Eur J Nutr (2008) 47:55–58
DOI 10.1007/s00394-008-0695-x

SHORT COMMUNICATION

Carmen Herrero-Barbudo
Fernando Granado-Lorencio
Begoña Olmedilla-Alonso

Suitability of 3-point *versus* 7-point postprandial retinyl palmitate AUC in human bioavailability studies

Received: 27 August 2007
Accepted: 17 January 2008
Published online: 25 January 2008

C. Herrero-Barbudo
F. Granado-Lorencio (✉)
B. Olmedilla-Alonso
Unidad de Vitaminas
Servicio de Bioquímica Clínica
Hospital Universitario Puerta de Hierro
28035 Madrid, Spain
Fax: +34-91/3737-667
E-Mail: fgranado.hpth@salud.madrid.org

B. Olmedilla-Alonso (Current address)
Dpto. de Nutrición y Metabolismo
Instituto del Frío-CSIC
Ciudad Universitaria
Madrid, Spain

Abstract *Background* Bioavailability is a critical feature in the assessment of the role of micronutrients in human health. Although postprandial behaviour does not predict long-term responses and acute responses, it is accepted that the study of triglyceride-rich lipoprotein fractions reflects newly absorbed lipids from recent meals. *Aim* To assess the predictive value of a 3-point *versus* 7-point post-prandial response (area under the curve) in nutrient bioavailability studies in humans. *Methods* We used results from a human bioavailability study ($n = 19$) that consisted of a single-dose pharmacokinetic assay involving three types of commercially available vitamin A and E fortified milk. *Results* Correlation coefficients between 3-point AUC (AUCp, predictive) *versus* 7-point AUC (AUCc, conventional) ranged between $r = 0.81$ ($P < 0.001$) for vitamin A-fortified skim milk and $r = 0.95$ ($P < 0.001$) for whole milk. Bland-Altman representations showed a good agreement between the two methods with 95% of the differences within the concordance limits. More than 90% of the subjects were correctly classified in the same or adjacent quartiles and he calculated relative absorption of vitamin A from the foods was, on average, <5% lower using the AUCp compared to that estimated using AUCc. *Conclusion* The use of the 3-point approach may be a reliable alternative to assess the relative postprandial lipid response in human bioavailability studies. Nevertheless, since this approach has been studied considering one nutrient (i.e. preformed vitamin A) and one type of food (i.e. milk), its applicability to other nutrients and foods should be tested.

Key words bio availability – vitamin A – human study – area under the curve

Introduction

Bioavailability is a critical feature in the assessment of the role of micronutrients in human health. Studies of the bioavailability of vitamins and related compounds (i.e. carotenoids) are difficult due to the endogenous presence of these substances in plasma and tissues, and most methods only yield information regarding the relative bioavailability (relative to a reference dose or control), not the absolute bioavailability of the vitamins [9].

Major challenges in the study of bioavailability include the release of these compounds from the food

matrix, micellization, the measurement of the plasma response and the inter-individual variability [6]. It is a well-known fact that humans can be divided into "responders" and "non-responders" according to their plasma response. Although postprandial behaviour does not predict long-term responses and acute responses (after a single dose) usually show larger inter-individual variability than chronic (multiple) intakes [6, 9], it is accepted that the study of triglyceride-rich lipoprotein fractions (TRL) reflects newly absorbed lipids from recent meals [2, 7, 10].

In vitro models based on human physiology have been developed as simple, inexpensive and reproducible tools to predict the bioavailability of different food components [5] (i.e. ascorbic acid, carotenoids, chlorophylls, polyphenols) but their potential predictive value regarding human absorption of phytochemicals should be validated in different in vivo situations [13]. In this respect, most of the in vivo studies rely on the statistical significance of the changes observed which, in turn, depend on the number of subjects involved and the magnitude of the change. Thus, a reliable and cost-efficient approach to assess the relative postprandial response would allow its applicability, in comparative terms, in human nutrient bioavailabilty studies using larger groups of subjects and using different foods while reducing the costs associated and minimizing the invasiveness of the method. Within this context, our aim was to assess the potencial predictive value (i.e. concordance and exchange value) of a 3-point postprandial response (area under the curve) approach in nutrient bioavailability studies in humans.

Subjects and methods

We approach the predictive value of the 3-point AUC using results from a human study to study the bioavailability of vitamins A and E from foods [12] that consisted of a single-dose pharmacokinetic assay involving three types of commercially available milk: whole milk, whole milk fortified with vitamins A and E and skim milk fortified with vitamins A and E. Nineteen apparently healthy volunteers (nine men and ten women) were enrolled in this bioavailability study and, for the three assays, a common breakfast, consisting of 430 ml of milk plus 10 unfortified biscuits, was provided. The three types of milk were consumed by all the subjects at 1-week intervals. The volunteers were asked to consume the breakfast after which blood samples were taken 90 min later and hourly for 5 hours. The response was evaluated by measuring the retinyl ester concentrations in the triacylglycerol-rich lipoprotein fractions (TRL) that were prepared from plasma (EDTA 7.5%) according to the protocol described by Griffiths et al. [10]. The study procedures were performed in accordance with the Ethical Committee for Clinical Investigation of Hospital Universitario Puerta de Hierro. Subjects were informed about the study and gave their written consent.

The areas under the curves (AUC) of the postprandial responses in TRL fractions versus time were calculated by the trapezoidal method after correction for baseline concentrations. The AUC were calculated using the conventional approach (AUCc) with all the samples collected (7-points; baseline, 1.5, 2.5, 3.5, 4.5, 5.5, 6.5 hours) and using 3 points (baseline, peak concentration and 6.5 hours), referred to as "predictive AUC" (AUCp) as previously reported [11]. For the 3-point curve, the time to the maximum concentration (3.5 hours) was established on the basis of previously bioavailability studies [3, 4, 14]. Percentages of relative absorption during the study period (6.5 hours) were calculated on the basis of the AUC values for retinyl esters in TRL fractions, correcting for plasma volume (assuming 4% body weight) [8], and expressed against the dose supplied for each type of milk, as determined by HPLC analysis [12].

There were no gender statistical differences regarding the AUC (using the trapezoidal method after correction for baseline concentrations) for the three types of milk (ANOVA) and, thus, the men and women were grouped for the subsequent analyses [12]. Pearson correlation coefficients were calculated and the concordance between AUCc and AUCp values was established using Bland-Altman diagrams and the predictive value by calculating linear regression equations. All calculations were performed for each type of milk consumed. Statistical significance was set at $P < 0.05$, and the analysis was performed with SPSS 13.0 statistical software for Windows (SPSS Inc., Chicago, IL).

Results

Correlation coefficients between AUCc and AUCp were $r = 0.952$ ($P < 0.001$) for whole milk, $r = 0.886$ ($P < 0.001$) for vitamin-A fortified whole milk and $r = 0.810$ ($P < 0.001$) for vitamin A-fortified skim milk. Bland-Altman representations (Figure 1) showed a good agreement between the two methods with 95% of the differences within the concordance limits. The predictive and exchange value of the 3-point AUCp is shown by the regression equations (Table 1). Overall, more than 90% of the subjects were correctly classified in the same or adjacent quartiles (17 or 18 out of the 19

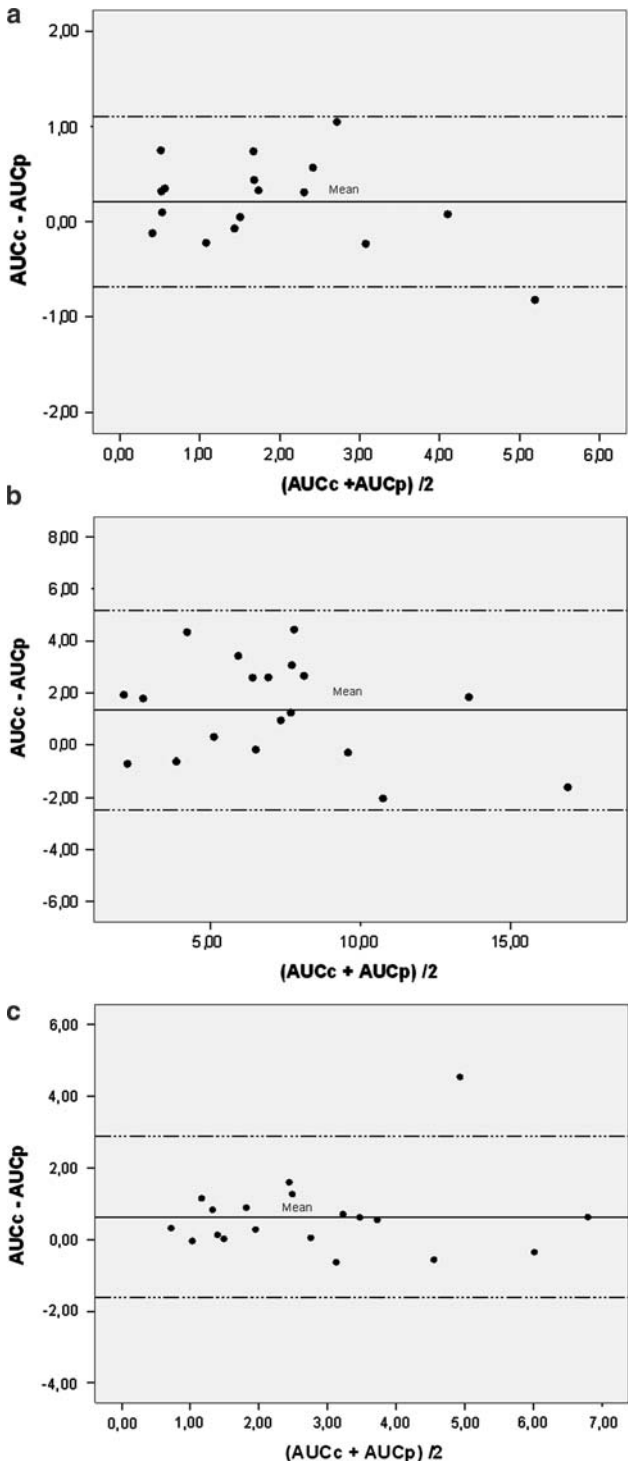

Fig. 1 Bland-Altman representations. Concordance between AUC of retinyl palmitate using 7-point *versus* 3-point measurements during the post-prandial response. **a** Whole milk; **b** vitamin A-fortified whole milk; **c** vitamin A-fortified skimmed milk

participants in each assay) and the calculated relative absorption of vitamin A was, on average, <5% lower using the 3-point AUCp (6–36%) compared to that estimated using 7-point AUCc (8–41%).

Table 1 Relationship between the postprandial responses measured using the conventional (7-point) and the predictive (3-point) approach

	Beta	Slope	Significance
Whole milk (n = 19)	0.475	0.850 *AUCp*	$P < 0.001$
Vitamin A-fortified whole milk (n = 19)	2.803	0.776 *AUCp*	$P < 0.001$
Vitamin A-fortified skim milk (n = 19)	0.957	0.872 *AUCp*	$P < 0.001$

Regression equations for conventional AUC as a function of predicted AUC (*AUCp*)

Discussion

The present results show that, as previously reported [11], a simplified approach can be used for the estimation of the post-prandial response. Compared to the more expensive and invasive conventional method, the use of a 3-point AUC may provide reliable information regarding the relative response of fat-soluble components in human bioavailability studies, both among subjects and foods, and especially, in comparative terms, i.e. assessing the effect of different matrices, absorption modifiers, processing methods, chemical forms, …

Although other simple approaches, i.e. a 1-point response, have been also used to monitor post-prandial responses upon nutrient intake [1, 3], these results have been interpreted in qualitative rather than quantitative terms. In this respect, the present study shows that a 3-point AUC may be used for quantitative purposes as well, since it can reliably the relative absorption, with <5% of deviation from a reference method, and also correctly classified >90% of the group according to their response. In addition, to the best of our knowledge, this is the first report to assess the validity of a brief approach to study in humans the bioavailability of nutrients from foods at achievable dietary levels and using different commercially available foods (i.e. whole versus fortified skim milks).

As previously mentioned, most *in vivo* bioavailability studies are performed in a small group of subjects and rely on the statistical significance of the changes observed which, in turn, depend on the number of subjects involved and the magnitude of the change. In this context, the use of the 3-point approach may be a reliable alternative to assess the relative postprandial response in human studies. This method is less invasive, reduces the number of samples to be processed and the costs (>50% in material, solvents and time) and, thus, makes it possible to increase the number of subjects, which, in turn, improves the reliability of the results. Nevertheless, it should be noted that this approach has been studied considering one

nutrient (i.e. preformed vitamin A) and one type of food (i.e. milk) and, therefore, its applicability to other nutrients and foods should be tested.

Acknowledgements This work was funded by the Fondo de Investigación Sanitaria (FIS 98/0386) and Instituto de Salud Carlos III (PI 051610), Spain.

References

1. Barua AB (1999) Intestinal absorption of epoxy-b-carotenes by humans. Biochem J 339:359–362
2. Biesalski HK (1997) Bioavailability of vitamin A. Eur J Clin Nutr 51(Suppl 1):S71–S75
3. Borel P, Grolier P, Mekki N, Boirie Y, Rochette Y, Le Roy B, Alexandre-Gouban MC, Lairon D, Azais-Braesco V (1998) Low and high responders to pharmacological doses of b-carotene: Proportion in the population, mechanisms involved and consequences on b-carotene metabolism. J Lipid Res 39:2250–2260
4. Cardinault N, Tyssandier V, Grolier P, Winklhofer-Roob BM, Ribalta J, Bouteloup-Demange C, Rock E, Borel P (2003) Comparison of the postprandial chylomicron carotenoid responses in young and older subjects. Eur J Nutr 42:315–323
5. Failla M, Chitchumroonchokchai C (2005) In vitro models as tools for screening the relative bioavailabilities of provitamin A carotenoids in foods. Technical Monographs Series 3. HarvestPlus
6. Faulks RM, Southon S (2005) Challenges to understand and measuring carotenoid bioavailability. Biochim Biophys Acta 170:95–100
7. Fielding B, Callow J, Owen RM, Samra J, Mathews DR, Frayn KN (1996) Postprandial lipemia: The origin of an early peak studied by specific dietary fatty acid intake during sequential meals. Am J Clin Nutr 63:36–41
8. Geigy JR (1962) Documenta Geigy. Scientific tables, 6th edn. Konrad Diem (ed) J.R. Geigy S.A., Basel, Switzerland, pp 547–548
9. Granado-Lorencio F, Olmedilla-Alonso B (2003) Bioavailability of vitamins. In: Vaquero MP, García-Arias T, Carbajal A, Sánchez-Muñiz FJ (eds) Bioavailability of micronutrients and minor dietary compounds. Metabolic and technological aspects. Recent research developments in nutrition. Research Singpost, Kerala 37/661(2):19–30
10. Griffiths AJ, Humphreys SM, Clark ML, Frayn K (1994) Immediate metabolic availability of dietary fat in combination with carbohydrates. Am J Clin Nutr 59:1025–1032
11. Guerci B, Paul JL, Hadjadj S, Durlach V, Verges B, Attia A, Girard-globa A, Drouin P (2001) Analysis of the postprandial lipid metabolism: use of a 3-point test. Diabetes Metab 27:449–457
12. Herrero-Barbudo C, Olmedilla-Alonso B, Granado-Lorencio F, Blanco-Navarro I (2006) Bioavailability of vitamins A and E from whole and vitamin-fortified milks in control subjects. Eur J Nutr 45(7):391–398
13. Oomen AG, Rompelberg CJM, Bruil MA, Dobbe CJG, Pereboom DPKH, Sips AJAM (2003) Development of an in vitro digestion model for estimating the bioaccessibility of soil contaminants. Arch Environ Contam Toxicol 44:281–287
14. Renuka KD, Silva R, Williams MC, Lovegrove JA (2001) Use of water miscible retinyl palmitate as markers of chylomicrones guves earlier peak response of plasma retinyl esters compared with oil-soluble retinyl palmitate. Br J Nutr 86:427–432