time (>75% of cases) when eating up to 30 gr of simple CHO with or without

fats and up to 20 gr of complex CHO without fats. We reported no Post-BG levels >250 mg/dl with any kind of unannounced snack up to 30 gr of CHO.

When eating simple CHO alone or ~10 gr of simple CHO with fats, there was a chance >40% of having the 2-hours BG inferior to the pre-meal one (ΔBG <0 mg/dl) (Table 3S). The mean for ΔBG was -1 mg/dl when eating ~10 gr of simple CHO, with a minimum of -62 mg/dl when eating ~30 gr of simple CHO.

Sensor-specific measures of glycemic control over 2-hour postprandial period

ΔBG was significantly associated with time in range (TIR, 70-180 mg/dl) and time above range (TAR, >180 mg/dl) in the 2 hours after the meals ($p<0.01$).

The mean TIR was ≥70% and TAR <25% in the 2 hours after the meals (except for the snack with 30 gr of mostly complex CHO with a mean TAR 30%) (Table 4S).

At ROC analysis for repeated measures, a cut-off of 23.6 gr of total CHO was found to determine a TIR≥70% with a sensitivity of 0.65 and a specificity of 0.75 or a TAR<25% with a sensitivity of 0.62 and a specificity of 0.76, respectively.

Mean glucose sensor was significantly higher when eating 20 or 30 grams of mostly complex CHO (alone or with fats) and 30 gr of simple CHO (with or without fats) compared to 10 gr of CHO (simple or mostly complex, with or without fats) or 20 gr of simple CHO (with or without fats) ($p<0.01$).

TIR was significantly lower and TAR significantly higher when eating 20 or 30 grams of mostly complex CHO and 30 gr of CHO (simple or mostly complex) with fats compared to 10 gr of CHO (simple or mostly complex, with or without fats) or 20 gr of simple CHO, or any type of CHO with fats ($p<0.01$).

TBR was significantly higher when eating 20 grams of simple CHO with fats compared to all other snacks ($p<0.01$).

Insulin delivery

The volume of insulin bolus delivered in the 2 hours after the unannounced snacks (both in U or U/kg) and the auto correction delivered (both in U or %) was significantly associated with CHO amount ($p<0.01$).

The volume of insulin bolus (in U) was significantly associated with age ($p=0.02$), but not when considering the insulin bolus per body weight (U/kg) ($p=0.37$). The auto correction delivered (both in U and in %) was not significantly associated with age ($p=0.08$ and $p=0.55$, respectively). We could not find any significant difference between insulin delivery and the duration of diabetes.

Supplementary Table S1. Data on unannounced snacks during the 12-day study (type of food, amounts and types of carbohydrates [CHO], amount of fats).

Day	**Type of snack**	**Total CHO** (grams)	**Complex CHO** (grams)	**Simple CHO** (grams)	**Fats** (grams)
1	Mostly complex CHO *(crackers)*	11.0	10.5	0.5	1.0
2		19.8	18.9	0.9	1.8
3		30.8	29.4	1.4	2.8
4	Only simple CHO *(juice)*	11.0	-	11.0	-
5		22.0	-	22.0	-
6		33.0	-	33.0	-
7	Mostly complex CHO + fats *(chocolate biscuits)*	11.8	6.8	5.0	4.0
8		23.6	13.6	10.0	8.0
9		29.5	17.0	12.5	10.0
10	Only simple CHO + fats *(chocolate bars)*	10.0	-	10.0	6.6
11		20.1	-	20.1	13.2
12		30.1	-	30.1	19.8

Supplementary Table S2. Characteristics of the study cohort. Data are reported as mean (±standard deviation) or number (%). GMI: glucose management indicator; TIR: time in range (70-180 mg/dl); TAR: time above range (>180 mg/dl); TBR: time below range (<70 mg/dl).

Females (n, %)	7 (50%)
Age, years	14.5 (±3.6)
Pre-pubertal (n, %)	3 (21%)
Pubertal (n, %)	3 (21%)
Post-pubertal (n, %)	8 (57%)
Disease duration, years	5.4 (±3.9)
Insulin daily requirement, U/kg/day	0.91 (±0.26)
Sensor-specific measures of glycemic control in the 2 weeks before study	
GMI, %	6.8 (±0.2)
TIR, %	75 (±7)
TAR, %	23 (±6)
TBR, %	2 (±2)
Mean glucose sensor, mg/dl	146 (±8)
Coefficient of variation, %	33.8 (±5.1)
Sensor wear, %	88 (±7)
Auto mode, %	86 (±18)
Bolus amount, %	57 (±9)
Auto correction, % on bolus amount	26 (±11)

Supplementary Table S3. Data on blood glucose and sensor-specific measures of glycemic control for the 2 hours after the unannounced snacks during the 12-day study. Data are reported as mean (±standard deviation) [min÷max]. Pre-BG: blood glucose before the meal; Post-BG: blood glucose 2 hours after the meal; ΔBG: difference between post- and pre-meal blood glucose; TAR: time above range (>180 mg/dL); TIR: time in range (70-180 mg/dL); TBR: time below range (<70 mg/dL).

Day	**Pre-BG** (mg/dl)	**Post-BG** (mg/dl)	**Post-BG ≥180 mg/dl** (%)	**ΔBG** (mg/dl)	**ΔBG ≥50 mg/dl** (%)	**ΔBG ≤0 mg/dl** (%)	**2-hour mean glucose sensor** (mg/dL)	**2-hour TAR** (%)	**2-hour TIR** (%)	**2-hour TBR** (%)
1	114 (±19) [83÷140]	125 (±25) [78÷159]	0%	11 (±19) [-33÷33]	0%	21%	130 (±28)	6 (±18)	94 (±18)	0 (±1)
2	110 (±18) [75÷144]	151 (±30) [83÷194]	21%	40 (±31) [-15÷91]	36%	7%	144 (±24)	24 (±38)	75 (±38)	1 (±2)
3	106 (±19) [72÷144]	181 (±42) [87÷230]	57%	75 (±43) [-9÷156]	71%	7%	155 (±21)	30 (±25)	70 (±25)	0 (±0)
4	113 (±16) [88÷146]	113 (±16) [77÷135]	0%	-1 (±17) [-29÷28]	0%	50%	122 (±10)	2 (±6)	99 (±6)	0 (±0)
5	113 (±19) [74÷139]	130 (±47) [77÷135]	14%	17 (±43) [-29÷28]	21%	43%	131 (±16)	5 (±9)	95 (±9)	0 (±0)
6	109 (±13) [83÷125]	124 (±31) [63÷182]	7%	15 (±31) [-62÷61]	14%	29%	141 (±18)	16 (±19)	84 (±19)	0 (±1)
7	110 (±13) [88÷132]	142 (±24) [91÷171]	0%	32 (±19) [-11÷55]	14%	7%	130 (±25)	9 (±18)	92 (±18)	0 (±0)
8	113 (±20) [88÷147]	171 (±27) [132÷218]	29%	58 (±34) [6÷116]	64%	0%	146 (±27)	12 (±17)	89 (±17)	0 (±0)
9	113 (±16) [90÷147]	166 (±39) [67÷218]	36%	53 (±38) [-27÷118]	57%	7%	154 (±19)	21 (±25)	79 (±25)	0 (±0)
10	116 (±20) [89÷147]	124 (±27) [86÷168]	0%	8 (±22) [-15÷58]	7%	43%	124 (±24)	5 (±17)	95 (±17)	0 (±0)
11	107 (±11) [86÷118]	149 (±28) [73÷185]	7%	42 (±32) [-42÷93]	43%	7%	130 (±25)	8 (±13)	90 (±16)	2 (±4)
12	99 (±15) [71÷130]	154 (±35) [112÷234]	14%	55 (±34) [5÷131]	50%	0%	143 (±25)	21 (±19)	79 (±19)	0 (±0)

Supplementary Table S4. Data regarding insulin delivery in the 2 hours after the unannounced snacks during the 12-day study. Data are reported as mean (±SD).

Day	**Type of snack**	**Bolus volume delivered** (U)	**Bolus delivered per weight** (U/kg)	**Auto correction delivered** (U)	**Auto correction rate** (%)
1	Mostly complex CHO *(crackers)*	3.0 (±1.7)	0.05 (±0.02)	0.7 (±0.7)	21 (±21)
2		3.8 (±2.0)	0.06 (±0.03)	1.5 (±1.1)	41 (±20)
3		4.3 (±2.2)	0.07 (±0.03)	2.3 (±1.9)	54 (±23)
4	Only simple CHO *(juice)*	3.1 (±1.6)	0.05 (±0.02)	0.6 (±0.4)	21 (±15)
5		2.9 (±1.5)	0.05 (±0.02)	1.0 (±0.7)	33 (±17)
6		3.4 (±2.0)	0.06 (±0.03)	1.8 (±1.2)	53 (±14)
7	Mostly complex CHO + fats *(chocolate biscuits)*	3.2 (±1.6)	0.05 (±0.02)	0.7 (±0.7)	25 (±21)
8		3.5 (±1.5)	0.06 (±0.02)	1.6 (±0.9)	44 (±21)
9		4.3 (±2.0)	0.07 (±0.02)	2.3 (±1.2)	53 (±16)
10	Only simple CHO + fats *(chocolate bars)*	2.9 (±1.6)	0.05 (±0.02)	1.0 (±1.2)	30 (±26)
11		4.1 (±2.3)	0.06 (±0.03)	1.7 (±2.1)	36 (±27)
12		3.8 (±1.7)	0.06 (±0.02)	1.9 (±1.5)	48 (±27)