Anorexigenic postprandial responses of PYY and GLP1 to slow ice cream consumption: preservation in obese adolescents, but not in obese adults

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Abstract

Objective: Eating slowly increases the postprandial responses of some anorexigenic gut hormones in healthy lean subjects. As the rate of food intake is positively associated with obesity, the aim of the study was to determine whether eating the same meal at different rates evokes different postprandial anorexigenic responses in obese adolescent and adult subjects.

Design and methods: Eighteen obese adolescents and adults were enrolled. A test meal was consumed on two different sessions by each subject, meal duration taking either 5 min (fast feeding) or 30 min (slow feeding). Circulating levels of glucagon-like peptide 1 (GLP1), peptide YY (PYY), glucose, insulin, and triglycerides were measured over 210 min. Visual analog scales were used to evaluate the subjective feelings of hunger and satiety.

Results: Fast feeding did not stimulate GLP1 release in obese adolescent and adults, whereas slow feeding increased circulating levels of GLP1 only in obese adolescents. Plasma PYY concentrations increased both in obese adolescents and in adults, irrespective of the eating rate, but slow feeding was more effective in stimulating PYY release in obese adolescents than in adults. Simultaneously, slow feeding evoked a higher satiety only in obese adolescents compared with fast feeding but not in obese adults. In obese adolescents, slow feeding decreased hunger (only at 210 min). Irrespective of the eating rate, postprandial responses of insulin and triglycerides were higher in obese adults than in obese adolescents. Conclusion: Slow feeding leads to higher concentrations of anorexigenic gut peptides and favors satiety in obese adolescents, but this physiological control of food intake is lost in obese adults.

Introduction

In the USA and Europe, rates of eating and food sizes (fast feeding) have considerably increased in the past decades (1, 2). In this context, recent studies have shown that eating large portions in a very short time enhances food intake and is thereby a risk factor for obesity (3, 4).

The lifestyle of the modern civilization facilitates diffusion of fast feeding and, consequently, high energy intake. A high eating rate, combined with distraction of attention from eating, is believed to undermine the capacity of the body to regulate its energy intake at healthy levels because these factors impair the congruent association between sensory signals and metabolic consequences. A number of studies have shown that foods eaten quickly lead to high food intake and low satiating effects because this type of feeding exposes the human body to insufficient sensory cues for satiety (5, 6).

In accordance, in healthy lean adults, eating at a physiologically moderate pace leads to a more pronounced anorexigenic gut peptide response than eating at a very fast rate. This is reflected in peptide YY (PYY) and glucagon-like peptide 1 (GLP1) responses (7), which, acting on the hypothalamus and brain stem, inhibit food intake and stimulate satiety (8).

Based on the foregoing arguments, sociopolitical and educational interventions directed at limiting eating rate and portion size might help lean consumers to reduce their food intake (2, 9). This becomes even more important for obese patients, who reportedly eat faster than lean individuals (10). Interestingly, in obese patients, eating rate has a positive correlation with adiposity (11), regardless of adjustment for nutrient intake (11) or subject age (obese children, adolescents, and adults) (12).

To the best of our knowledge, postprandial concentrations of anorexigenic peptides have not been assessed.
in blood of obese patients in the context of different rates of eating behavior. As the obese state tends to worsen in an age-dependent manner (13), an intriguing possibility is that eating more slowly will induce more satiety and higher concentrations of anorexigenic peptides only in an early phase of obesity (e.g. obese adolescents), a physiological control of food intake that would be lost after many years of the obese state and the related insulin resistance (e.g. obese adults). Hence, this study examined possible differences in the postprandial responses of GLP1, PYY, glucose, insulin, and triglycerides when identical meals were consumed in two separate sessions of different duration (fast feeding vs slow feeding) by two groups of obese patients, i.e. adolescents and adults.

Materials and methods

Subjects

Obese adolescents (age, 16.7 ± 0.4 years; F/M, 6/3; BMI, 37.2 ± 2.4 kg/m²) and adults (age, 30.1 ± 3.2 years; F/M, 7/2; BMI, 44.1 ± 1.9 kg/m²) were recruited from the Istituto Auxologico Italiano, IRCCS, Verbania, Italy, to which they were admitted for a multidisciplinary weight reduction intervention. All adults were obese since pubertal age. Exclusion criteria included previous diagnosis of any disease affecting the endocrine system and metabolism (apart from obesity), chronic use of medications (including oral contraceptives) affecting metabolism and/or appetite, ≥ 5.0 kg weight change during the 3 months preceding study participation, pregnancy, allergies to or stated dislike of the components of the test meal (see below), and clinically diagnosed eating disorder or a score of ≥ 20 on the Eating Attitudes Test (14). All females were eumenorrheic, apart from a single adult woman who suffered from hypoligomenorrhea, in whom a diagnosis of polycystic ovary syndrome was ruled out. The two experimental groups (obese adolescents and adults) were socioeconomically homogenous when evaluated by a specific questionnaire (15). The study was approved by the Local Ethics Committee. All subjects participated in this study after providing their free and informed consent. In cases when the subject was < 18 years old, consent was provided by his/her parents.

Study design

Anthropometric characteristics were evaluated during the screening period. BMI was calculated from measured height and weight. Fat-free mass (FFM) and fat mass (FM) were evaluated by bioelectrical impedance analysis (Human-IM Scan, DS-Medigroup, Milan, Italy).

The experimental period comprised two separate tests occurring on nonconsecutive days over at least 2 weeks. The tests (fast feeding or slow feeding) were performed in a random order. After a 12-h fast, subjects began each of the two tests at the same period, between 0800 and 0830 h. The subjects were instructed to adhere to the diet and physical activity patterns of the day preceding each test and to abstain from strenuous physical exertion, caffeine, and smoking during the 12-h fast. Twenty-four-hour diet and activity recalls were completed during each test to ensure compliance with these instructions. To maintain a stable daily caloric intake of the in-hospital patients, the amount of foods administered at lunch and dinner of the experimental days was proportionally reduced to account for the calories of the test meal.

During each of the two tests, an intravenous cannula for blood sampling was inserted into a superficial forearm vein and kept patent by isotonic saline water infusion. All subjects consumed the same test meal of 10 kcal/kg ice cream for a maximum of 510 g (85 g, 59% of kcal fat, 33% carbohydrates, and 8% protein; lipid composition for 100 g of the product: monounsaturated fatty acids, 3.49 g; polyunsaturated fatty acids, 0.45 g; saturated fatty acids, 7.48 g; and cholesterol, 91 mg) at different rates. In one session (fast feeding), the entire meal was consumed within 5 min, whereas in the other (slow feeding) it was divided into six equal portions, which were given to the subject every 5 min and consumed within 30 min. Subjects were instructed to finish each portion in < 1 min to maintain a uniform rate of meal ingestion. Qualitative and quantitative characteristics of the test meal were chosen to elicit robust responses in PYY and GLP1 in lean subjects (our personal experience and reference (7)).

Blood samples for the measurement of glucose, insulin, triglycerides, PYY, and GLP1 were drawn before the meal and at 30-min intervals after the beginning of meal consumption until the end of the session 210 min later. Visual analog scales (VASs) for assessment of the subjective feelings of hunger and satiety were completed before the meal and at 30-min intervals after meal termination until the end of the study session.

Biochemical assays

Total plasma PYY level, including both PYY1–36 and PYY3–36, was measured by a commercially available RIA for PYY (Millipore, Saint Charles, MO, USA). The sensitivity of the method was 10 pg/ml; intra- and interassay coefficients of variation (CV) were 2.9 and 7.1% respectively.

Total plasma GLP1 level, including GLP17–36 amide, GLP17–37, GLP19–36 amide, GLP19–37, GLP11–36 amide, and GLP11–37, was measured, after an extraction procedure, by RIA (Millipore). A DPP-4 inhibitor was added to tubes to prevent the breakdown of GLP1. The sensitivity of the method was 3 pmol/l; intra- and interassay CV were 2.9 and 7.1% respectively.

Serum insulin concentration was determined by chemiluminescent immunometric assay using a
commercial kit (Immulite 2000, DPC, Los Angeles, CA, USA). The sensitivity of the method was 2 μIU/ml; intra- and interassay CV were 22–38% and 14–23% respectively.

Serum glucose level was measured by the glucose oxidase enzymatic method (Roche Diagnostics). Serum triglycerides levels were quantified by a colorimetric enzymatic method (Wako Chemical GmbH, Neuss, Germany).

**Statistical analysis**

Results are reported as mean ± S.E.M. Homeostasis model of assessment of insulin resistance (HOMA-IR) was calculated by the formula: (insulin (μIU/ml) × glucose (mg/dl))/405. The responses in glucose, insulin, triglycerides, PYY, and GLP1 and VASs for hunger and satiety were evaluated as absolute values and also as area under the curve (AUC) of postprandial measurements using the trapezoid rule for each session (fast feeding and slow feeding).

Despite a female prevalence in the recruited population, there were no statistical differences in any of the investigated parameters in each group between females and males. Thus, data of all subjects of the same group were pooled.

All parameters (glucose, insulin, triglycerides, PYY, GLP1, and VASs for hunger and satiety) were compared within each group (obese adolescents or adults) and among the two eating rates (fast feeding and slow feeding) using a two-way ANOVA with repeated measures followed by the post hoc Bonferroni’s test. One-way ANOVA was used to compare the AUCs of the four groups (obese adolescents and adults × fast feeding and slow feeding). The other demographic, clinical, metabolic, and hormonal characteristics of the study subjects were analyzed by Student’s t-test for unpaired data. Correlations were evaluated by calculation of Pearson’s product-moment coefficients (for all data). A stepwise multiple regression was used to test the influence of age, weight, BMI, and HOMA-IR on the investigated parameters in each group between females and males. Thus, data of all subjects of the same group were pooled.

Irrespective of the eating rate, food intake significantly increased circulating levels of PYY in both groups (P < 0.05; Fig. 1). Interestingly, plasma GLP1 concentrations were higher after slow feeding than fast feeding in obese adolescents at 90, 120, 150, 180, and 210 min postprandially (P < 0.05); the same increases in plasma GLP1 levels were recorded at all time points in fast and slowly fed obese adults (P < 0.05) (Fig. 1). When obese adolescents were slowly fed, plasma PYY levels were significantly higher than values stimulated by fast feeding at 90, 120, and 180 min; a statistically significant difference was present in obese adults only at 120 min (P < 0.05; Fig. 1). In contrast to obese adults, in obese adolescents, the AUC for GLP1 response over 210 min was significantly higher after slow feeding than fast feeding (P < 0.05); in addition, slow feeding induced a significantly higher GLP1 response in obese adolescents than in obese adults (P < 0.05) (Fig. 2). There were no differences in the AUCs for postprandial PYY responses over 210 min in obese adolescents and adults, when fast or slowly fed (inter- and intra-groups comparisons; Fig. 2).

Irrespective of the eating rate, there were significant decreases and increases in hunger and satiety VAS

**Results**

Baseline demographic, clinical, hormonal, and metabolic characteristics of the study subjects are reported in Table 1. Only slow feeding significantly increased plasma concentrations of GLP1 in obese adolescents (P < 0.05), whereas neither fast nor slow feeding significantly changed plasma levels of GLP1 in obese adults (Fig. 1). Irrespective of the eating rate, food intake significantly increased circulating levels of PYY in both groups (P < 0.05; Fig. 1). Interestingly, plasma GLP1 concentrations were higher after slow feeding than fast feeding in obese adolescents at 90, 120, 150, 180, and 210 min postprandially (P < 0.05); the same increases in plasma GLP1 levels were recorded at all time points in fast and slowly fed obese adults (P < 0.05) (Fig. 1). When obese adolescents were slowly fed, plasma PYY levels were significantly higher than values stimulated by fast feeding at 90, 120, and 180 min; a statistically significant difference was present in obese adults only at 120 min (P < 0.05; Fig. 1). In contrast to obese adults, in obese adolescents, the AUC for GLP1 response over 210 min was significantly higher after slow feeding than fast feeding (P < 0.05); in addition, slow feeding induced a significantly higher GLP1 response in obese adolescents than in obese adults (P < 0.05) (Fig. 2). There were no differences in the AUCs for postprandial PYY responses over 210 min in obese adolescents and adults, when fast or slowly fed (inter- and intra-groups comparisons; Fig. 2).

Irrespective of the eating rate, there were significant decreases and increases in hunger and satiety VAS

| Age (years) | 16.7±0.4 | 30.1±3.2* |
| Gender (F/M) | 6/3 | 7/2 |
| BMI (kg/m²) | 37.2±2.4 | 44.1±1.9* |
| FFM (%) | 56.4±2.2 | 48.3±2.3* |
| FM (%) | 43.6±2.2 | 51.7±2.3* |
| GLP1 (pmol/l) | 13.5±2.4 | 18.0±5.6 |
| PYY (pg/ml) | 85.5±4.4 | 90.3±7.1 |
| Triglycerides (mg/dl) | 81.6±1.5 | 88.2±1.6* |
| Glucose (mg/dl) | 65.6±4.2 | 101.9±14.2* |
| Insulin (μIU/ml) | 8.1±1.7 | 14.0±1.9* |
| HOMA-IR | 1.6±0.4 | 3.1±0.5* |

*P < 0.05 vs obese adolescents. FFM, fat-free mass; FM, fat mass; GLP1, glucagon-like peptide 1; PYY, peptide YY.
ratings respectively after food intake in both groups \((P < 0.05; \text{Fig. 3})\). The hunger VAS rating was significantly lower at 210 min in slowly fed obese adolescents, when compared with that recorded after fast feeding \((P < 0.05)\); there was no difference in obese adults \((\text{Fig. 3})\). The satiety VAS rating was higher in obese adolescents after slow feeding than fast feeding from 120 min to the end of the study \((210 \text{ min}; P < 0.05)\); there were the same increases in the satiety VAS rating for all time points in fast and slowly fed obese adults \((\text{Fig. 3})\). There were no significant differences in AUCs for hunger and satiety VAS ratings over 210 min between the two groups at different eating pace \((\text{intra-} \text{and inter-group comparisons; Fig. 2})\).

Irrespective of the eating rate, there were significant increases in serum levels of glucose and insulin after food intake in both groups, whereas levels of triglycerides significantly increased only in obese adolescents \((P < 0.05; \text{Fig. 4})\). There were the same increases in serum concentrations of glucose, insulin, and triglycerides at all time points in both obese adolescents and adults, fast and slowly fed \((\text{Fig. 4})\). There were significant differences in AUCs for serum levels of insulin and triglycerides over 210 min between the two groups when fast or slowly fed \((P < 0.05; \text{Fig. 5})\).

To assess whether postprandial changes in circulating PYY and GLP1 after slow and fast feeding, expressed as differences in maximum percent increases from preprandial values, were related to age, weight, BMI, and HOMA-IR, a stepwise multiple regression with PYY or GLP1 as dependent variable and age, weight, BMI, and HOMA-IR as independent variables was run. This analysis showed that across all the subjects, no significant effect emerged between these variables when the two subject groups \((\text{adolescents} \text{ and adults})\) were assessed separately or together.

As expected, BMI and FM were positively correlated with baseline serum levels of insulin \((r = 0.70, r = 0.67 \text{ respectively, } P < 0.05)\); baseline serum levels of glucose were positively correlated with those of insulin \((r = 0.48, P < 0.05)\); furthermore, there were positive correlations between BMI and FM \((r = 0.64, P < 0.05)\) or baseline serum levels of triglycerides \((r = 0.43, P < 0.05)\). HOMA-IR was positively correlated with BMI \((r = 0.68, P < 0.01)\), FM \((r = 0.65, P < 0.01)\), glucose \((r = 0.56, P < 0.01)\), and insulin \((r = 0.99, P < 0.01)\). Despite the negative correlation between plasma levels of PYY \((\text{but not GLP1})\) and FM \((r = -0.41, P < 0.05)\), there were no correlations between AUCs of GLP1 or PYY to slow feeding and fast feeding and BMI or FM. Interestingly, baseline serum levels of glucose, insulin, and HOMA-IR were negatively correlated with AUCs of GLP1 \((r = -0.66, r = -0.49, \text{ and } r = -0.54 \text{ respectively, } P < 0.05)\) or PYY \((r = -0.40, r = -0.42, \text{ and } r = -0.47 \text{ respectively} \) to slow feeding, but not to fast feeding; AUC of PYY \((\text{but not GLP1})\) to slow feeding was positively correlated with that to fast feeding \((r = 0.61, P < 0.05)\).

**Discussion**

The anorexigenic peptides GLP1 and PYY are released in response to nutrient ingestion from endocrine L cells, most densely located in the distal ileum \((16, 17, 18, 19, 20, 21)\). Importantly, administration of GLP1 or PYY reduced energy intake in both lean and overweight subjects \((22)\). The hypothalamus and the brainstem are thought to be the most relevant areas of satiating and hypophagic effects of peripheral GLP1 and PYY \((23, 24)\).

In this study, eating at a physiologically slow pace \((\text{slow feeding})\) led to a more pronounced anorexigenic peptide response than eating fast \((\text{fast feeding})\) in obese

![Figure 2](https://www.eje-online.org)

**Figure 2** Values of area under the curve \((\text{AUC})\) over 210 min for plasma concentrations of GLP1 and PYY and VAS ratings of hunger and satiety in obese adolescents and adults administered with a 10 kcal/kg meal at different rate \((5 \text{ min for fast feeding and 30 min for slow feeding})\). Values are expressed as mean \(\pm \text{S.E.M.} *P < 0.05\).

![Figure 3](https://www.eje-online.org)

**Figure 3** Changes of VAS ratings of hunger and satiety after a 10 kcal/kg meal eaten in 5 min \((\text{fast feeding})\) or 30 min \((\text{slow feeding})\) by obese adolescents or adults. Both meals were administered at 0 min. Values are expressed as mean \(\pm \text{S.E.M.} *P < 0.05 \text{ vs the corresponding time point of fast feeding (satiety) or slow feeding (hunger)}; \times P < 0.05 \text{ vs the corresponding baseline value (T0)}\).
adolescents, whereas obese adults showed only negligible differences. In particular, while fast feeding did not stimulate GLP1 release in either group, only in obese adolescents was slow feeding capable of increasing circulating levels of GLP1. Furthermore, compared with the baseline condition, plasma PYY concentrations were increased by food intake irrespective of the eating pace. Finally, slow feeding was more effective in stimulating PYY release in obese adolescents than in obese adults.

Obesity has been associated with attenuation of the postprandial responses of GLP1 and PYY (22, 25). In addition, a negative correlation has been reported between circulating levels of these anorexigenic peptides and adiposity, evaluated as BMI and FM (26, 27). In this study, despite a significant difference in BMI between the two groups, there was no correlation between postprandial responses in GLP1 and PYY and BMI or FM. Furthermore, a stepwise multiple regression showed no influence of BMI on postprandial changes in circulating PYY and GLP1 after slow and fast feeding. Although the small sample size may have impeded to unveil any statistically significant difference, these findings would imply a causative role to the long-lasting obesity state in adults (about 15–20 years), disrupting the physiological control of GLP1 and PYY postprandial elevation which, on the contrary, would be maintained in obese adolescents as in lean adults (7). Analogously, changes in the concentrations of the adipokines, e.g. leptin and adiponectin, occurred with worsening and persistence of obesity over time (13). Further studies are needed to demonstrate the involvement of these adipokines (or other factors?) in the loss of increased anorexigenic peptide production after a slow meal in obese adults.

In this study, slow feeding evoked a higher satiety in obese adolescents but not in obese adults. In addition, in obese adolescents, there was a significant decrease in hunger after slow feeding (at 210 min). These findings are fully congruent with the potent anorexigenic responses of GLP1 and PYY to slow feeding in obese adolescents.

In our opinion, though VAS is the best tool to evaluate proneness to feeding or satiety, it remains a subjective expression of a person’s perceptions. Therefore, VAS may not have been sensitive enough to discern subtle differences in obese adults administered with food at different paces (28). Nonetheless, irrespective of the eating pace, food intake markedly reduced or increased hunger and satiety respectively, also in obese adults, when the two parameters were compared with the baseline conditions. Thus, the unchanged hunger and satiety in this group after fast and slow feeding could be interpreted as the inability of slow feeding to enhance plasma rise of GLP1 and PYY and to stimulate a valid anorexigenic response at central level. Noteworthy, despite significant differences in postprandial GLP1 and PYY levels or satiety and hunger ratings between obese adolescents and adults, there was no significant difference in PYY response or hunger and satiety perception when assessed by AUC. This might be due to the long-lasting evaluation of the postprandial responses (210 min), which was entirely used for the calculation of AUC values, and to the intersubject variability, which impeded to obtain a statistically significant difference.

Given that PYY is released within 15 min of food intake, this must occur before the ingested nutrients reach the distal small intestine and colon (where the greatest concentrations of PYY are present). Therefore, initial postprandial release of PYY is likely to be under neural control. Further release of PYY is observed when the nutrients arrive to the distal gut and the release is particularly stimulated by a high-fat diet (21, 22). Also GLP1 shows a biphasic response after a meal, the first peak occurring before nutrients enter the distal gut and is augmented by a high-carbohydrate meal (29).

In this study, the enhancement of GLP1 and PYY responses after slow feeding in obese adolescents appeared at 90 min. This finding seems to detract the contribution of neural control to the anorexigenic effects of slow feeding. The hypothesis that fast feeding provides brief periods of sensory exposure and insufficient cues for satiation has to be reevaluated taking into account the findings of this study (5, 6).

Multiple studies have shown the association of pediatric and adolescent obesity with obesity in adults.
Overweight children/adolescents are more prone to becoming overweight adults, especially at higher BMIs (30). Almost half of overweight adults have been overweight adolescents (31). Obesity during adolescence is associated with many adverse health consequences (32), and dietary habits, physical inactivity, and rates and degree of obesity become worse with the transition from adolescence into adulthood (33). A rise in insulin resistance has also been reported with the persistence of the obese state, as well as with the worsening of various components of the metabolic syndrome (34, 35). These considerations are supported by the metabolic findings of our study, and particularly the postprandial insulin and triglycerides responses were higher in obese adults than in obese adolescents. As time of insulin resistance is not equal to time of obesity exposure, further studies are necessary to demonstrate the involvement of age-related worsening of insulin resistance in the ineffectiveness of slow feeding to increase the postprandial anorexigenic response in obese adults. Anyway, it is noteworthy recalling that, in this study, baseline serum levels of glucose and insulin, as well as HOMA-IR, were negatively correlated with postprandial rises of GLP1 and PYY to slow feeding, but not to fast feeding. Furthermore, a bidirectional interaction has been reported to exist between insulin and the anorexigenic gut peptides such as GLP1 and PYY (36, 37).

Some limitations of this study have to be mentioned. First, there was no control group formed by lean adolescents and adults, who would have allowed a better dissection of the effects of body weight and age on the studied parameters. So, the slight difference in BMI between both groups might have been a potential confounder in the interpretation of the results of this study. Secondly, though there were no statistically significant differences in all investigated parameters between females and males in each group, there was a female preponderance in the recruited population. Therefore, the results of this study should be confirmed in a larger number of sex-matched subjects. Thirdly, the composition of the test meal did not correspond to the standard recommended diet; thus, a different test meal might have provided different results. Nonetheless, we chose ice cream because it is a qualitatively homogenous and highly palatable meal that may be easily administered in an experimental context and quickly consumed (only 5 min).

In conclusion, this study demonstrates that eating the same meal over 30 min instead of 5 min leads to higher concentrations of anorexigenic gut peptides and favors satiety in obese adolescents, whereas this physiological control of food intake is disrupted in obese adults. Therefore, slow feeding might become a dietary behavior to be inserted in any educational program for pediatric obesity (38). Before extending the results to clinical practice, because of the inevitability of the artificial environment of any clinical study, the qualitative composition of the administered meal, and the female predominance in the study population, long-term clinical studies in obese children/adolescents (possibly in a home or school setting) are mandatory to demonstrate the efficacy of this (absolutely inexpensive and safe) preventive intervention.

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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**Figure 5** Values of AUC over 210 min for serum levels of glucose, insulin, and triglycerides in obese adolescents and adults administered with a 10 kcal/kg meal at different rate (5 min for fast feeding or 30 min for slow feeding). Values are expressed as mean ± S.E.M. *P < 0.05.
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Author contribution statement
A E Rigamonti designed the study, A Sartorio, E Compri, and N Marazzi enrolled the subjects and performed the experiments. F Agosti elaborated the database and, together with M Guanta, performed the analysis of all the parameters. A E Rigamonti analyzed the data and, together with A Sartorio, wrote the manuscript. E E Müller contributed to data interpretation and discussion writing. All authors contributed to the manuscript revision.

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