Ordered 24-Hrs Recalls Associate with Systematic and Order-Specific Differences in Reported Energy and Carbohydrate Intakes by Individuals with Obesity: A methodological Approach in A Cross-Sectional Study

Costela Lacrimioara Serban
Universitatea de Medicina si Farmacie Victor Babes din Timisoara
Iulia Teodora Perva
Universitatea de Medicina si Farmacie Victor Babes din Timisoara
Adela Chirita-Emandi (adela.chirita@umft.ro)
Universitatea de Medicina si Farmacie Victor Babes din Timisoara
Alexandra Sima
Universitatea de Medicina si Farmacie Victor Babes din Timisoara
Nicoleta Andreescu
Universitatea de Medicina si Farmacie Victor Babes din Timisoara
Salomeia Putnoky
Universitatea de Medicina si Farmacie Victor Babes din Timisoara
Mihai Dinu Niculescu
Universitatea de Medicina si Farmacie Victor Babes din Timisoara
Maria Puiu
Universitatea de Medicina si Farmacie Victor Babes din Timisoara

Research

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Abstract

Background. In depth understanding of dietary patterns of individuals with obesity is needed in practice and research, in order to support dietitians and physicians in the design and implementation of nutritional management. We aimed to analyze the consistency of energy, macro- and micronutrients reported intakes in four non-consecutive 24-hrs dietary recalls using information collected in the NutriGen Study (ClinicalTrials.gov, NCT02837367).

Methods. Data included reported food intakes from 388 adults who completed four 24-hrs recalls.

Results. Analyses indicated a significant decrease between the first and subsequent evaluations in regard to energy and several nutrients, with a systematic decrease in reporting energy and carbohydrates for the second evaluation. When excluding the second 24-hrs recall from the average, this new evaluation induced significant increases in the averages for reported energy, carbohydrates, and almost all micronutrients, indicating that the second recall is a point of controversy for whether to be included or not in further analyses.

Conclusion. This study identified systematic differences in energy and carbohydrate reporting in the second recall, when four ordered 24-hour recalls were administered to adults with obesity. More studies are needed to identify the source of these differences, in order to ascertain whether reporting, training bias, or behavioral changes are responsible for such differences, and whether a time point with systematic differences should be included or not in further analyses.

Introduction

24-hour dietary recalls provide metrics of food intake, necessary in studies that link nutrient intakes to diseases or other health-related outcomes. The multiple pass 24-hrs recall method used for capturing food intake has been proven to be a reliable method for evaluation in individuals with obesity (1, 2). Usually, several 24-hrs recalls are needed, in order to provide enough days to capture intra-individual intake variations. The number of 24-hrs recalls recommended varies, depending on the outcome of the study, ranging from a minimum of two - for the comparison of protein and potassium intake between European countries (3), to a maximum of 10–15 days when assessing the diet across a 6 months period (4). Jackson et all (5) suggested a maximum of eight 24-hrs recalls in overweight and obese population to reduce random error.

Underreporting of food intakes has been acknowledged as a source of bias and has been associated with higher BMI or body fat percent, feminine gender, social desirability (6–8). Even though dietary restraint practices were related to lower energy and fat reporting, it did not modify the accuracy of the method (9). However, little is known about whether the order of the subsequent recalls, in itself, is a factor to be accounted for when assessing bias, or whether the order of the recalls would induce behavior changes to the interviewed individuals.

The purpose of this study was to evaluate the consistency of reported energy, macro-, and micronutrient intakes in a series of four 24-hrs recalls in adults with obesity, under medical surveillance for various obesity-associated diseases.

Methods

Recruitment of subjects

Data represents food intakes collected with a broader scope within the Nutrigen Study (ClinicalTrials.gov NCT02837367), undertaken in Timișoara, Romania. Details of inclusion/exclusion criteria, selection of participants, dietary assessment and coding of nutrients were presented elsewhere (10). Briefly, 197 men and 212 women were recruited, among patients with obesity and associated diseases, who were under medical surveillance and treatment for these conditions at the time of recruitment. A total of 1587 24-hrs dietary recalls were collected, representing up to 4 days of food intake assessment for each participant. For the purpose of this research we have selected 388 patients with complete sets of four evaluations. Of the four dietary recalls, three recalls were performed during work weekdays (Monday to Friday), and one in a day of weekend (Saturday or Sunday). Food intakes obtained from the recalls were converted into nutrient intakes using Nutritioapp (https://nutritioapp.com) (11), a web-application using data from both the USDA Food and Nutrient Database for Dietary Studies, and from European and Romanian databases (10).

No subjects were under any dietary intervention during this component of the study.

Data analysis

Data analysis was performed using the IBM-SPSS version 25 software (IBM, Armonk, New York, U.S.A.). Descriptive summary measures of central tendency (mean) and of dispersion (standard deviation) were computed for numerical variables. Proportions of weekend days/work weekdays in each evaluation set were compared using chi-square, and statistical significance was adjusted using the Bonferroni method. Up to three 24-hrs recalls of weekdays were also averaged and further used in analysis. Differences between weekend and averaged weekday intakes were calculated for energy, macro-, and micronutrient intakes. Averaged nutrients of four ordered 24-hrs recalls and averaged nutrient intakes of three 24-hrs (resulting from the exclusion of the second evaluation) were also used in analyses.

For non-parametric paired data, Wilcoxon Signed Ranks Test was used. For prediction of the difference of energy intakes between weekdays and weekend, a linear regression model was used. Series of daily intakes were compared using general linear model repeated measures design, using the gender of participants as factor. Results of pairwise comparisons were adjusted by Sidak method. All results were adjusted for false discovery rate (FDR), using an online tool (https://tools.carbocation.com/FDR) (12), which adjusted the p values to the number of comparisons per research hypothesis. After adjustments, p-values < 0.05 were considered statistically significant.

Results

The cohort had a mean age of 53.2 +/- 12.1 years (51.1 +/- 13.5 for males and 55.2 +/- 10.2 for females) and a mean body mass index (BMI) of 36.7 +/- 5.6 kg/m^2 (35.5 +/- 5.2 kg/m^2 for males and 37.9 +/- 5.7 kg/m^2 for females).

Order of 24-hrs recalls and nutrient intakes

In Table 1 are presented, separately for four ordered 24-hrs recalls, the mean intakes of energy and of 36 macro- and micronutrients. Significant differences were found between evaluations, after FDR correction, for energy, carbohydrates, total sugar, vitamin C, calcium, fiber, folates and potassium. Total sugars, vitamin C and folates, showed differences between recalls 1 and 4, while the other nutrients indicated differences between recalls 1 and 2. Figure 1 illustrates the mean values for energy and macronutrients, for each recall, while Fig. 2 indicates the mean values for the micronutrients found to have significantly different intakes between recalls. Gender was tested as a between-subjects factor. All interactions between gender and time factors were not statistically significant ($p > 0.05$).

Table 1

Mean intakes of energy and of 36 macro- and micronutrients from 24-hrs dietary recalls (n = 388 individuals with obesity)

Nutrients	First evaluation	Second evaluation	Third evaluation	Fourth evaluation
Energy (kcal)	1641.70 +/- 827.57 [a]	1480.37 +/- 779.81 [b]	1578.28 +/- 786.16 [a,b]	1561.65 +/- 846.51 [a,b]
Protein (g)	78.14 +/- 37.51 [a]	74.37 +/- 41.41 [a]	76.27 +/- 39.23 [a]	77.43 +/- 40.34 [a]
Fat (g)	68.02 +/- 45.02 [a]	62.22 +/- 43.11 [a]	64.73 +/- 44.16 [a]	65.74 +/- 46.34 [a]
Carbohydrates (g)	182.12 +/- 99.45 [a]	161.51 +/- 89.84 [b]	174.48 +/- 91.60 [a,b]	166.89 +/- 97.52 [a,b]
Vitamin C (mg)	74.04 +/- 88.67 [a]	57.59 +/- 69.31 [a,b]	58.20 +/- 75.90 [a,b]	55.13 +/- 62.05 [b]
Vitamin D (IU)	67.59 +/- 88.21 [a]	65.63 +/- 126.74 [a]	82.35 +/- 163.61 [a]	70.19 +/- 112.51 [a]
Vitamin A (µg)	602.40 +/- 1204.79 [a]	599.05 +/- 1072.80 [a]	594.02 +/- 1120.83 [a]	499.59 +/- 854.51 [a]
Iron (mg)	12.26 +/- 7.01 [a]	11.27 +/- 7.27 [a]	11.78 +/- 7.24 [a]	11.41 +/- 7.98 [a]
Calcium (mg)	1078.16 +/- 745.97 [a]	931.23 +/- 657.66 [b]	1013.85 +/- 674.06 [a,b]	955.35 +/- 647.87 [a,b]
Magnesium (mg)	241.84 +/- 161.02 [a]	223.18 +/- 146.18 [a]	224.66 +/- 131.09 [a]	216.59 +/- 142.44 [a]
Total water (g)	2704.52 +/- 1122.72 [a]	2528.83 +/- 1011.37 [a]	2664.27 +/- 951.65 [a]	2615.81 +/- 1039.72 [a]
Fiber (g)	17.33 +/- 10.21 [a]	15.40 +/- 9.55 [b]	16.18 +/- 11.28 [a,b]	15.76 +/- 11.59 [a,b]
Vitamin K (µg)	96.73 +/- 180.25 [a]	77.95 +/- 132.81 [a]	76.56 +/- 132.14 [a]	70.51 +/- 101.25 [a]
Thiamine (mg)	1.48 +/- 0.92 [a]	1.37 +/- 0.92 [a]	1.38 +/- 0.88 [a]	1.38 +/- 1.01 [a]
Riboflavin (mg)	1.33 +/- 0.84 [a]	1.24 +/- 0.89 [a]	1.27 +/- 0.87 [a]	1.16 +/- 0.85 [a]

General Linear model – repeated measures design with gender as between subjects' factor.

Post-hoc tests adjusted with Sidak method and for false discovery rate

Values with different letters in superscript denote statistical significance, after FDR adjustment.

Nutrients	First evaluation	Second evaluation	Third evaluation	Fourth evaluation
Niacin (mg)	17.18 +/- 11.95 [a]	16.85 +/- 13.58 [a]	16.99 +/- 12.66 [a]	16.24 +/- 11.12 [a]
Vitamin B6 (mg)	1.39 +/- 0.99 [a]	1.36 +/- 1.12 [a]	1.33 +/- 0.96 [a]	1.34 +/- 0.96 [a]
Folates (µg)	300.73 +/- 222.25 [a]	260.07 +/- 205.57 [a,b]	268.95 +/- 204.25 [a,b]	251.26 +/- 180.85 [b]
Vitamin B12 (µg)	3.58 +/- 5.28 [a]	3.88 +/- 6.30 [a]	4.04 +/- 6.57 [a]	3.77 +/- 5.89 [a]
Pantothenic acid (mg)	4.08 +/- 2.41 [a]	3.94 +/- 2.84 [a]	3.97 +/- 2.55 [a]	3.64 +/- 2.33 [a]
Betaine (mg)	32.03 +/- 171.72 [a]	14.87 +/- 32.58 [a]	18.88 +/- 96.51 [a]	14.07 +/- 26.09 [a]
Choline (mg)	250.33 +/- 160.41 [a]	224.69 +/- 177.09 [a]	244.99 +/- 181.95 [a]	253.31 +/- 189.80 [a]
Copper (mg)	1.02 +/- 0.67 [a]	0.94 +/- 0.63 [a]	0.94 +/- 0.53 [a]	0.91 +/- 0.63 [a]
Fluor (µg)	444.12 +/- 625.22 [a]	386.19 +/- 555.25 [a]	403.44 +/- 579.09 [a]	336.35 +/- 526.32 [a]
Phosphor (mg)	952.38 +/- 547.91 [a]	886.50 +/- 543.55 [a]	917.36 +/- 520.42 [a]	884.47 +/- 544.43 [a]
Manganese (mg)	1.99 +/- 1.85 [a]	2.41 +/- 12.70 [a]	1.98 +/- 3.27 [a]	2.12 +/- 6.59 [a]
Selenium (µg)	80.46 +/- 53.81 [a]	77.96 +/- 55.34 [a]	82.12 +/- 54.71 [a]	77.99 +/- 53.29 [a]
Zinc (mg)	7.08 +/- 4.35 [a]	6.74 +/- 5.01 [a]	6.74 +/- 4.35 [a]	6.65 +/- 5.00 [a]
Potassium (mg)	2327.06 +/- 1042.88 [a]	2081.36 +/- 1011.08 [b]	2179.68 +/- 1002.34 [a,b]	2153.82 +/- 1117.42 [a,b]
Sodium (mg)	3428.53 +/- 2300.20 [a]	3072.57 +/- 2087.39 [a]	3246.28 +/- 2041.39 [a]	3212.74 +/- 2044.13 [a]
EPA (mg)	0.03 +/- 0.11 [a]	0.04 +/- 0.14 [a]	0.05 +/- 0.22 [a]	0.03 +/- 0.12 [a]
DHA (mg)	.06 +/- 0.18 [a]	0.07 +/- 0.22 [a]	0.08 +/- 0.33 [a]	0.07 +/- 0.23 [a]

General Linear model – repeated measures design with gender as between subjects' factor.

Post-hoc tests adjusted with Sidak method and for false discovery rate

Values with different letters in superscript denote statistical significance, after FDR adjustment.

Nutrients	First evaluation	Second evaluation	Third evaluation	Fourth evaluation
Vitamin E (g)	4.85 +/- 4.46 [a]	4.84 +/- 4.74 [a]	4.52 +/- 3.82 [a]	4.32 +/- 4.05 [a]
LA (g)	3.07 +/- 5.38 [a]	2.73 +/- 5.06 [a]	3.23 +/- 4.70 [a]	3.09 +/- 4.74 [a]
ALA (g)	0.30 +/- 0.59 [a]	0.28 +/- 0.59 [a]	0.34 +/- 0.58 [a]	0.32 +/- 0.54 [a]
Fatty acids total saturated (g)	24.01 +/- 18.65 [a]	21.37 +/- 16.80 [a]	22.60 +/- 17.57 [a]	22.46 +/- 17.70 [a]
Sugars total (g)	52.72 +/- 41.56 [a]	46.32 +/- 37.71 [a,b]	49.43 +/- 46.39 [a,b]	44.32 +/- 39.54 [b]
General Linear model – repeated measures design with gender as between subjects' factor.				
Post-hoc tests adjusted with Sidak method and for false discovery rate				
Values with different letters in superscript denote statistical significance, after FDR adjustment.				

In addition to differences already presented, for nutrients such as vitamin C, total water, folates, sodium and total sugars, initially significant decreases in second recall were observed, but these became insignificant when corrected for FDR (data not shown). No significant increases were observed for any nutrient variable.

Pairwise comparisons between second and third evaluation, or third and fourth evaluation, indicated no significant differences for energy, macro- or micronutrient reported intakes.

Week-end vs week-day intakes

We tested whether the presence of weekend days was balanced between ordered recalls, in order to identify a potential bias that could be associated with the differences previously identified. Weekend days represented 12.0% of the first evaluation, 10.8% of second evaluation, 9.4% of third evaluation, and 57.7% of forth evaluation. Excepting the fourth evaluation which had a significantly higher proportion of weekend days as compared with previous evaluations ($p > 0.0083$), between first, second or third evaluation, no significant differences were observed after Bonferroni correction ($p > 0.0083$). Analysis within each evaluation revealed no significant differences between week-end and weekdays intakes for the first and third day of evaluation, higher intake of vitamin A in the weekend versus work days in the second, and higher intake of docosahexaenoic acid (DHA) in weekends as compared to work days in the fourth recall, after FDR correction (results not shown).

Using weekend intakes versus the mean intake values of the 3 weekdays for energy and nutrients, significant interactions were observed between the type of day and the gender of participants. Therefore, the analysis in Table 2 is presented stratified by gender. In males, higher intakes of energy were reported in weekends as compared to weekday intakes, but when corrected for FDR it did no longer reach the

threshold for significance. Higher but non-significant intakes were observed for all macronutrients. In contrast, for vitamin C and vitamin K, significantly higher intakes were observed in weekdays.

Table 2

Weekend vs weekday nutrient intakes stratified by gender (N = 388 individuals with obesity)

Nutrients	Males (n1 = 189)			Females (n2 = 199)		
	Weekend intake	Weekday intake	Sig.*	Weekend intake	Weekday intake	Sig.*
Energy (kcal)	1985.3 +/- 871.3	1802.0 +/- 697.5	0.013	1282.4 +/- 507.5	1303.8 +/- 501.6	0.920
Protein (g)	93.8 +/- 44.5	87.5 +/- 29.9	0.102	63.3 +/- 28.8	64.9 +/- 25.6	0.594
Fat (g)	85.6 +/- 57.7	76.2 +/- 35.9	0.083	52.6 +/- 29.2	52.7 +/- 28.9	0.905
Carbohydrates (g)	205.7 +/- 128.1	192.7 +/- 78.8	0.542	146.2 +/- 64.4	148.7 +/- 57.3	0.667
Fat (%)	37.4 +/- 11.4	37.5 +/- 7.9	0.961	35.6 +/- 11.2	35.2 +/- 8.2	0.438
Carbohydrates (%)	42.4 +/- 11.1	43.3 +/- 8.7	0.142	46.9 +/- 13.8	46.7 +/- 9.9	0.996
Vitamin C (mg)	64.8 +/- 82.4	69.7 +/- 56.0	**0.002****	53.3 +/- 61.8	54.2 +/- 41.7	0.293
Vitamin D (IU)	86.8 +/- 140.3	87.2 +/- 94.3	0.195	54.0 +/- 80.6	58.2 +/- 72.1	0.119
Vitamin A (µg)	561.3 +/- 826.8	675.4 +/- 815.4	0.014	409.3 +/- 367.7	515.4 +/- 349.8	**0.001****
Iron (mg)	13.5 +/- 9.5	13.3 +/- 5.4	0.254	9.4 +/- 5.0	10.1 +/- 4.6	0.033
Calcium (mg)	1060.0 +/- 755.2	1115.3 +/- 529.2	0073	779.6 +/- 499.9	903.5 +/- 483.3	**0.002****
Magnesium (mg)	258.9 +/- 155.9	261.6 +/- 117.1	0.299	186.1 +/- 115.4	194.9 +/- 85.7	0.061
Total water (g)	2788.2 +/- 1161.4	2828.7 +/- 818.5	0.386	2378.5 +/- 936.4	2413.8 +/- 730.3	0.576
Fiber (g)	18.9 +/- 14.8	18.1 +/- 8.1	0.377	13.7 +/- 6.9	14.4 +/- 6.4	0.417
Vitamin K (µg)	77.1 +/- 103.3	96.8 +/- 109.3	**0.001****	69.3 +/- 119.9	65.6 +/- 79.8	0.508

**Wilcoxon Signed Ranks Test*

***Significant using a false discovery rate of 0.05*

Sig., significance

Nutrients	Males (n1 = 189)			Females (n2 = 199)		
	Weekend intake	Weekday intake	Sig.*	Weekend intake	Weekday intake	Sig.*
Thiamine (mg)	1.7 +/- 1.1	1.6 +/- 0.7	0.173	1.1 +/- 0.7	1.2 +/- 0.6	0.242
Riboflavin (mg)	1.5 +/- 1.1	1.4 +/- 0.6	0.214	1.0 +/- 0.7	1.1 +/- 0.5	**0.004****
Niacin (mg)	20.8 +/- 13.8	19.6 +/- 9.3	0.418	13.5 +/- 8.6	13.9 +/- 7.4	0.404
Vitamin B6 (mg)	1.7 +/- 1.1	1.6 +/- 0.7	0.531	1.1 +/- 0.7	1.1 +/- 0.6	0.466
Folates (µg)	295.4 +/- 205.7	316.8 +/- 164.5	0.067	204.3 +/- 122.7	231.1 +/- 121.4	**0.004****
Vitamin B12 (µg)	4.5 +/- 6.1	4.6 +/- 4.3	0.294	3.0 +/- 5.5	3.0 +/- 3.1	0.029
Pantothenic acid (mg)	4.5 +/- 2.6	4.3 +/- 1.8	0.768	3.3 +/- 2.0	3.5 +/- 1.6	0.061
Betaine (mg)	19.7 +/- 37.5	22.4 +/- 66.1	0.194	13.7 +/- 27.5	19.1 +/- 86.2	0.422
Choline (mg)	298.8 +/- 213.2	278.1 +/- 136.7	0.696	205.7 +/- 125.2	204.9 +/- 100.5	0.678
Copper (mg)	1.1 +/- 0.7	1.1 +/- 0.5	0.386	0.8 +/- 0.6	0.8 +/- 0.3	0.089
Fluor (µg)	438.9 +/- 626.0	434.7 +/- 465.4	0.073	353.0 +/- 493.8	343.8 +/- 402.3	0.608
Phosphor (mg)	1117.9 +/- 637.4	1031.2 +/- 434.0	0.041	747.8 +/- 379.6	778.6 +/- 334.7	0.288
Manganese (mg)	2.3 +/- 2.7	2.1 +/- 1.8	0.231	2.4 +/- 9.1	2.0 +/- 6.0	0.882
Selenium (µg)	100.5 +/- 56.5	92.6 +/- 38.8	0.111	63.5 +/- 41.5	65.4 +/- 33.1	0.506
Zinc (mg)	8.3 +/- 5.7	7.9 +/- 3.3	0.846	5.5 +/- 3.6	5.7 +/- 2.8	0.373
Potassium (mg)	4093.2 +/- 2661.3	3783.7 +/- 1696.0	0.250	2580.6 +/- 1428.7	2653.7 +/- 1195.2	0.611
Sodium (mg)	2517.0 +/- 1276.0	2406.3 +/- 836.9	0.791	1951.1 +/- 810.4	1936.5 +/- 618.9	0.498
EPA (mg)	0.0 +/- 0.1	0.0 +/- 0.1	0.741	0.0 +/- 0.1	0.0 +/- 0.1	0.020

**Wilcoxon Signed Ranks Test*

***Significant using a false discovery rate of 0.05*

Sig., significance

Nutrients	Males (n1 = 189)			Females (n2 = 199)		
	Weekend intake	Weekday intake	Sig.*	Weekend intake	Weekday intake	Sig.*
DHA (mg)	0.1 +/- 0.2	0.1 +/- 0.2	0.717	0.1 +/- 0.3	0.0 +/- 0.1	0.029
Vitamin E (g)	5.4 +/- 5.3	5.0 +/- 3.2	0.764	4.3 +/- 4.5	4.0 +/- 2.2	0.072
LA (g)	4.0 +/- 5.0	3.8 +/- 4.1	0.669	2.5 +/- 4.1	2.1 +/- 2.3	0.348
ALA (g)	0.4 +/- 0.6	0.4 +/- 0.5	0.827	0.3 +/- 0.5	0.2 +/- 0.3	0.333
Fatty acids total saturated (g)	29.3 +/- 22.7	26.3 +/- 14.4	0.306	16.9 +/- 10.5	19.1 +/- 12.4	0.084
Sugars total (g)	46.7+/-43.9	49.7 +/- 32.2	0.270	47.3 +/- 38.5	45.3 +/- 26.6	0.490
**Wilcoxon Signed Ranks Test*						
***Significant using a false discovery rate of 0.05*						
Sig., significance						

In females, no significant differences were noted in weekend intakes as compared to weekday intakes for energy and all macronutrients. For micronutrients, higher intakes of vitamin A, riboflavin, folates and calcium during weekday were observed, when compared to weekends.

A linear regression model was built to predict the difference of energy intake between weekend and weekday intakes, using the difference of micronutrient intakes between weekend and weekday intakes, gender of participants, and controlling for age and BMI. The results indicated a significant contribution of gender and of differences in intakes for carbohydrates and fats. The highest contribution to the difference in energy had the difference of fat intakes (g), which accounted alone for 36.5% for the variation in energy, followed closely by the difference in carbohydrates (g), which accounted alone for 28% of variation in energy (Table 3).

Table 3

Unstandardized coefficients and 95% CI for the prediction of the difference in calories from weekends to work week days

Independent variables	Unstandardized Coefficients		95.0% Confidence Interval for B
	B	Std. Error	
carbohydrates intake difference we-wd	4.363	0.109	4.148; 4.578
fat intake difference we-wd	10.304	0.225	9.862; 10.746
Gender Female	-41.909	18.714	-78.718; -5.100
Dependent Variable: energy difference between weekend days and weekdays (we-wd).			
Predictors in the Model: fat intake difference we-wd, carbohydrates intake difference we-wd, gender; controlling for Age and body mass index (BMI); Constant = 61.6			

Exclusion of the second 24-hrs recall

Because the second evaluation was identified to have differences in reported intakes of energy and carbohydrates when compared to the first evaluation, we sought to evaluate whether, by omitting the second evaluation, significant changes could be identified when compared to the means across all four evaluations. Table 4 indicates the difference of mean intakes for the complete set of recalls and for mean intakes of recalls from which the second 24-hrs recall was excluded. Results indicated differences in intakes for energy and many macro and micronutrients (Table 4), with size effects (r) ranging from small (0.1–0.25) to small-to-medium effect size (0.26–0.29) (13, 14).

Table 4

Mean differences between including three and four 24-hrs recalls (N = 388 subjects)

Nutrient	Mean of 3 recalls minus mean of four recalls	Significance *	Size effect (r)
Energy (kcal)	27.5 +/- 154.1	< 0.001**	0.19***
Protein (g)	0.7 +/- 9.0	0.010**	0.13***
Fat (g)	1.0 +/- 9.5	0.006**	0.14***
Carbohydrates (g)	3.1 +/- 19.8	0.000**	0.23***
Fat (%)	-0.1 +/- 3.1	0.887	0.01
Carbohydrates (%)	0.0 +/- 3.2	0.917	0.01
Vitamin C (mg)	1.2 +/- 18.2	0.001**	0.16
Vitamin D (IU)	1.8 +/- 33.4	< 0.001**	0.26****
Vitamin A (µg)	-8.6 +/- 292.0	0.304	0.05
Iron (mg)	0.1 +/- 1.7	0.001**	0.16***
Calcium (mg)	20.8 +/- 152.6	0.000**	0.19***
Magnesium (mg)	1.0 +/- 33.4	0.041	0.10
Total water (g)	31.3 +/- 218.2	0.064	0.09
Fiber (g)	0.2 +/- 2.4	0.001**	0.17***
Vitamin K (µg)	0.8 +/- 33.5	0.001**	0.17***
Thiamine (mg)	0.0 +/- 0.2	0.029**	0.11***
Riboflavin (mg)	0.0 +/- 0.2	0.064	0.09
Niacin (mg)	0.0 +/- 3.2	0.196	0.06
Vitamin B6 (mg)	0.0 +/- 0.3	0.158	0.07
Folates (µg)	3.2 +/- 49.9	0.000**	0.19***
Vitamin B12 (µg)	0.0 +/- 1.6	0.000**	0.17***
Pantothenic acid (mg)	0.0 +/- 0.7	0.309	0.05

* Wilcoxon Signed Ranks Test

**Significant using FDR of 0.05

***small size effect

****small-to-medium size effect

Nutrient	Mean of 3 recalls minus mean of four recalls	Significance *	Size effect (r)
Betaine (mg)	1.7 +/- 21.1	0.000**	0.26****
Choline (mg)	5.9 +/- 44.7	0.000**	0.23***
Copper (mg)	0.0 +/- 0.1	0.031**	0.11***
Fluor (µg)	1.9 +/- 112.7	0.052	0.10
Phosphor (mg)	7.8 +/- 117.4	0.041	0.10
Manganese (mg)	-0.1 +/- 2.6	0.018**	0.12***
Selenium (µg)	0.5 +/- 13.3	0.241	0.06
Zinc (mg)	0.0 +/- 1.2	0.059	0.09
Potassium (mg)	53.9 +/- 446.0	0.000**	0.18***
Sodium (mg)	33.5 +/- 225.1	0.001**	0.17***
EPA (mg)	0.0 +/- 0.0	0.000**	0.18***
DHA (mg)	0.0 +/- 0.1	0.000**	0.19***
Vitamin E (g)	-0.1 +/- 1.1	0.180	0.07
LA (g)	0.1 +/- 1.3	0.000**	0.22***
ALA (g)	0.0 +/- 0.1	0.000**	0.26****
Fatty acids total saturated (g)	0.4 +/- 3.6	0.000**	0.19***
Sugars total	0.6 +/- 8.7	0.004**	0.15***
* Wilcoxon Signed Ranks Test			
**Significant using FDR of 0.05			
***small size effect			
****small-to-medium size effect			

Discussion

The current study aimed to investigate the potential bias induced by the order of 24-hrs recalls, in a population of obese men and women, who were at the time of the investigation under medical supervision, under *ad libitum* diets of their choice (no dietary intervention).

The reduction in reporting, or a true reduction of intakes in consecutive days of evaluation, has been reported in some occasions (15), but has never been approached in obese individuals yet, to the best of

our knowledge. In our dataset, energy and carbohydrates intakes (in which a reduction of reporting was observed), and fat and protein intakes (in which similar intakes were observed), support the results found by *Arab et al* in series of eight days of evaluation (15). The reduction of reporting in series of 24-hrs recalls could be explained by subjects becoming aware of their intakes in the process of declaring the intakes, also known as training bias (the “big brother” effect). These findings could also be due to reporting fatigue, or could reflect genuine changes in eating habits, induced by the fact that subjects become more aware about their diets and the importance of healthy eating. Underreporting of energy intakes was previously associated with dissatisfaction with body image and dieting practices, especially in women (7).

In this study, the only differences in energy and macronutrient reporting (carbohydrates) were identified between the first two recalls (Table 1 and Fig. 1). These systematic differences, specific to second recall, prompted us to ascertain whether the second recall could play a role in modifying the average intakes, if this time point would be eliminated from the averages constructed using all recalls. The exclusion of the second recall led to the occurrence of differences in energy and intakes for many nutrients, when compared to averages across all four recalls.

In this context, several questions deserve more scrutiny: 1) If a systematic bias exists in the reported values of one specific ordered recall, and this bias cannot be ascertained to other known factors, should this recall be included in further analyses?; 2) If this specific recall is eliminated from calculations, how does this change the reported energy and nutrient intakes that are considered further in the subsequent analyses? Our study suggests that the elimination of the recall identified with order-specific bias would significantly change the reported intakes, and this could potentially create further methodological issues when intake data would be used for further analyses (Table 4).

On the other hand, this study has also identified lower intakes for several micronutrients (vitamin C, calcium, fiber, folates, potassium), and for total sugars, which were specific for either the second or fourth recalls (Table 1 **and** Fig. 2). Due to the design of our study, it is difficult to speculate whether such differences are truly systematic and specific to the order of recalls, or if, within the FDR limits, these are spurious.

The origin of differences in energy and carbohydrate intakes, specific to the second recall, is not clear. One hypothesis could be that such differences reflect true differences in intakes. The follow-up recalls (second to fourth) were performed, for each individual, in different days of the week, with no obvious reason to consider that such differences could arise from a different distribution between weekends and work week days. Therefore our analysis indicated that these differences could not be ascertained to the distribution of weekends in first two recalls. However, we did identify differences in some of the nutrient intakes between weekend days and work days (Table 2), which could be due to differences in eating habits. Studies performed in other countries reported that the day of the week had little impact on the variance of reported values (4, 16). Recently, Gibson et al discussed many causes of misreporting and

measurement errors in self-administered 24-hour recalls (17), However, our recalls were all administered by an interviewer.

Another hypothesis is that the decrease in energy and carbohydrate reported intakes, in the second recall, could reflect underreporting of intakes due to training bias. Although the 24-hrs recall method has the lowest underreporting bias (18) as compared with other food intake capturing methods, it is known that this method is still prone to misreporting energy intakes by up to 15% (18, 19).

Another reason for the systematic differences observed between the first two recalls could be due to changes in eating behavior, which then subsides or diminishes during subsequent recalls (third and fourth).

Although the weekend/weekday discrepancies had been tackled before in studies aiming children (20, 21), young women (22), middle life women (23) or general population (24), this assessment has not been previously done in an obese population, when looking at the systematic differences between the order of recalls. In this population, the increase in the energy difference between weekend and weekday intake was related to an increase in both carbohydrates and fats, indicating higher non-specific macronutrient food intake in weekend.

The trend observed in men (Tables 2 and 4) was similar to other results published, suggesting that weekend intakes are higher than weekday intakes, with mean differences in energy of 195.1+/-832.1 kcal, in fat of 0.9+/-30.8 g, and in carbohydrates of 13.1+/-102.1 g, but not reaching significance threshold in our sample after FDR correction (24–26). For females there was no difference observed between weekdays and weekends, with mean differences in energy − 2.1+/-503.6 kcal, fat 10.2+/-52.4 g and carbohydrates − 1.1+/-69.6 g, which is in contrast to results published in other studies, where similar trends as in males were seen (23, 24). Vitamin K and vitamin C intakes were lower in weekend for men, and vitamin A and riboflavin, folates and calcium had lower intakes during weekend in women, denoting potentially a lower quality diet during weekend for both men and women, in agreement with other published results (22–25, 27, 28).

In order to improve the overall quality of ordered 24-hrs recalls used to capture the energy and nutrient intake, and to reduce the exacerbation of bias observed between different sessions, our study suggests the possibility of systematic differences in the reported intakes for energy and macronutrients, which can be specific to the second reporting session. Whether these differences are due to true lower intakes or due to reporting bias, this study indicates that repeated 24-hr recalls can inherently present systematic differences between specific sessions. In our study, these differences were specific to the second session.

The results obtained when comparing the average of four 24-hrs recalls with the average of three evaluations made up from the same 24-hrs recalls (minus the second evaluation) indicated an increase of the averaged energy intake, all macronutrient intakes and most of micronutrients, with small or small to medium size effects (Table 4).

To overcome this potential bias, we suggest that, prior to averaging specific intakes across all sessions of reporting, a preliminary analysis should be done in order to identify if a certain time point had systematic, order-specific differences form all other time points. Once identified, a decision should be made about whether to include this time point or not in further analyses for nutrient intakes.

One of the limitations of this study was that the true intake of nutrients was not assessed, nor biomarkers of available nutrients were available, so the true cause of the differences found for the second time point could not be identified. Another limitation was that the study did not use a control sample and, therefore, one cannot ascertain whether such differences were specific or not to individuals with obesity and associated morbidities.

Conclusion

We performed a study with 388 participants, where dietary intakes were investigated using four 24-hrs recalls. Systematic differences of reported intakes (energy and carbohydrates) were identified for the second session of reporting, when compared to first session. Preliminary analysis of potential differences between time points should identify whether session-specific bias exists, and whether such sessions should be included or not in further analyses.

Abbreviations

BMI – body mass index

DHA – Docosahexaenoic acid

FDR – false discovery rate

USDA – United States Department of Agriculture

Declarations

Ethics approval and consent to participate:

The study received ethics approval from the Scientific Research Ethics Committee Board of the Victor Babes University of Medicine and Pharmacy Timisoara, and was conducted in accordance with the Helsinki Declaration. Before any study procedure, all participants signed the informed consent.

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Availability of data and materials:

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Competing interests:

The authors declare no conflict of interest.

Authors contribution:

Conceptualization, C.L.S. and M.D.N.; methodology, M.D.N. and M.P.; validation, C.L.S., I.T.P., and S.P.; formal analysis, C.L.S.; investigation, A.S.; data curation, A.C.-E.; writing—original draft preparation, C.L.S.; writing—review and editing, M.D.N., A.C.-E. and M.P.; project administration, M.P.; funding acquisition, M.D.N.

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Figures

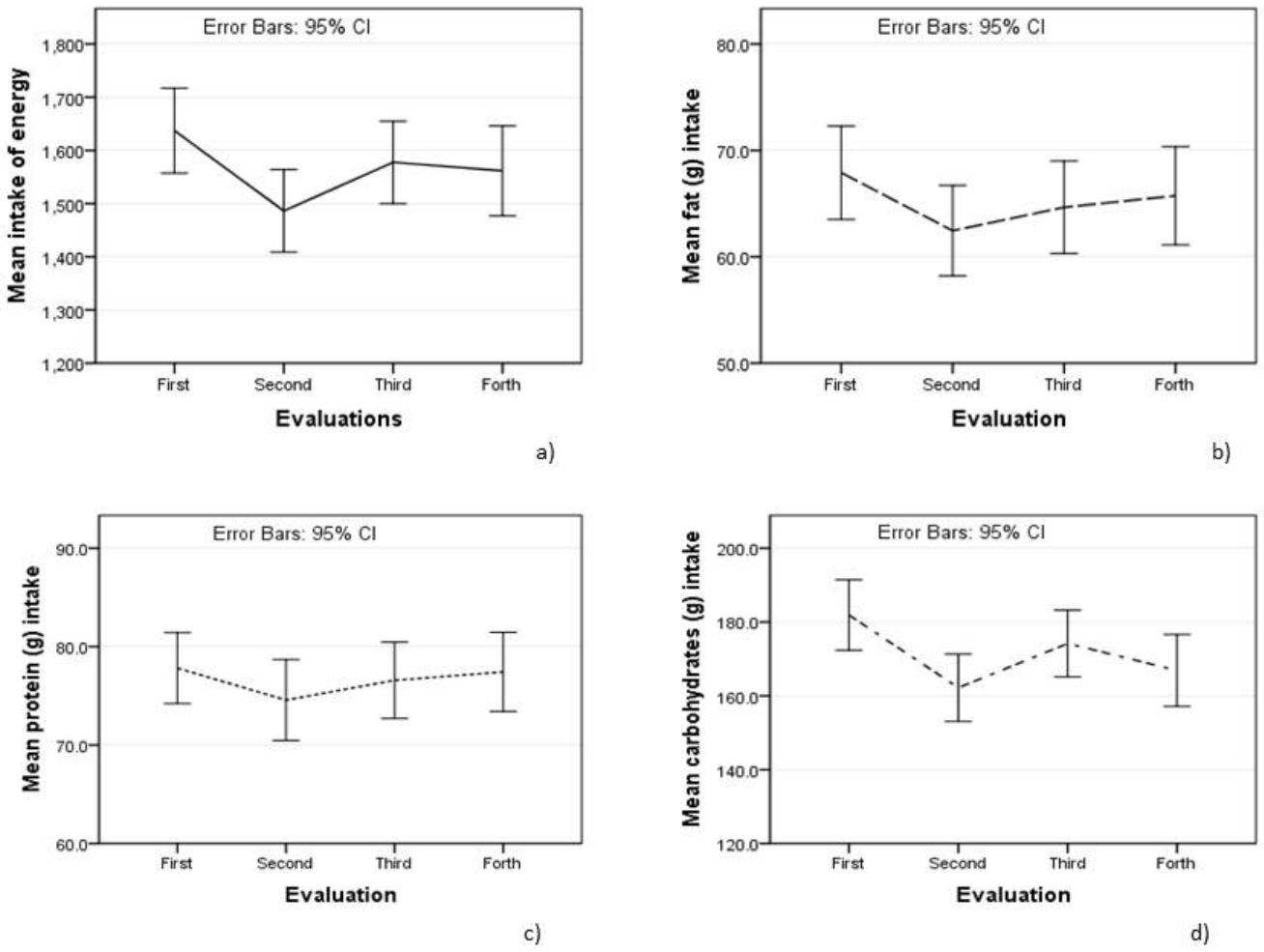

Figure 1

Differences in energy and macronutrient intakes between four evaluations of 24-hrs intake (N=388 individuals with obesity) Legend: a) energy intake (Kcal) trend b) fat intake (g) trend c) protein intake (g) d) carbohydrate intake (*) significantly lower as compared to initial evaluation (after FDR correction)

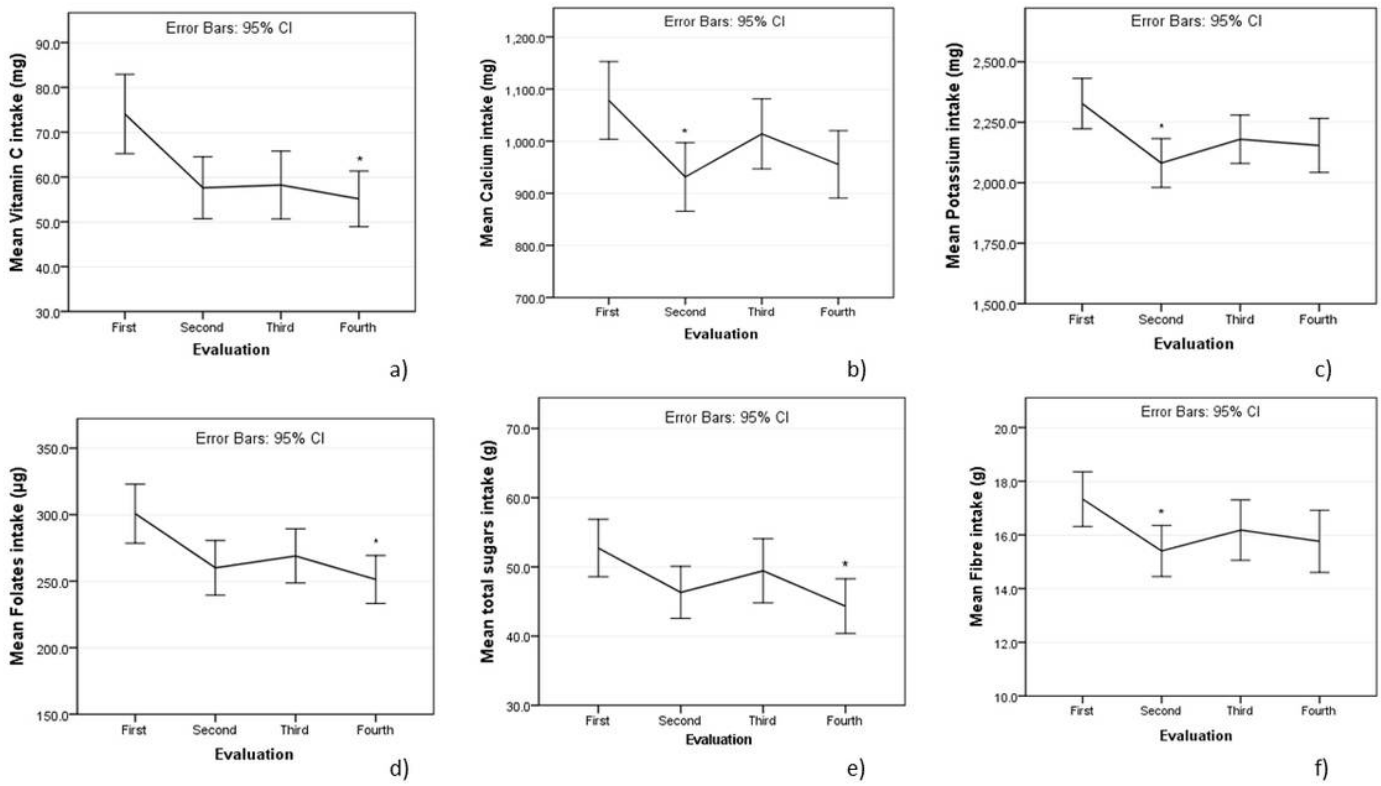

Figure 2

Differences in micronutrient intakes between four evaluations of 24-hrs intake (N=388 individuals with obesity) Legend: a) Mean Vitamin C intake (mg) b) Mean Calcium intake (mg) c) Mean Potassium intake (g) d) Mean Folates intake (µg) e) Mean Total sugars intake (mg) f) Mean Fiber intake (mg) (*) significantly lower as compared to initial evaluation (after FDR correction)