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Klinik der Universität München

Direktor: Prof. Dr. Dr. Christoph Klein

Chrono-Nutrition - The impact of regularity, time-of-day and frequency of energy and energy from macronutrient consumption on body mass index in children. Results of the Childhood Obesity Project Study

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Vanessa Jäger

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Berichterstatter:	Prof. Dr. med. Dr. hc. Berthold Koletzko
Mitberichterstatter:	Prof. Dr. Olaf Adam
	PD. Dr. Julia Seiderer-Nack
Mitbetreuung durch den promovierten Mitarbeiter:	Dr. med. Veit Grote, M.Sc.
Dekan:	Prof. Dr. med. Thomas Gudermann
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List of Abbreviations

BMI	Body Mass Index
CHOP	Childhood Obesity Project
CoDA	Compositional data analysis
EF	Eating frequency
EO	Eating occasion
SCN	Suprachiasmatic nuclei

1. Introduction

1.1 Background

Overweight and obesity prevention in childhood is of high public health relevance as it is associated with many adverse short- and long-term health consequences [1]. The prevalence of children with overweight and obesity has been levelled off at a high rate in Germany in recent years [2]. Worldwide, the childhood obesity prevalence has been stagnated or even increased [3]. The reasons for the obesity development are likely multifactorial, but diet is one important and modifiable factor [4].

It has been suggested that dietary patterns of our modern society have been changing due to a higher exposure to artificial light and hence, food intake and bed times have been delayed to later time points [5]. Furthermore, regularity and time of food intake may be less consistent, varying from day to day [6] such as breakfast skipping, eating out or night eating. These characteristics of a modern diet may contribute to the high overweight and obesity prevalence. Those thoughts along with the observation that timing of food intake resulted in greater weight loss in overweight adults [7] have led to the definition of a new and increasing research area in nutritional sciences called Chrono-Nutrition. Chrono-Nutrition does not only take into account “what” and “how much” is eaten, but “when” food is eaten and it is the intersection between two domains, namely chronobiology and nutritional sciences [8,9]. Not all dietary aspects of a modern lifestyle are similarly attributable in children compared to adults. However, the updated position paper of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition [4] includes, apart from dietary factors, also food habits and lifestyle factors for preventing childhood obesity. This emphasizes the possible role of dietary habits and lifestyle factors such as breakfast skipping or a regular overall eating pattern with meals and snacks in preventing childhood obesity.

Chrono-Nutrition is based on chronobiologic findings that circadian rhythms are responsible for our daily biological rhythms, which synchronize physiological and behavioural aspects to our 24-hour day. External cues such as the light-dark cycle provide signals to synchronize the circadian clocks (biological timing system) [8]. The main pacemaker (central clock) is located in the suprachiasmatic nuclei (SCN) in the hypothalamus and it is stimulated by light received through our eyes. In addition, there are further clocks in peripheral tissues and in the brain, which are linked to the central clock and hence, to the light-dark cycle. This system of central and peripheral clocks regulate rhythmic control of almost the entire body such as behavioural (e.g. sleeping/wakefulness, feeding/fasting) or physiological factors (e.g. hormone release). However, peripheral clocks can be additionally stimulated by factors independent from the SCN to adapt to our surrounding such as feeding time [6,8,10]. It has been suggested that not synchronized peripheral and central clocks, behaviour and SCN or behaviour and peripheral clocks may increase the risk of overweight/obesity and other health related outcomes [5,9]. Thus, the time of energy and energy from macronutrient intakes aligned to the circadian rhythms of metabolic processes may be beneficial for body weight control and general metabolic health [9]. For instance, it has been shown that insulin and glucose exhibit a circadian rhythm with a reduced insulin sensitivity and glucose tolerance throughout the day and a nadir in the evening [11,12]. According to this theory, higher food intakes in the morning might be beneficial for weight control and general health, whereas higher food intakes in the evening might be less beneficial as indicated by epidemiological studies in adults [13,14]. Besides that, irregular eating pattern or eating at different temporal times may negatively affect body weight control. Circadian rhythms have been evolved to facilitate adaption of all living organisms to the daily change from day to night. Circadian rhythms are predictable as they are repeated daily and provide regularity to all organisms [8,15]. Irregular or inconsistent eating pattern varying in time or frequency, which are not aligned with metabolic processes, may contradict these assumptions. Thus, Chrono-Nutrition generally comprises three dimensions: regularity, time-of-day and frequency of eating [6,15,16]. Some authors additionally suggest duration of the eating period and duration of the fasting period, respectively, as the fourth dimension of Chrono-Nutrition [8]. However, we did not include the aspect of “duration” as it was not applicable in children, especially in young children where night feeding is common.

Regularity of eating as the first dimension of Chrono-Nutrition is usually examined in epidemiological studies as irregular consumption of meals and snacks on health outcomes (meals and snacks are summarized to eating occasions (EO) in the following). Particular in children, breakfast skipping has been examined in previous studies with conflicting results. Some studies found a beneficial effect of regular breakfast consumption on body mass index (BMI) and body composition [17], but others did not [18]. A systematic review by Monzani et al. [19] concluded that further studies are needed to examine the association between breakfast skipping and weight due to heterogeneous results of existing studies using standardized methods. However, the regularity of other EOs than breakfast have rarely been investigated in children.

The second dimension of Chrono-Nutrition, the time-of-day of eating on overweight development in children has been investigated in only a small number of studies. The available studies reported varying results. In particular, higher food intakes at supper [20] or evening/night snack [21,22] have been associated with higher weight in children; higher food intake at breakfast [23] has been associated with neutral effects, whereas higher food intake at lunch was associated with lower overweight risk [24]. Time of macronutrient intake has been rarely examined. One study found a higher fat intake at lunch to be detrimental for weight status [21], but another study found that lower fat and carbohydrate intake in the morning to be associated with a higher body fat mass index [25]. These contradicting results indicate the need for further studies, particularly of studies with longitudinal design to decrease the probability of chance findings.

Eating frequency (EF) as the third dimension of Chrono-Nutrition has been described in relation to weight development in children in a relatively large number of studies. A meta-analysis examined the association between EF and overweight in children and adolescents and found a higher EF (highest vs. lowest EF category) to be beneficial on body weight, which was significant only in boys [26]. The studies included in the meta-analysis are all cross-sectional and either use 24h-recalls or questionnaires, mainly filled out by parents. EF is a behaviour, which is likely to vary from day to day and hence, several days of dietary reporting are needed to receive a glimpse of a regular eating habit.

In summary, the studies reported above indicate clearly that for each of the three dimensions of Chrono-Nutrition more high-quality evidence is needed. As clinical trials are often difficult to conduct in nutritional epidemiology, particularly for eating behaviour (e.g. difficulties in blinding) [27,28], longitudinal studies often represent the highest evidence. As the regularity, time of eating and frequency are all aspects related to eating behaviour, data collected over several days are needed to mimic and visualize regular eating patterns. Furthermore, intake from one EO is likely to influence intake of subsequent or prior EOs. For instance, if a snack between meals is eaten, the following meal ought to be smaller than without that snack. Hence, it is important to examine the dietary intake over a complete 24 hour day.

As described in the beginning, the “when” of food intake is similarly important in Chrono-Nutrition compared to the “what” and “how much” was eaten as the “what” and “how much” represent the quality and quantity of foods consumed. We examined the “what” was eaten by looking at the macronutrient composition, namely the total protein, total fat and total carbohydrate intake. As indicated above, only very few studies have examined the timing of macronutrient intake on overweight development in children [21,25] and most of the studies focused on a particular EO such as regularity of breakfast consumption and nutrient intake [29]. The “how much” was eaten has been examined by looking at the energy intake consumed at each EO. The “what” and “how much” of food intake are inherently linked to “when” was eaten and hence, are part of the Chrono-Nutrition definition [6].

We have limited our analyses to infants and children as this is a sensitive time period, when dietary habits [30], but also overweight and obesity start to develop [2], which may provide potential for prevention and public health interventions. Most of the research on Chrono-Nutrition has been reported in adults, but it is not yet known, if results obtained from adults apply similarly to children. Furthermore, we have limited our analyses to examine overweight and obesity by BMI in children, as BMI is a good indicator for overweight development. However, BMI is not a diagnostic tool and does not discriminate between body compositions with higher or lower body fat contents [31]. As Chrono-Nutrition and particularly, the analysis of Chrono-Nutrition in children is in the beginning, we believe that the BMI is a good first indicator to examine, if a potential association exist.

There are potential factors, which might bias or modify the association between Chrono-Nutrition and BMI in children, besides quality and quantity of food consumed. Possible factors were identified by literature research and expert opinions and include the age of children, country of residence, sex and feeding mode. Firstly, the age of investigated children is an important factor as younger compared to older children have different dietary and developmental needs, which probably influence the regularity, frequency and distribution of EOs throughout the day. For instance, infants receive breastmilk or formula as sole food during the first 4- to 6-months of life. After that, complementary foods are added as an addition. Twelve-months-olds are able to eat similar foods as the other family members and with increasing age, children acquire further motor, cognitive, social and language skills, which may influence their eating behaviour [32]. In older children, the influence of peers might influence the eating behaviour as children want to be similar to their peer group [33]. Secondly, the country of residence is a further probably important factor as it is known that time of eating, kind of foods consumed and the importance of specific EOs are different in different European countries [34]. Thirdly, sex of the children might be a modifying factor as previous studies have been reported that for instance, girls tend to skip breakfast more often than boys [19]. Fourthly, the choice of feeding mode (breastfeeding vs. formula feeding) might be an influential factor as for instance gastric emptying is faster in breastfed compared to formula-fed infants, probably due to a different nutrient composition [35]. This might have effects on the EF and the distribution of EOs throughout the day.

The available evidence outlined, indicate the need for examining the potential association between Chrono-Nutrition in children and BMI development. In particular, longitudinal studies, which mimic a regular eating pattern over a time period of several, complete 24h-days in children are needed. Furthermore, it is important to take into account all aspects of Chrono-Nutrition ("when", "what" and "how much" was eaten) and to consider potential influential factors to receive coherent results.

1.2 Outline

The overarching aim of this cumulative thesis is to examine the effect of Chrono-Nutrition with its dimensions regularity, time-of-day and frequency of energy and macronutrient consumption on BMI in children. Secondary aims of this thesis are to examine the modifying factors country of residence, developmental age, sex and feeding mode in relation to Chrono-Nutrition and BMI in children. For all analyses, data from the European Childhood Obesity Project (CHOP) [36] are used. This cumulative thesis is based on three publications listed as follows:

(I) **Jaeger, V.**, Koletzko, B., Luque, V., Ferré, N., Gruszfeld, D., Gradowska, K., Verduci, E.,Zuccotti, G.V., Xhonneux, A., Poncelet, P., Grote, V. Distribution of energy and macronutrient intakes across eating occasions in European children from 3 to 8 years of age: The EU Childhood Obesity Project Study. Eur J Nutr 62, 165–174 (2023). https://doi.org/10.1007/s00394-022-02944-6

(II) **Jaeger, V.**, Koletzko, B., Luque, V., Gispert-Llauradó, M., Gruszfeld, D., Socha, P., Verduci, E., Zuccotti, G.V., Etienne, L., Grote, V. Time of Dietary Energy and Nutrient Intake and Body Mass Index in Children: Compositional Data Analysis from the Childhood Obesity Project (CHOP) Trial. Nutrients, 14, 4356 (2022). https://doi.org/10.3390/nu14204356

(III) **Jaeger, V.**, Koletzko, B., Luque, V., Gruszfeld, D., Verduci, E., Xhonneux, A., Grote, V. Eating Frequency in European Children from 1 to 96 Months of Age: Results of the Childhood Obesity Project Study. Nutrients, 15, 984 (2023). https://doi.org/10.3390/nu15040984

The overall and secondary aims are discussed in the three listed publications, whereby in each publication a different aspect of Chrono-Nutrition is covered. In the following, the specific aims of the publications are detailed. A graphical summary with all aspects of Chrono-Nutrition and the contribution of each publication to the overall aim is presented in Figure 1. The full publications and the contribution of the applicant to the publications are provided in chapters 4 to 6.

(I) The aims of publication **I** are to examine the total energy intake and energy intake from macronutrient (total fat, carbohydrate and protein) at different EOs over the day in children aged 3 to 8 years in five European countries. Furthermore, differences between country of residence, sex and age groups as well as skipping of EOs are examined.

(II) The aims of publication **II** are to examine the time-of-day energy and energy from macronutrient (total carbohydrate, protein, and fat) intakes in relation to the overweight status of European children aged 3 to 8 years. In addition, the effect of meal timing by analysing the (re-) distribution of energy and energy from macronutrients throughout the day (breakfast, lunch, supper, and snacks) on BMI z-score were examined in a longitudinal study.

(III) The aims of publication **III** are to study the EF of European children during the age from 1 to 96 months of age. Moreover, the EF trajectory evolution with age, during the time-of-day, and potential associations with infant feeding mode (breastfeeding vs. non-breastfeeding), sex, country of residence and weight status were examined.

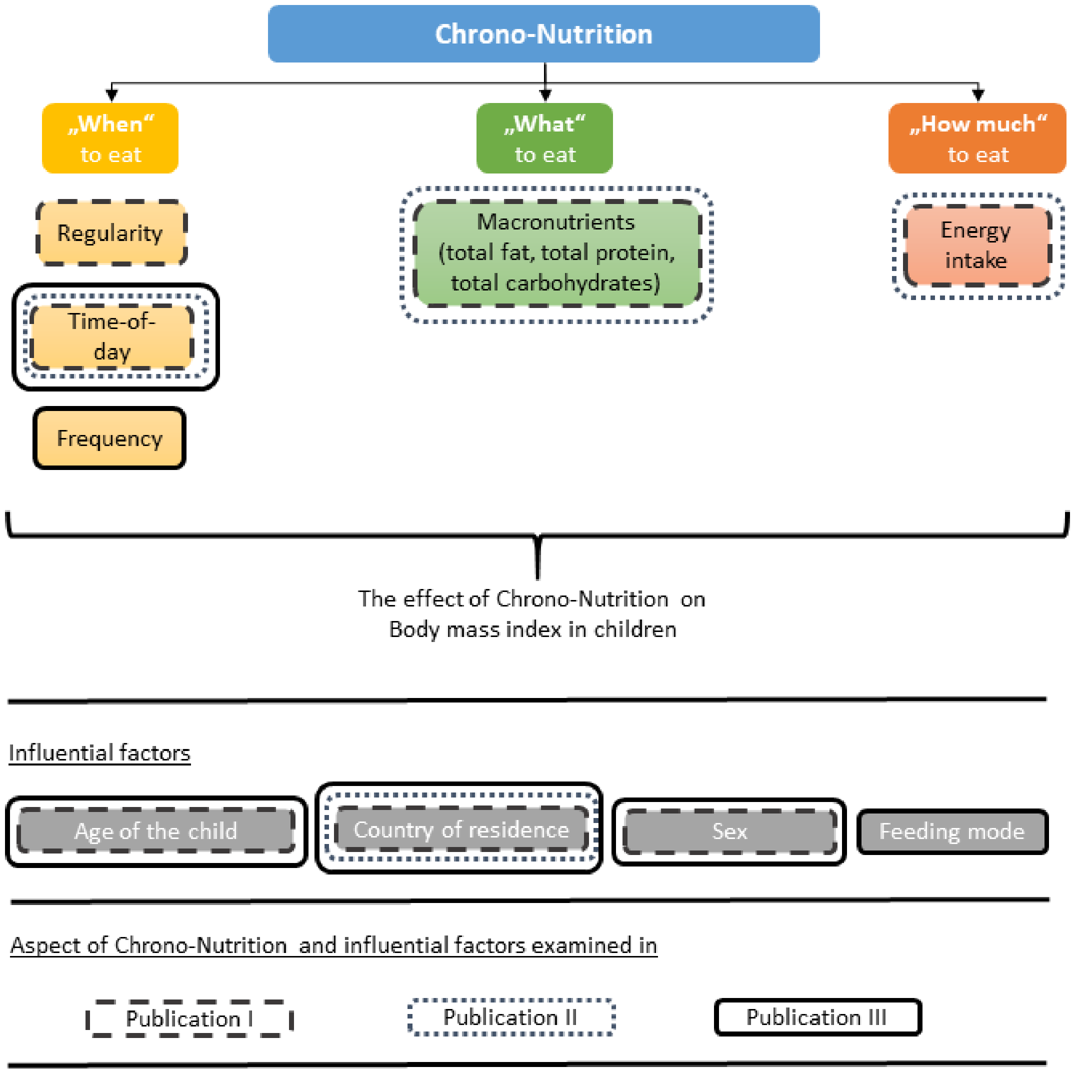

Figure 1: Overview of factors examined within this cumulative thesis and overview of how these factors were assigned to each of the three publications. First level: columns of Chrono-Nutrition ("when", "what" and "how much" was eaten) with the dimensions regularity, time-of-day and frequency of eating; second level: identified and examined influential factors; third level: publications.

2. Summary

Epidemiological studies have increasingly shown that the time of food intake might be similarly important for the development of overweight than the amount and composition of foods consumed. Within this thesis, I have aimed to examine the effect of Chrono-Nutrition with its dimensions regularity, time-of-day and frequency of eating on the risk of developing overweight in European children. In addition, I have examined factors, which have the potential to influence the association between Chrono-Nutrition and BMI in children. These influential factors are the feeding mode during the first year of life, country of residence, age evolution and sex. This thesis is based on three publications, with each publication comprising one of the three dimensions of Chrono-Nutrition and several potentially influential factors. Publication **I** examined the regularity of eating and the distribution of energy and energy from macronutrients throughout the day; publication **II** investigated the daytime effect of energy intake and energy intake from macronutrients on BMI; publication **III** examined the frequency of eating, its distribution throughout the day and the possible association between frequency of eating and overweight development.

We used for all publications data of the CHOP study. The CHOP study was initiated as a randomized clinical trial including an additional observational study arm of breastfed children. The study comprised in total 1678 healthy children from Belgium, Germany, Italy, Poland and Spain recruited during the first two months of life. The infants were followed-up in regular time intervals until 11 years of age. In all publications we used valid nutritional data collected as three-day weighed and/or estimated dietary records. The dietary records were collected monthly during the first nine months of life and at 12, 18, 24, 36, 48, 60, 72 and 96 months of age. The time of eating was differently defined before study start in infants/toddlers and in children to collect eating patterns for both age ranges and to be able to compare eating patterns between countries. The time of eating was entered as exact time until 24 months of age and thereafter as pre-defined categories (breakfast, lunch, supper and snacks during morning, afternoon and evening). The daytime distribution of meals and snacks as well as the EF have been examined by adding all daily EOs averaged over three days. The regularity of food intake has been identified by the frequency of each EOs in the three-day dietary records. In addition to dietary records, we used all time points from anthropometric measurements (BMI and BMI cut-offs, respectively), which were available at 24, 36, 48, 60 and 72 months of age. Influential factors were collected by questionnaire. We used for all analyses mixed effects models, as mixed effects models are capable of handling dependencies, which arise by measuring the same subject repeatedly. The mixed effects models were adapted for each research question. In publication **I**, we used mixed effects beta regression as the outcome variable was present as continuous proportional data, while in publication **III**, we used generalized additive mixed effects models to estimate non-linear models. In publication **II**, we combined mixed effects models with compositional data analysis (CoDA) to investigate firstly, the effect of time-of-day of eating separately from the energy intake and secondly, to investigate all EOs simultaneously. CoDA is a method, which haven´t been used in nutritional epidemiology previously. All models were assessed with respect to BMI development in children, if statistically plausible. We performed several sensitivity analyses for each model to verify the robustness of the results. Statistical model assumptions have been checked by inspection of residuals. All analyses were performed in R Studio.

Depending on the research question, data have been comprised from 740 (publication **I**), 729 (publication **II**) or 1244 (publication **III**) subjects aged between 36 and 96 months of age (publications **I** and **II**) and 1 and 96 months (publication **III**), respectively. The composition of the study population in all publications is similar: children originate to 25% each from Spain and Italy, followed by 20% from Poland as well as 15% each from Germany and Belgium. European children showed a regular eating pattern of meals with the highest daily intake at lunch, followed by supper, breakfast and snacks. Lunch and supper were mainly composed of protein and fat intakes, whereas a high portion of carbohydrates characterized breakfast and snacks. Children being overweight or normal-weight showed different proportional distributions of energy and energy from macronutrients at meals and snacks. Children being overweight consumed higher proportional intakes of energy and energy from macronutrients at lunch and less at snacks. However, the differences related to the overweight development in children were no longer significant, when the effect of time-of-day was disentangled from the energy intake. Furthermore, no statistical significant differences in EF was observed between children being overweight and normal-weight. From the potential influential factors examined, country of residence was the most often observed influential factor, as the proportional distribution of each EO, EF and the daytime of eating was different in the countries examined. The child development influenced Chrono-Nutrition, where children at older ages proportionally ate on average more at lunch and supper, and less during snack times compared to younger age children. Similarly, the EF decreased significantly from 1 to 96 months of age. The EF of breastfed compared to non-breastfed infants was higher in the first year of life. No differences between boys and girls were observed in any of the three dimensions of Chrono-Nutrition.

The results of the here presented thesis suggest that the effect of Chrono-Nutrition on overweight development in children is of minor relevance. In particular, EF contributes to the total daily energy intake and is a notable dietary factor relevant for the overweight development. However, our results suggests that this seems not to be true in children. It might be related due to child growth and development. Children have higher nutritional requirements and relatively higher energy needs than adults, while gastric capacity is restricted at the same time, which demand children to eat more frequently. Overall, by taking into account, influential factors, particularly country of residence, it seems that cultural factors shape the time and frequency of EOs as well as the daytime distribution of energy intake and energy obtained from macronutrients. Cultural factors seem to be more important than purely physiological factors to determine the (temporal) eating pattern in children.

3. Zusammenfassung

Epidemiologische studien haben vermehrt gezeigt, dass der Zeitpunkt des Essens ebenso einen Einfluss auf die Übergewichtsentwicklung haben könnte, wie die Menge und die Zusammensetzung der Nahrung. In dieser Arbeit wurden der Effekt der Chrono-Ernährung mit den Dimensionen Regularität, Zeitpunkt und Häufigkeit des Essens auf das Risiko der Übergewichtsentwicklung bei europäischen Kindern untersucht. Zudem wurden Faktoren, die die mögliche Assoziation zwischen Chrono-Ernährung und BMI bei Kindern beeinflussen könnten, überprüft. Die untersuchten Einflussfaktoren sind die Ernährungsmethode im ersten Lebensjahr, der Wohnsitz, die Altersentwicklung und das Geschlecht. Diese Arbeit umfasst drei Publikationen, die jeweils einen Aspekt der Chrono-Ernährung sowie mehrere Einflussfaktoren beinhalten. Publikation **I** untersucht die Regelmäßigkeit des Essens sowie die tageszeitliche Verteilung der Nahrungsaufnahme; Publikation **II** untersucht die Tageszeit der Nahrungsaufnahme auf den BMI; Publikation **III** untersuchte die Häufigkeit des Essens, deren Verteilung über den Tag und die mögliche Assoziation zwischen der Häufigkeit des Essens und der Übergewichtsentwicklung.

Für alle Publikationen wurden Daten des Childhood Obesity Projects (CHOP) verwendet. Die CHOP-Studie wurde 2002 als eine klinisch randomisierte Studie initiiert und enthielt einen zusätzlichen Studienarm aus beobachteten gestillten Kindern. Insgesamt wurden 1.678 gesunde Säuglinge aus Belgien, Deutschland, Italien, Polen und Spanien in den ersten beiden Lebensmonaten rekrutiert. Die Säuglinge wurden in regelmäßigen Abständen zu Studienbesuchen bis elf Jahren eingeladen. Für alle Publikationen wurden Daten von Probanden mit validen Ernährungsdaten verwendet, die anhand von gewogenen und/oder geschätzten Tagesprotokollen an drei Tagen erhoben wurden. Die Ernährungsprotokolle wurden in den ersten neun Monaten monatlich, sowie im Alter von 12, 18, 24, 36, 48, 60, 72 und 96 Monaten erhoben. Der Zeitpunkt der Essensaufnahme wurde zu Studienbeginn unterschiedlich für Säuglinge/Kleinkinder und Kinder definiert um das Essverhalten in beiden Altersbereichen und zwischen den Ländern vergleichbar zu machen. Bis 24 Monate wurden exakte Uhrzeiten angegeben und ab 36 Monate wurden vordefinierte Kategorien verwendet (Frühstück, Mittagessen, Abendessen und Zwischenmahlzeiten während des Vormittags, des Nachmittags und des Abends). Um die tageszeitliche Verteilung der Haupt- und Zwischenmahlzeiten (nachfolgend: Essgelegenheiten) sowie die Esshäufigkeit zu untersuchen, wurden alle Essgelegenheiten eines jeden Tages aufsummiert und der Durchschnitt über die drei Tage gebildet. Die Regelmäßigkeit des Essens wurde anhand der Häufigkeit der jeweiligen Essgelegenheiten in den einzelnen Protokollen ermittelt. Neben Zeitpunkten mit Ernährungsprotokollen wurden Zeitpunkte mit anthropometrischen Messungen (BMI bzw. BMI-Cut-offs) berücksichtigt, die mit 24, 36, 48, 60, 72 und 96 Monaten erhoben wurden. Die Einflussfaktoren wurden via Fragebogen erhoben. Für alle Publikationen wurden gemischte Modelle („Mixed effects models“) verwendet, da gemischte Modelle Abhängigkeiten berücksichtigen können, die bei wiederholten Messungen der gleichen Probanden auftreten. Die gemischten Modelle wurden an die jeweiligen Fragestellungen angepasst. In Publikation **I** wurden gemischte Beta-Regressionsmodelle verwendet, da die Ergebnisvariable in stetigen prozentualen Werten vorlag, während in

Publikation **III** generalisierte additive gemischte Modelle verwendet wurden, um nichtlineare Modelle untersuchen zu können. In Publikation **II** wurden gemischte Modelle mit der zusammengesetzten Datenanalyse kombiniert, um einerseits den Effekt der Tageszeit getrennt vom Effekt der Energiezufuhr und andererseits alle Essgelegenheiten simultan untersuchen zu können. Die zusammengesetzte Datenanalyse ist eine Methode, die in der Ernährungsepidemiologie zuvor noch nicht verwendet wurde. Alle Modelle wurden, wenn statistisch plausibel, hinsichtlich BMI Entwicklung bei Kindern analysiert. Für alle Modelle wurden verschiedene Sensitivitätsanalysen durchgeführt, um die Robustheit der Ergebnisse zu prüfen. Die statistischen Modellannahmen wurden durch die grafische Inspektion der Residuen überprüft. Alle Analysen wurden in R Studio durchgeführt.

Je nach Fragestellung wurden Daten von 740 (Publikation **I**), 729 (Publikation **II**) oder 1,244 (Publikation **III**) Probanden im Alter zwischen 36 und 96 Monaten (Publikationen **I** und **II**) bzw. 1 und 96 Monaten (Publikation **III**) untersucht. Die Zusammensetzung der Studienpopulationen in allen Publikationen ist ähnlich: Die Kinder stammten zu jeweils 25% aus Spanien und Italien, gefolgt von Polen (20%), Deutschland und Belgien (jeweils 15%). Europäische Kinder zeigten ein regelmäßiges Essverhalten bei den Hauptmahlzeiten auf mit der höchsten täglichen Zufuhr zum Mittagessen, gefolgt vom Abendessen, Frühstück und den Zwischenmahlzeiten. Mittag- und Abendessen setzten sich überwiegend aus Fetten und Proteinen zusammen, während das Frühstück und die Zwischenmahlzeiten durch einen hohen Anteil an Kohlenhydraten geprägt waren. Kinder mit Übergewicht und Normalgewicht zeigten unterschiedliche proportionale Verteilungen an Energie und Energie von Makronährstoffen zu den Essgelegenheiten. Kinder mit Übergewicht verzehrten proportional mehr Energie und Energie von Makronährstoffen zum Mittagessen und weniger zu den Zwischenmahlzeiten. Jedoch waren die Unterschiede auf die Übergewichtsentwicklung bei Kindern nicht mehr statistisch signifikant, wenn der Effekt der Tageszeit von der Energiezufuhr getrennt betrachtet wurde. Des Weiteren konnten keine statistisch signifikanten Unterschiede in der Esshäufigkeit zwischen Kindern mit Übergewicht und Normalgewicht beobachtet werden. Von den untersuchten Einflussfaktoren war der Wohnsitz der Kinder der am häufigste beobachtete Einflussfaktor. Länderunterschiede wurden beobachtet bei der proportionalen Verteilung der jeweiligen Essgelegenheiten, der Esshäufigkeit und der Tageszeit zu denen die Essgelegenheiten verzehrt wurden. Die Entwicklung der Kinder beeinflusst die Chrono-Ernährung, wobei ältere Kinder im Schnitt proportional mehr zu Mittag- und Abendessen und weniger zu den Zwischenmahlzeiten verzehrten als im jüngeren Alter. Zudem verringerte sich die Esshäufigkeit von 1 zu 96 Monaten signifikant. Die Esshäufigkeit von gestillten im Vergleich zu nicht gestillten Säuglingen war im ersten Lebensjahr höher. Unterschiede zwischen Jungen und Mädchen konnten in keiner der drei Dimensionen von Chrono-Ernährung beobachtet werden.

Die Ergebnisse der vorliegenden Arbeit implizieren, dass der Effekt der Chrono-Ernährung auf die Übergewichtsentwicklung bei Kindern eine untergeordnete Rolle spielt. Insbesondere die Esshäufigkeit ist durch den Beitrag zur Gesamtenergiezufuhr ein relevanter ernährungsbezogener Faktor für die Übergewichtsentwicklung. Unsere Ergebnisse deuten allerdings darauf hin, dass dies bei Kindern nicht der Fall zu sein scheint. Dies könnte auf das kindliche Wachstum und Entwicklung zurückzuführen sein. Kinder haben einen erhöhten Nährstoffbedarf und einen relativ höheren Energiebedarf bei gleichzeitig eingeschränkter gastrischer Kapazität als Erwachsene, weshalb Kinder häufiger essen müssen. Wenn Einflussfaktoren, insbesondere die Herkunft der Kinder, auf das zeitliche Essverhalten berücksichtigt werden, scheinen kulturelle Faktoren den Zeitpunkt und die Häufigkeit der Essgelegenheiten sowie die tageszeitliche Verteilung von Energie und Energie von Makronährstoffen zu formen. Kulturelle Faktoren scheinen somit wichtiger zu sein als rein physiologische Faktoren, um das (zeitliche) Essverhalten von Kindern zu bestimmen.

4. Publication I

Jaeger, V., Koletzko, B., Luque, V., Ferré, N., Gruszfeld, D., Gradowska, K., Verduci, E.,Zuccotti, G.V., Xhonneux, A., Poncelet, P., Grote, V. Distribution of energy and macronutrient intakes across eating occasions in European children from 3 to 8 years of age: The EU Childhood Obesity Project Study. Eur J Nutr 62, 165–174 (2023).

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I contributed to this publication by conceptualizing the research question, selecting the methodology, performing the formal statistical analysis and interpreting the results. Furthermore, I have prepared the original draft, created the figures and tables, incorporated feedback from my supervisors and co-authors and I was responsible for the submission to Eur J Nutr and the peer-review process.

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ORIGINAL CONTRIBUTION

Distribution of energy and macronutrient intakes across eating occasions in European children from 3 to 8 years of age: The EU Childhood Obesity Project Study

Vanessa Jaeger[1] · Berthold Koletzko[1] · Veronica Luque[2,3] · Natàlia Ferré[2] · Dariusz Gruszfeld[4] · Kinga Gradowska[4] · Elvira Verduci[5] · Gian Vincenzo Zuccotti[5] · Annick Xhonneux[6] · Pascale Poncelet[7] · Veit Grote[1]

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Abstract

Purpose We aimed to characterize the distribution of energy and macronutrient intakes across eating occasions (EO) in European children from preschool to school age.

Methods Data from 3-day weighed food records were collected from children at ages 3, 4, 5, 6 and 8 years from Belgium, Germany, Italy, Poland and Spain. Food intakes were assigned to EO based on country-specific daytimes for breakfast, lunch, supper and snacks (morning, afternoon). The average energy and nutrient intakes were expressed as percentage of total energy intake (%E). Nutrients were additionally expressed as percentage per EO (%E_{EO}). Foods were assigned to food groups; variation in intake was calculated via coefficient of variation (CV). We analyzed age trends in diurnal intake using mixed-effects beta regression.

Results The 740 healthy children included in the analysis consumed the largest proportion of daily energy at lunch (31%E ± 8, M ± SD) and supper (26%E ± 8), followed by breakfast (19%E ± 7) and snacks [afternoon (16%E ± 8); morning (8%E ± 7)], with the most variable intake at morning snack (CV = 0.9). The nutrient composition at lunch and supper was highest for fat (36 ± 9%E_{Lunch}; 39 ± 11%E_{Supper}) and protein (18 ± 5%E_{Lunch}; 18 ± 6%E_{Supper}) and at breakfast and snacks for carbohydrates (54 ± 12%$E_{Breakfast}$; 62 ± 12%E_{Snacks}). High-sugar content foods were consumed in relatively large proportions at breakfast and snacks. Food intakes varied significantly with age, with lower snack intakes at later ages ($p < 0.001$).

Conclusion Possibly unhealthy EOs with high-fat intakes and high-sugar-content foods were observed. Changes in nutrient composition of EOs may be beneficial for health.

Trial registry: ClinicalTrials.gov: NCT00338689; 19/June/2006.

Keywords Children · Chrono-nutrition · Time-varying · Energy · Macronutrients · Dietary pattern

Abbreviations

BMI Body Mass Index
CV Coefficient of variation
EO Eating occasion
TEI Total energy intake

✉ Berthold Koletzko
berthold.koletzko@med.uni-muenchen.de

1 Division of Metabolic and Nutritional Medicine, Department of Paediatrics, Dr. von Hauner Children's Hospital, LMU University Hospitals, LMU - Ludwig-Maximilians-Universität Munich, Lindwurmstr. 4, 80337 Munich, Germany

2 Paediatrics Research Unit, Universitat Rovira I Virgili, IISPV, Reus, Spain

3 Serra Hunter Fellow, Universitat Rovira I Virgili, IISPV, Reus, Spain

4 Neonatal Department and Neonatal Intensive Care Unit, Children's Memorial Health Institute, Warsaw, Poland

5 Department of Paediatrics, Vittore Buzzi Children's Hospital, University of Milan, Milan, Italy

6 Centre Hospitalier Chretien St. Vincent, Rocourt, Liège-Rocourt, Belgium

7 Department of Paediatrics, University Children's Hospital, Queen Fabiola, Université Libre de Bruxelles, Brussels, Belgium

Introduction

Chrono-nutrition is an emerging field in nutrition science which does not only study what is eaten but also takes the timing, regularity and frequency of eating into account [1]. Research in children and adolescents has focused on breakfast skipping [2, 3] or late night eating [4, 5] and its possible health effects.

The diet of young children is markedly influenced by the family's eating behaviour [6]. Eating behaviour develops already during the first and second year of life, particularly after introduction of complementary feeding, and is likely to track into mid-childhood and older ages [7]. Infants and young children typically eat more frequently than older children and adults [8]. When children get older, eating occasions (EO) in western countries are usually three main meals per day (breakfast, lunch and supper) and an additional one-to-three snacks between main meals. The quantity and composition of food as well as the time when food is consumed varies markedly across countries [9–11]. Furthermore, first results of studies examining time-varying food intake suggest that dietary intakes at different times of the day might have differential effects on weight gain [12, 13].

Studies investigating eating behaviour in children have focused mainly on one [3, 14] or few EOs [15]. In particular, breakfast consumption and its health consequences have been studied [16, 17]. Food and nutrient intake at one meal is affected by the meal and snacks eaten before. Also, how food intake is distributed within a day may vary with age. Better understanding of eating habits across the day might contribute to strategies for improving dietary habits and health in children. Therefore, we aimed to describe the energy and macronutrient (total fat, carbohydrate and protein) intake at different EOs over the day in children aged 3 to 8 years in five European countries. Differences between countries, sex and age groups and skipping of EOs are analyzed.

Materials and methods

Study design and population

This study used dietary data collected as part of the European Childhood Obesity Project (CHOP) randomized intervention trial. The intervention examined the effect of different protein content in infant formula on growth and later obesity risk [18, 19]; apart from the intervention groups, one-third of all included children were fully breastfed for at least 3 months of life. Between 2002 and 2004, 1678 infants in Belgium (Liège and Brussels), Germany (Munich and Nuremburg), Italy (Milano), Poland (Warsaw) and Spain (Tarragona and Reus) were enrolled during their first 8 weeks of life (median 2 weeks) and followed up until 11 years of age. Only healthy, full-term and singleton infants were included in the trial. The trial was conducted in accordance with the Declaration of Helsinki, approved by the ethical committee from each participating site, and registered (NCT00338689, clinicaltrials.gov). Parents provided written informed consent before enrolment. At the age of 8 years, children also provided assent to further follow-up.

Dietary assessment

Dietary data with predefined EOs were collected at the ages 3, 4, 5, 6 and 8 years using weighed and estimated dietary records for three consecutive days (2 weekdays and 1 weekend day) completed by parents or caretakers. Food and leftovers were weighed using food scales (Unica 66006; Soehnle, Murrhardt, Germany). In case no weighing was possible, an atlas of food pictures was used to support the estimation of portion sizes.

Dietary records were entered in a software specifically developed and designed for this study. Nutritional products and their content were primarily based on the German food composition database BLS 2.3 (Bundeslebensmittelschlüssel) and were enriched by information from product labels, manufacturers or national food databases of participating countries, if necessary. For analysis, nutritional values of products were updated to BLS 3.01. Quality checks of collected dietary records were executed by trained dieticians on several stages following standard operating procedures [20, 21]; all records were checked by a dietician together with the parents at each study visit. Foods and beverages reported by parents were assigned to food groups and summarized to main groups. A list with the types of foods in each food group are detailed in the Supplementary Table S1.

Misreporting of energy intake was considered based on the ratio of reported energy intake and estimated energy requirements as described in detail elsewhere [22]. Misreports were identified but not excluded as recommended [23].

Eating occasions (EO)

An EO is any event when foods or beverages are consumed. The term EO is a neutral definition to examine eating patterns including meals and snacks. In this analysis, EOs were predefined (before data collection) according to typical time slots and typical foods in each country in 3 meals (breakfast, lunch and supper) and 3 snacks (morning, afternoon and evening). All food intakes were then assigned to one of the six EOs above. Thus, also country-specific distinctions of times of EOs on weekends and weekdays were taken into account.

Skipping of EOs was defined as subjects who have not eaten an EO throughout all days of a food record. Intake from subjects skipping an EO was included in the analysis with zero calories.

Covariates

At each time point, weight and height were measured by trained study personal. Sex, smoking during pregnancy and parental education were collected by questionnaire at baseline. The latter was classified according to the International Standard Classification of Education [24] into low, medium and high level of education. Maternal body mass index [BMI; weight (kg)/height (m^2)] was calculated from self-reported pre-pregnancy weight and from height measured at baseline. BMI was classified in weight groups according to the World Health Organisation [25].

Statistical analysis

Subjects with dietary data on at least one out of five time points (3, 4, 5, 6 and 8 years) were included in the analysis as described previously [18]. The average intake (kcal/day) was calculated for energy and energy from macronutrients (carbohydrate, protein and fat) from the dietary records for each time point. Each nutrient was expressed either as percentage contribution towards total daily energy intake (TEI;%E) or as its percentage contribution towards total energy intake for each EO (%E_{EO}). The nutrient composition in%E_{EO} facilitated the assessment of nutrient composition at each EO independent of the amount of energy each EO is contributing towards TEI. The average intakes for energy and energy from nutrients were summarized by age and country unless otherwise indicated.

Data are presented as arithmetic mean (standard deviation) for continuous variables and as counts (%) for categorical variables. CV (standard deviation/mean) was used to assess variability independent of size of meal consumed.

Age effects for energy and nutrient intakes were estimated using mixed-effects beta regression which is suitable to model continuous proportional outcomes with repeated measurements. Intake from subjects skipping an EO was transformed according to $(Y\times(n\text{-}1)+0.5)/n$, where n is the sample size and Y energy intake, as recommended [26]. Age effects for afternoon and morning snacks were combined to "snacks" due to a higher number of subjects skipping an EO. Mixed-effects models with a logit link were used with child-specific random intercept and slope over age. Age was added as fixed effects either as continuous variable or in case of non-linearity as piecewise linear splines (energy and fat: splines for lunch with knots at 5 and 6 years; carbohydrate: splines for lunch with knot at 5 years; protein: splines for lunch with knot at 4 year and snacks with knot at 5 years). Energy and energy from nutrients for each EO (% and %E, respectively) were used as outcomes. The models were adjusted for misreporting, TEI, country and the interaction of TEI and country. The interaction was included as the increase in TEI over age differed by country. For macronutrients TEI was replaced by energy from total carbohydrate (kcal), total protein (kcal) and total fat (kcal), respectively. Model specifications were checked graphically by depiction of residuals. Sensitivity analyses were performed by exclusion of 2.5% of highest and lowest intake for each EO and energy or energy from nutrients. Regression results were plotted using predicted values. Sex differences in energy and nutrient intakes (in %E) at each EO were tested by t-test with food intakes averaged over age for each subject to avoid dependent observations. All statistical analysis were performed by R studio version 4.0.4 [27], and the package "glmmTMB", "DHARMa" and "ggeffects" were applied. Statistical testing was defined as significant for $p<0.05$ with Bonferroni adjustment.

Results

We included 740 healthy subjects (53% girls) with 2563 food records in this analysis. Subjects participated on average in 3.5 out of 5 time points, whereas 33% of subjects participated in all 5 time points. 97% of all food protocols were recorded for 3 days as intended. Most of the subjects were recruited in Spain (28%), followed by Italy (27%), Poland (17%), Germany (14%) and Belgium (13%). The average participation rate was highest in Italy (4 out of 5 visits) and lowest in Poland (2 out of 5 time points). A third of the children belonged to the initial observational breastfed group and 68% to the intervention group. Half of subject parents had a medium education level and participation rate at all 5 time points was higher if parents had a medium or high education level. Maternal pre-pregnancy BMI was classified as normal-weight in 63% of the mothers, as overweight in 20% and obese in 8% of all mothers. One-third of the mothers smoked during pregnancy. Twenty-four percent of the subjects were at risk of overweight or obesity with a BMI z-score above 1, with highest rate at 8 years of age (30% of subjects). Average EO frequency decreased from 3 to 8 years from 5.7 EOs to 5.1 EOs per day (excluding EOs consisting of water or unsweetened tea).

The mean dietary intake for the study population stratified by age group and the total population is described in Table 1. The average daily energy consumption increased from 1160 kcal at 3 years to 1547 kcal at 8 years, while the energy intake per kg body weight decreased considerably, but the contribution of macronutrients to energy were

Table 1 Mean energy and macronutrient intake of children aged 3 to 8 years

Age in years	3 ($n=570$)	4 ($n=533$)	5 ($n=489$)	6 ($n=525$)	8 ($n=446$)	Total ($N=740$)[a]
Energy						
Kcal/day	1160 ± 240	1268 ± 235	1343 ± 249	1432 ± 249	1547 ± 288	1340 ± 284
Kcal/kg[b]	80 ± 18	76 ± 16	71 ± 15	66 ± 14	56 ± 13	70 ± 17
Carbohydrates						
g/day	150 ± 46	160 ± 37	172 ± 49	183 ± 43	192 ± 49	170 ± 47
%E[c]	52 ± 16	51 ± 10	51 ± 13	51 ± 10	50 ± 11	51 ± 12
Protein						
g/day	45 ± 12	48 ± 12	50 ± 13	54 ± 13	59 ± 15	51 ± 14
%E[c]	16 ± 3	15 ± 3	15 ± 3	15 ± 3	15 ± 3	15 ± 3
Fat						
g/day	45 ± 12	50 ± 13	53 ± 13	56 ± 14	62 ± 17	53 ± 15
%E[c]	35 ± 6	35 ± 6	35 ± 6	35 ± 6	36 ± 6	35 ± 6

Values are presented as mean ± standard deviation.

[a]740 subjects with 2563 observations in total

[b]48 subjects had no weight measurements

[c]Percentage values are based on total energy intake per day

very similar for each age group. The highest average total energy was consumed in Poland (1449 ± 239 kcal) followed by Spain (1403 ± 293 kcal), Italy (1305 ± 273 kcal), Belgium (1253 ± 250 kcal) and Germany (1237 ± 297 kcal). The total amount of energy from carbohydrate was highest in Germany (756 ± 352 kcal; 61%E) and for protein (246 ± 59 kcal; 18%E) and fat (559 ± 149 kcal; 40%E) in Spain (Supplementary Table 2 and 3). Dietary data were identified as under-reported in 13% and as over-reported in 12% of all observations. The number of plausible reports was

Table 2 Energy and macronutrient intake by eating occasion in percentage of daily total energy intake and of total energy intake from macronutrient with coefficient of variation (CV) in 740 children with food protocols ($N=2563$) at 3, 4, 5, 6, and 8 years of age and from 5 countries (Belgium, Germany, Italy, Poland, and Spain)

	Energy		Carbohydrates		Protein		Fat	
	Mean ± SD (%)	CV	Mean ± SD (%E)	CV	Mean ± SD (%E)	CV	Mean ± SD (%E)	CV
Breakfast	18.9 ± 6.8	0.4	20.3 ± 8.0	0.4	17.7 ± 7.5	0.4	17.9 ± 8.7	0.5
Morning snack	7.9 ± 7.4	0.9	9.9 ± 8.8	0.9	5.3 ± 6.1	1.1	6.3 ± 7.6	1.2
Lunch	30.9 ± 8.4	0.3	28.0 ± 9.2	0.3	36.5 ± 10.3	0.3	32.4 ± 11.1	0.3
Afternoon snack	16.4 ± 7.6	0.5	19.9 ± 8.9	0.4	10.4 ± 6.4	0.6	14.3 ± 9.1	0.6
Supper	25.8 ± 8.1	0.3	22.0 ± 8.9	0.4	30.0 ± 10.5	0.4	29.0 ± 11.1	0.4

CV coefficient of variation, *SD* standard deviation

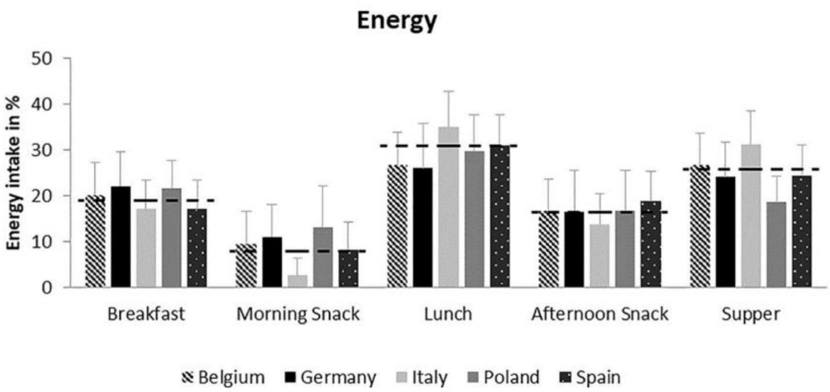

Fig. 1 Average energy intake in percentage of total intake per eating occasion in children followed at 3, 4, 5, 6, and 8 years of age in Belgium ($n=97$), Germany ($n=106$), Italy ($n=201$), Poland ($n=126$), and Spain ($n=210$). The dashed lines indicate the mean of each eating occasion across all countries ($N=740$)

highest in Germany with 81% of dietary records. Highest percentages of over-reporting and under-reporting were seen in Poland (16% and 11% of dietary records, respectively) and Spain (14% and 13% of dietary records, respectively).

The relative contribution of energy and energy from macronutrients at each eating occasion is summarized in Table 2. Most energy was consumed at lunch, followed by supper and breakfast. The snack during afternoon provided more energy than the morning snack. Snack intakes contributed 24% of TEI and, hence, a greater contribution than breakfast (19%). The highest variation in mean intake (CV) was seen at morning snacks, especially for protein and fat.

In Fig. 1 the average intake of energy is displayed for each country. Breakfast (22 ± 6%) was larger than supper (19 ± 6%) in Poland, whereas in Belgium, lunch and supper were consumed with similar size. Morning snack in Italy was very small (3 ± 4%), but lunch (35 ± 8%) and supper (26 ± 7%) were higher than in the other countries.

Overall and country-specific average nutrient distribution for each EO over age is depicted in Fig. 2. Lunch and supper were characterized by high intakes of fat (lunch: $36.3 \pm 9.1\%E_{Lunch}$; supper:$39.1 \pm 10.9\%E_{Supper}$) and protein (lunch: $18.1 \pm 5.2\%E_{Lunch}$; supper: $17.7 \pm 5.6\%E_{Supper}$), whereas breakfast and snacks were characterized by high proportions of carbohydrates (breakfast: $53.7 \pm 11.7\%E_{Breakfast}$; snacks: $61.9 \pm 12.2\%E_{Snacks}$). In Spain, carbohydrate intakes were less than in the other countries, especially at lunch ($39\%E_{Lunch}$), supper ($34\%E_{Supper}$) and in snacks ($55\%E_{Snacks}$). In contrast, in Spain, more protein-containing foods were consumed at lunch ($21\%E_{Lunch}$), supper ($21\%E_{Supper}$) and snacks ($12\%E_{Snacks}$) than in most other countries. Greater amounts of fat-containing foods were more often consumed in Spain at all EOs and in Poland at breakfast ($37\%E_{Breakfast}$) and lunch ($39\%E_{Lunch}$) than in the remaining countries.

The most frequently consumed food groups per EO are presented in Table 3. Breakfast was characterized by high-sugar-content products, milk products and cereals; snacks had similar characteristics, but fruits and beverages were often additionally consumed. Vegetables, cereals, fats and meat were typically eaten at lunch and supper.

In Table 4, the frequency of skipping an EO is presented. Morning snack showed the highest frequency of skipping. Around 24% of dietary records reported no morning snack. All other EOs showed only a small number of skipping.

Age trends are shown in Fig. 3. Predicted energy intake at snacks decreased significantly from 26 to 22% from 3 to 8 years ($p < 0.001$), along with a decreasing carbohydrate intake ($p < 0.001$). Predicted intake of energy from carbohydrate at lunch increased significantly over time from 24 to 28% ($p < 0.001$), whereas predicted intakes of energy from protein and fat at breakfast decreased from 19° to 17% and

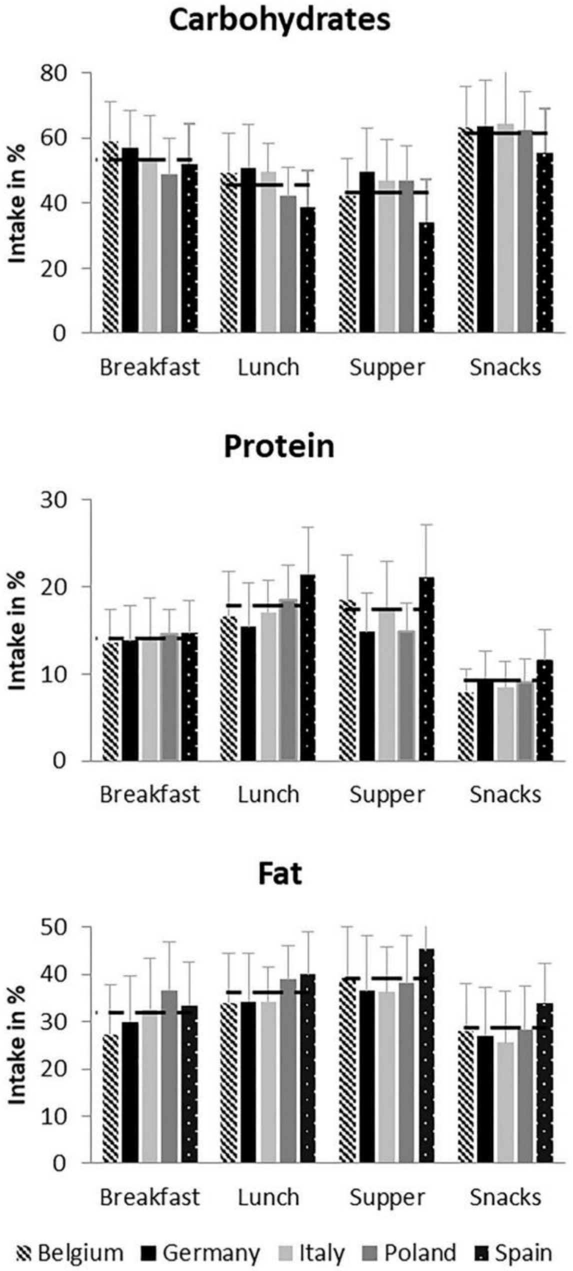

Fig. 2 Average macronutrient intake as percentage of total energy intake per eating occasion (%EO) of children followed at 3, 4, 5, 6, and 8 years of age in Belgium ($n = 97$), Germany ($n = 106$), Italy ($n = 201$), Poland ($n = 126$), and Spain ($n = 210$). The dashed lines indicate the mean of each eating occasion across all countries

from 19 to 16%, respectively ($p < 0.001$). Numerical data to all figures are presented in the Supplementary Tables 4, 5, 6 and 7. Sensitivity analyses yielded similar results, except for

Table 3 Frequency of the five most often consumed food groups as percentage of total food groups at each eating occasion

Breakfast		Morning snack		Lunch		Afternoon snack		Supper	
Food group	%	Food group	%	Food group	%	Food group	%	Food group	%
High-sugar-content products	31	High-sugar-content products	22	Vegetables & vegetable products	17	High-sugar-content products	30	Cereals	16
Milk & milk products	30	Cereals	16	Cereals	16	Milk & milk products	16	Fats	16
Cereals	21	Beverages	14	Fats	16	Beverages	14	Milk & milk products	14
Fats	6	Fruits	14	Meat	11	Cereals	13	Meat	12
Beverages	5	Milk & Milk products	12	Milk & milk products	11	Fruits	11	Vegetables & vegetable products	12

Food groups and food items are listed in Supplementary online material—Table S1. Data of 2563 dietary records are used from 740 children at 3, 4, 5, 6, and 8 years of age and from 5 countries (Belgium, Germany, Italy, Poland, and Spain)

Table 4 Frequency of skipping an eating occasion on all record days based on total number of dietary protocols ($N_{protocol}=2563$) of all comprised subjects ($N=740$) and time points (3 to 8 years)

	$N_{protocol}$	%
Breakfast	14	0.5
Morning snack	607	23.7
Lunch	3	0.1
Afternoon snack	40	1.6
Supper	3	0.1

energy intake at lunch and supper with significantly higher intakes at later ages ($p<0.0125$; data not shown).

Boys and girls differed in energy and energy from macronutrient intakes, with boys over all ages consuming on average 73.6 kcal more than girls. However, no relative difference was noticed at each EO (all $p>0.05$; data not shown).

Discussion

The time-related distribution of energy and macronutrient intakes throughout the day of 740 healthy European children followed from 3 to 8 years of age showed highest mean energy intakes at lunch and supper. Breakfast and snacks had high proportions of carbohydrates, whereas lunch and supper were characterized by high-fat and protein intakes. Food intakes changed with age, with lower intakes for snacks and higher intakes for lunch and supper at later ages. No sex differences between EOs were observed.

The average energy intake consumed in this study population was in line with the recommended intakes (RI) for energy and nutrients defined by the European Food Safety Authority [28], except for total fat. For children aged 4 years and older, a total fat intake of 20–35%E is recommended [29, 30]. In the current cohort, total fat intake was at the upper limit or above the recommended range. Fat intakes above RI might contribute to a high-energy intake and to promotion of weight gain and an increased risk of overweight and obesity [29]. In this study population, energy intake from fat was highest during lunch and supper, where particular opportunities for reducing or changing fat intake may exist. Especially in Spain, fat %E was above the reference intake, and correspondently, carbohydrate %E was at the lower end of the recommended range, which agrees with result of another study examining Spanish children and adolescents [31]. The Spanish diet as part of the Mediterranean diet is usually characterized by high total fat intakes with high consumption of olive oil and a low proportion of saturated fats [32]. A Mediterranean diet supplemented with olive oil or nuts was found to be beneficial for health [33], and thus, the high fat intake Spanish children may not be an indicator for an unhealthy eating pattern. Since the fat profile was not examined, the present study does not support conclusions about quality of fat intake.

The timing, frequency and kind of food consumed depend on social and cultural aspects [9]. Furthermore, there is no unique definition of what is considered a meal or a snack [9, 34]. These aspects need to be taken into consideration when results of time-varying food intake are compared. A similar energy and nutrient (%E) distribution as in our study was also reported in other reports on Spanish children and adolescents [12] and Belgian adolescents [35]. However, Belgian children observed in our study consumed foods at lunch and supper of similar size. The different distribution could be due to the different age groups studied. In other countries not examined in our analysis, energy intake for lunch was reported lower than for supper in New Zealand [15, 36] and USA [37].

Snacking in younger children was prevalent in our study and contributed 24% of TEI. In other studies, snacking contributed from 20 to 24% [35] to 30.5% [38]. Vatanparast et al. [39] reported that younger children do not only snack more frequently but also consume more energy at snacks than adults. Snacking is an easy way for children to achieve

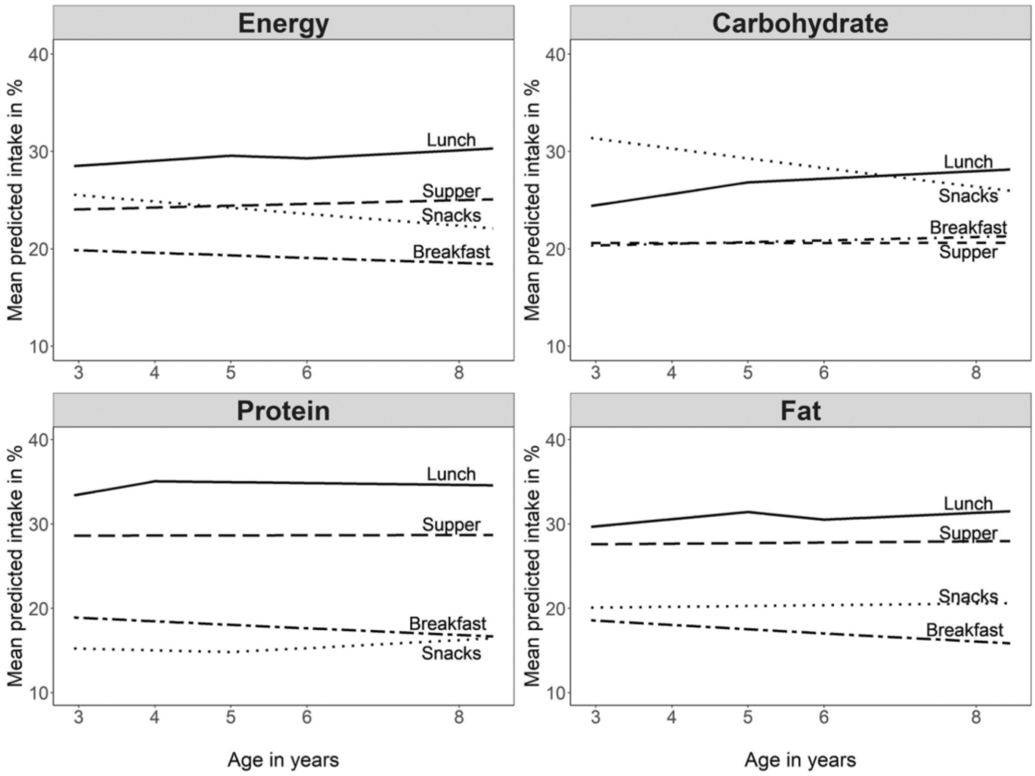

Fig. 3 Mean predicted percentage energy and macronutrient energy percentages for eating occasions by age in children followed at 3, 4, 5, 6, and 8 years of age ($N=740$). Data are based on beta regression (logit link) and applied to generalized linear mixed-effects models adjusted for misreporting, total energy intake (TEI), country and interaction of country, and TEI (for nutrients, TEI replaced by total nutrient intake)

energy balance. Young children have the highest energy and nutrient requirements based on their body weight than in any other phase of the lifespan [40]. Therefore, snacking may help young children to meet their relatively high-energy needs when gastric capacity is limited. The high carbohydrate portion at snacks ($\%E_{Snacks}$) related to frequent consumption of high-sugar-content foods observed in our study population seems to be concerning; however, total carbohydrate intake (%E) is within the recommended intake ranges of 45–60%E [30].

Although energy intake between girls and boys varied, we found no proportional differences either in daily energy intake or in the macronutrient distribution at each EO. Given that the energy difference between girls and boys was largest at 8 years of age, differences might evolve in older age groups. However, other studies also observed no sex differences in time-varying energy intake [15, 41].

Skipping of an EO is usually determined through questionnaires [2] or dietary records [14, 42]. Breakfast skipping in children was investigated in several previous studies, and a wide variation in prevalence was observed [43]. The variation in prevalence is also influenced by different definitions of breakfast skipping. When comparing our results with studies using a similar definition [3], breakfast skipping in children tends to be low. The percentage of persons skipping breakfast seems to increase in adolescence and adulthood [3, 14, 42].

Eating behaviour and macronutrient composition of EOs by age have been reported. A cross-sectional study performed in the UK showed similar energy and nutrient intakes during the life course (children, adolescents, adults and older adults) for breakfast [42]. Another cross-sectional study in New Zealand children found a larger proportion of daily energy intake during evening and a smaller proportion during afternoon in older children (11–14 years) compared to younger children (5–10 years) [15]. However, to our knowledge, age trends in a longitudinal setting and with the use of inferential statistics have not been examined. The decreased snack intake at later ages might be in line with decreased energy requirements based on body weight where less-frequent intakes are needed and more is eaten at main meals. Additionally, we found statistically significant results for breakfast (protein and fat); the effect sizes over time are small (e.g., mean predicted values vary from 3 to 8 years between 2 and 3 percentage points) indicating relatively stable eating habits over time.

Strength and limitations

The strength of this study is its longitudinal design with a follow-up of children from 3 to 8 years of age. This design enabled us to study between and within subject variation. For data collection, 3-day weighed dietary protocols following standardized procedures were used, which allowed for a more accurate description of dietary intake than other methods. Furthermore, the present study comprised children from five European countries which increased the external validity of the study results and allowed studying cultural variation of eating habits. On the contrary, different cultural eating habits and time slots for typical EOs were challenging to standardize. All EOs were named the same for each country, although actual time points of the consumed meal might differ greatly. These standardisations were necessary for data analysis, but might hamper comparison between countries and eating pattern.

Conclusion

The present study examined diurnal energy and energy from macronutrient intakes from early childhood to school age and revealed additional insights in the eating behaviour of children. Food was consumed regularly throughout the day and size of EOs seemed to be relatively stable at each age with highest intakes at lunch and supper. Possibly unhealthy EOs with high-fat intakes at lunch and supper and high-sugar-content foods at breakfast and snacks were observed, which may indicate potential for intervention. Practical approaches for a healthier diet may be achieved by substituting high-sugar-content foods with fruits, and by increasing the vegetable consumption at lunch and supper. Future studies should consider taking more detailed characterization of diet quality and critical nutrients across eating occasions into account.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00394-022-02944-6.

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Author contributions VJ analyzed the data, drafted, and finalized the manuscript. VL, NF, DG, KG, EV, GZ, AX, and PP conducted the study, entered data at study sites, and critically reviewed the manuscript. BK designed the research and critically reviewed the manuscript. VG designed the research, participated in the data analysis, and critically reviewed the manuscript. All authors read and approved the final manuscript.

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Declarations

Conflict of interest The authors report no conflicts of interest in relation to the content of this article.

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References

1. Almoosawi S, Vingeliene S, Gachon F, Voortman T, Palla L, Johnston JD, Van Dam RM, Darimont C, Karagounis LG (2019) Chronotype: implications for epidemiologic studies on chrono-nutrition and cardiometabolic health. Adv Nutr (Bethesda, Md) 10(1):30–42. https://doi.org/10.1093/advances/nmy070
2. Küpers LK, de Pijper JJ, Sauer PJJ, Stolk RP, Corpeleijn E (2014) Skipping breakfast and overweight in 2- and 5-year-old Dutch children—the GECKO Drenthe cohort. Int J Obes 38(4):569–571. https://doi.org/10.1038/ijo.2013.194
3. Smith KJ, Breslin MC, McNaughton SA, Gall SL, Blizzard L, Venn AJ (2017) Skipping breakfast among Australian children and adolescents; findings from the 2011–12 national nutrition and physical activity survey. Aust N Z J Public Health 41(6):572–578. https://doi.org/10.1111/1753-6405.12715
4. Diederichs T, Rossbach S, Herder C, Alexy U, Buyken AE (2016) Relevance of morning and evening energy and macronutrient intake during childhood for body composition in early adolescence. Nutrients. https://doi.org/10.3390/nu8110716
5. Coulthard JD, Pot GK (2016) The timing of the evening meal: how is this associated with weight status in UK children? Br J Nutr 115(9):1616–1622. https://doi.org/10.1017/S0007114516000635
6. Emmett PM, Jones LR, Northstone K (2015) Dietary patterns in the avon longitudinal study of parents and children. Nutr Rev 73(Suppl 3):207–230. https://doi.org/10.1093/nutrit/nuv055
7. Luque V, Escribano J, Closa-Monasterolo R, Zaragoza-Jordana M, Ferré N, Grote V, Koletzko B, Totzauer M, Verduci E, ReDionigi A, Gruszfeld D, Socha P, Rousseaux D, Moretti M, Oddy W, Ambrosini GL (2018) Unhealthy dietary patterns established in infancy track to mid-childhood: the EU childhood obesity project. J Nutr 148(5):752–759. https://doi.org/10.1093/jn/nxy025
8. Lobstein T, Baur L, Uauy R (2004) Obesity in children and young people: a crisis in public health. Obes Rev 5(Suppl 1):4–104. https://doi.org/10.1111/j.1467-789X.2004.00133.x
9. Chiva M (1997) Cultural aspects of meals and meal frequency. Br J Nutr 77(Suppl 1):S21-28. https://doi.org/10.1079/bjn19970101
10. Bellisle F (2014) Meals and snacking, diet quality and energy balance. Physiol Behav 134:38–43. https://doi.org/10.1016/j.physbeh.2014.03.010
11. Almoosawi S, Vingeliene S, Karagounis LG, Pot GK (2016) Chrono-nutrition: a review of current evidence from observational studies on global trends in time-of-day of energy intake and its association with obesity. Proc Nutr Soc 75(4):487–500. https://doi.org/10.1017/s0029665116000306
12. Vilela S, Oliveira A, Severo M, Lopes C (2019) Chrono-nutrition: the relationship between time-of-day energy and macronutrient intake and children's body weight status. J Biol Rhythms 34(3):332–342. https://doi.org/10.1177/0748730419838908
13. Palla L, Almoosawi S (2019) Diurnal patterns of energy intake derived via principal component analysis and their relationship with adiposity measures in adolescents: results from the national diet and nutrition Survey RP (2008–2012). Nutrients 11(2):422. https://doi.org/10.3390/nu11020422
14. Coulthard JD, Palla L, Pot GK (2017) Breakfast consumption and nutrient intakes in 4–18-year-olds: UK National Diet and Nutrition Survey Rolling Programme (2008–2012). Br J Nutr 118(4):280–290. https://doi.org/10.1017/S0007114517001714
15. Rockell JE, Skidmore PML, Parnell WR, Wilson N (2011) What children eat during afternoons and evenings: is it important? Public Health Nutr 14(3):557–562. https://doi.org/10.1017/S1368980010003277
16. Sievert K, Hussain SM, Page MJ, Wang Y, Hughes HJ, Malek M, Cicuttini FM (2019) Effect of breakfast on weight and energy intake: systematic review and meta-analysis of randomised controlled trials. BMJ 364:l42. https://doi.org/10.1136/bmj.l42
17. Ma X, Chen Q, Pu Y, Guo M, Jiang Z, Huang W, Long Y, Xu Y (2020) Skipping breakfast is associated with overweight and obesity: a systematic review and meta-analysis. Obes Res Clin Pract. https://doi.org/10.1016/j.orcp.2019.12.002
18. Koletzko B, von Kries R, Closa R, Escribano J, Scaglioni S, Giovannini M, Beyer J, Demmelmair H, Gruszfeld D, Dobrzanska A, Sengier A, Langhendries JP, Rolland Cachera MF, Grote V (2009) Lower protein in infant formula is associated with lower weight up to age 2 y: a randomized clinical trial. Am J Clin Nutr 89(6):1836–1845. https://doi.org/10.3945/ajcn.2008.27091
19. Weber M, Grote V, Closa-Monasterolo R, Escribano J, Langhendries JP, Dain E, Giovannini M, Verduci E, Gruszfeld D, Socha P, Koletzko B, European Childhood Obesity Trial Study G (2014) Lower protein content in infant formula reduces BMI and obesity risk at school age: follow-up of a randomized trial. Am J Clin Nutr 99(5):1041–1051. https://doi.org/10.3945/ajcn.113.064071
20. Luque V, Escribano J, Mendez-Riera G, Schiess S, Koletzko B, Verduci E, Stolarczyk A, Martin F, Closa-Monasterolo R (2013) Methodological approaches for dietary intake assessment in formula-fed infants. J Pediatr Gastroenterol Nutr 56(3):320–327. https://doi.org/10.1097/MPG.0b013e3182779a60
21. Verwied-Jorky S, Schiess S, Luque V, Grote V, Scaglioni S, Vecchi F, Martin F, Stolarczyk A, Koletzko B, European Childhood Obesity P (2011) Methodology for longitudinal assessment of nutrient intake and dietary habits in early childhood in a transnational multicenter study. J Pediatr Gastroenterol Nutr 52(1):96–102. https://doi.org/10.1097/MPG.0b013e3181f28d33
22. Gomes D, Luque V, Xhonneux A, Verduci E, Socha P, Koletzko B, Berger U, Grote V (2018) A simple method for identification of misreporting of energy intake from infancy to school age: results from a longitudinal study. Clin Nutr 37(3):1053–1060. https://doi.org/10.1016/j.clnu.2017.05.003
23. European Food Safety Authority (2014) Guidance on the EU Menu methodology. EFSA J 12(12):3944. https://doi.org/10.2903/j.efsa.2014.3944
24. UNESCO United Nations Educational SaCO (2003) International standard classification of education, ISCED 1997. Advances in cross-national comparison: a European working book for demographic and socio-economic variables

25. World Health Organization (2021) Body mass index - BMI. https://www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi. Accessed 12 Nov 2021
26. Smithson M, Verkuilen J (2006) A better lemon squeezer? Maximum-likelihood regression with beta-distributed dependent variables. Psychol Methods 11(1):54–71. https://doi.org/10.1037/1082-989x.11.1.54
27. R Core Team (2017) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
28. EFSA Panel on Dietetic Products Nutrition and Allergies (2013) Scientific opinion on dietary reference values for energy. EFSA J 11(1):3005. https://doi.org/10.2903/j.efsa.2013.3005
29. EFSA Panel on Dietetic Products Nutrition and Allergies (2010) Scientific opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. EFSA J 8(3):1461. https://doi.org/10.2903/j.efsa.2010.1461
30. EFSA Panel on Dietetic Products Nutrition and Allergies (2010) Scientific opinion on dietary reference values for carbohydrates and dietary fibre. EFSA J 8(3):1462. https://doi.org/10.2903/j.efsa.2010.1462
31. López-Sobaler AM, Aparicio A, Rubio J, Marcos V, Sanchidrián R, Santos S, Pérez-Farinós N, Dal-Re MÁ, Villar-Villalba C, Yusta-Boyo MJ, Robledo T, Castrodeza-Sanz JJ, Ortega RM (2019) Adequacy of usual macronutrient intake and macronutrient distribution in children and adolescents in Spain: A National Dietary Survey on the Child and Adolescent Population, ENALIA 2013–2014. Euro J Nutr 58(2):705–719. https://doi.org/10.1007/s00394-018-1676-3
32. Davis C, Bryan J, Hodgson J, Murphy K (2015) Definition of the mediterranean diet: a literature review. Nutrients 7(11):9139–9153. https://doi.org/10.3390/nu7115459
33. Estruch R, Ros E, Salas-Salvadó J, Covas M-I, Corella D, Arós F, Gómez-Gracia E, Ruiz-Gutiérrez V, Fiol M, Lapetra J, Lamuela-Raventos RM, Serra-Majem L, Pintó X, Basora J, Muñoz MA, Sorlí JV, Martínez JA, Martínez-González MA (2013) Primary prevention of cardiovascular disease with a mediterranean diet. N Engl J Med 368(14):1279–1290. https://doi.org/10.1056/NEJMoa1200303
34. Leech RM, Worsley A, Timperio A, McNaughton SA (2015) Understanding meal patterns: definitions, methodology and impact on nutrient intake and diet quality. Nutr Res Rev 28(1):1–21. https://doi.org/10.1017/s0954422414000262
35. Matthys C, De Henauw S, Devos C, De Backer G (2003) Estimated energy intake, macronutrient intake and meal pattern of Flemish adolescents. Eur J Clin Nutr 57(2):366–375. https://doi.org/10.1038/sj.ejcn.1601533
36. Regan A, Parnell W, Gray A, Wilson N (2008) New Zealand children's dietary intakes during school hours. Nutr Diet 65(3):205–210. https://doi.org/10.1111/j.1747-0080.2008.00288.x
37. Mathias KC, Almoosawi S, Karagounis LG (2017) Protein and energy intakes are skewed toward the evening among children and adolescents in the United States: NHANES 2013–2014. J Nutr 147(6):1160–1166. https://doi.org/10.3945/jn.116.245621
38. Fayet-Moore F, Peters V, McConnell A, Petocz P, Eldridge AL (2017) Weekday snacking prevalence, frequency, and energy contribution have increased while foods consumed during snacking have shifted among Australian children and adolescents: 1995, 2007 and 2011–12 National Nutrition Surveys. Nutr J 16(1):65. https://doi.org/10.1186/s12937-017-0288-8
39. Vatanparast H, Islam N, Patil RP, Shafiee M, Smith J, Whiting S (2019) Snack consumption patterns among Canadians. Nutrients. https://doi.org/10.3390/nu11051152
40. Wang Z (2012) High ratio of resting energy expenditure to body mass in childhood and adolescence: a mechanistic model. Am J Hum Biol 24(4):460–467. https://doi.org/10.1002/ajhb.22246
41. Maffeis C, Provera S, Filippi L, Sidoti G, Schena S, Pinelli L, Tatò L (2000) Distribution of food intake as a risk factor for childhood obesity. Int J Obes Relat Metab Disord 24(1):75–80. https://doi.org/10.1038/sj.ijo.0801088
42. Gaal S, Kerr MA, Ward M, McNulty H, Livingstone MBE (2018) Breakfast consumption in the UK: patterns, nutrient intake and diet quality. A Study from the International Breakfast Research Initiative Group. Nutrients 10(8):999. https://doi.org/10.3390/nu10080999
43. Monzani A, Ricotti R, Caputo M, Solito A, Archero F, Bellone S, Prodam F (2019) A systematic review of the association of skipping breakfast with weight and cardiometabolic risk factors in children and adolescents. What should we better investigate in the future? Nutrients 11(2):387. https://doi.org/10.3390/nu11020387

5. Publication II

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I contributed to this publication by conceptualizing the research question, selecting the methodology, performing the formal statistical analysis and interpreting the results. In particular, I searched for suitable statistical models to examine firstly, the timing of food intake separately from its caloric intake and secondly, to examine the dependency of several variables simultaneously. To my knowledge, no such statistical method has been used in nutritional epidemiology previously. I found a method called “compositional data analysis” and I acquired the knowledge to use this method and implemented the models in R. Moreover, I have prepared the original draft, created the figures and tables, incorporated feedback from my supervisors and co-authors and I was responsible for the submission to Nutrients and the peer-review process.

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Article

Time of Dietary Energy and Nutrient Intake and Body Mass Index in Children: Compositional Data Analysis from the Childhood Obesity Project (CHOP) Trial

Vanessa Jaeger [1], Berthold Koletzko [1,*], Veronica Luque [2,3], Mariona Gispert-Llauradó [2], Dariusz Gruszfeld [4], Piotr Socha [5], Elvira Verduci [6], Gian Vincenzo Zuccotti [6], Louise Etienne [7] and Veit Grote [1]

[1] Division of Metabolic and Nutritional Medicine, Department of Pediatrics, Dr. von Hauner Children's Hospital, University Hospital, LMU Munich, 80337 Munich, Germany
[2] Paediatrics Research Unit, Universitat Rovira I Virgili-IISPV, 43201 Reus, Spain
[3] Serra Hunter Fellow, Universitat Rovira I Virgili-IISPV, 43201 Reus, Spain
[4] Neonatal Department and Neonatal Intensive Care Unit, Children's Memorial Health Institute, 04-730 Warsaw, Poland
[5] Department of Gastroenterology, Hepatology, Nutritional Disorders and Pediatrics, Children's Memorial Health Institute, 04-730 Warsaw, Poland
[6] Department of Paediatrics, Vittore Buzzi Children's Hospital, University of Milan, 2012 Milan, Italy
[7] Groupe Santé CHC, Bd. Patience et Beaujonc 2—(B), 4000 Liège, Belgium
* Correspondence: berthold.koletzko@med.uni-muenchen.de

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Abstract: Meal timing is suggested to influence the obesity risk in children. Our aim was to analyse the effect of energy and nutrient distributions at eating occasions (EO), including breakfast, lunch, supper, and snacks, on the BMI z-score (zBMI) during childhood in 729 healthy children. BMI and three-day dietary protocols were obtained at 3, 4, 5, 6, and 8 years of age, and dietary data were analysed as the percentage of the mean total energy intake (TEI; %E). Intakes at EOs were transformed via an isometric log–ratio transformation and added as exposure variables to linear mixed-effects models. Stratified analyses by country and recategorization of EOs by adding intake from snacks to respective meals for further analyses were performed. The exclusion of subjects with less than three observations and the exclusion of subjects who skipped one EO or consumed 5% energy or less at one EO were examined in sensitivity analyses. Around 23% of the children were overweight at a given time point. Overweight and normal-weight children showed different distributions of dietary intakes over the day; overweight children consumed higher intakes at lunch and lower intakes of snacks. However, no significant effects of timing of EOs on zBMI were found in regression analyses.

Keywords: chrono-nutrition; overweight; eating pattern; compositional data analysis; children; macronutrients; energy; meal timing

1. Introduction

Childhood obesity is a major public health concern with detrimental consequences for health and wellbeing [1,2] whereby diet is a decisive and modifiable factor contributing to obesity. Childhood represents an age when food preferences and dietary patterns develop, and hence, it is a time window with particular preventive potential [3,4].

Increasingly, it has been reported that not only what and how much is eaten, but also when food is eaten may matter. Chrono-nutrition refers to this hypothesis and is based on the interaction between biological rhythms, temporal eating patterns, and its influence on metabolic health [5,6]. The hypothesis is driven by the assumption that food intake at different times of the day may have beneficial or detrimental effects on weight and other health conditions [7,8]. In particular, higher food intake at supper [9] or evening/night snacks have been associated with adverse effects on weight status in children [10,11], whereas the contribution of breakfast to TEI was reported to have neutral effects [12]. Higher food intake at lunch was associated with a lower overweight risk [13]. Only limited data from studies in children and adolescents investigating the effects of the time of macronutrient intake are available. One study found that lower proportions of nutrients in the afternoon and evening are associated with a lower body weight [10], whereas another study found that low fat and high carbohydrate (CHO) intake in the morning are associated with a higher body fat mass index [14].

A systematic review by Almoosawi et al. [7] examined the effects of the time of day of energy intake and obesity and concluded that further studies are needed to establish a potential association. Specifically, studies are needed which examine simultaneously all EOs throughout the day, as other EOs may affect the intake at one EO. Previous studies examined different EOs throughout the day but analyzed only one EO at a time with respect to being overweight/obese in regression analyses [12], probably due to collinearity issues between EOs. Thus, we propose compositional data analysis, which is widely used in other disciplines (e.g., geology) but is rarely used in nutritional epidemiology [15]. Compositional data analysis is specifically designed to simultaneously analyze the effects of different parts of a total (here: parts are intakes at EOs which add to the TEI) on an outcome. A further asset of compositional data analysis is the possibility to disentangle the effects of meal timing from the total energy or nutrient intake, and thus, may reveal estimates that are more accurate. By compositional data analysis, we are able to examine the effect of meal timing by shifting energy or energy from nutrients from one EO to another. For instance, for a given TEI, how is the BMI affected if more energy is distributed to supper and less to the remaining EOs?

The aims of the following study are, firstly, to describe the time-of-day energy and energy from nutrient intakes (CHO, protein, and fat) in relation to being overweight in European children aged 3 to 8 years. Secondly, we aim to examine the effect of meal timing by analyzing the (re-) distribution of energy and energy from nutrients throughout the day (breakfast, lunch, supper, and snacks) on zBMI in a longitudinal study.

2. Materials and Methods

2.1. Study Design and Population

This analysis was based on data from the Childhood Obesity Project Trial, a randomized controlled trial that recruited 1678 healthy, full-term infants in the first 8 weeks of life from 2002 to 2004. The aim of the intervention was to examine the effect of different protein content in infant formula consumed during the first year of life on later obesity risk [16,17]. An observational arm of breastfed children was also included in the study. The infants were recruited in Belgium, Germany, Italy, Poland, and Spain. Legal guardians of the participating infants provided written informed consent prior to study enrollment. For the analysis of this paper, data from the follow-up visits performed during 2005 to 2012 at the ages of 3, 4, 5, 6, and 8 years were used. The trial was conducted in accordance with the Declaration of Helsinki, approved by the ethical committee from each study site, and registered at clinicaltrials.gov (NCT00338689).

2.2. Dietary Assessment

Parents or caretakers provided weighted and estimated dietary records over three consecutive days (2 weekdays and 1 weekend day). Food and leftovers were weighed using food scales (Unica 66006; Soehnle, Murrhardt, Germany). If weighing foods was not possible, an atlas with food pictures was used to support estimations of portion sizes.

Dietary records were entered in a database specifically created for this study, with data mainly based on the German food composition database BLS 2.3 (Bundeslebensmittelschlüssel). When necessary, the database was enriched by information from product labels, manufacturers, or national food databases of participating countries. Nutritional values of products were updated to BLS 3.01 for analysis. Trained dieticians discussed open issues in protocols with participants and executed quality checks of the collected dietary records following standard operating procedures.

An EO is defined as any occasion where food or beverages are consumed. Predefined categories with typical country-specific time slots were used for this study to enter protocol data according to the following EOs: breakfast, lunch, and supper for meals as well as morning, afternoon, and evening for snacks. Country-specific differences in times of EOs between weekdays and weekends were considered.

The misreporting of energy intake was calculated by comparing individual cut-offs with the ratio of reported energy intake to estimated energy requirements [18]. Dietary records were assumed to be implausible when the individual ratio fell below or above these cut-offs, but were not excluded from the analysis as previously recommended [19].

2.3. Anthropometric Measurement (Outcome)

At each time point, weight and height were measured twice while wearing only light clothes following standard operating procedures based on the WHO growth reference study [20]. Measurements were performed using the same weight scale (SECA 702) and stadiometer (SECA 242) at each study site. Body mass index (BMI = weight (kg)/height (m)^2) was calculated and standardized for age and sex (zBMI) according to the WHO reference population [20,21]. Children were classified as overweight or obese according to the cut-offs defined by the International Obesity Task Force (IOTF) [22]. The IOTF, in contrast to the WHO, provide a coherent cut-off for the entire observation period from 3 to 8 years.

2.4. Statistical Analysis

Anthropometric and/or dietary data were obtained at 3, 4, 5, 6, and 8 years of age. All subjects with measurements for both anthropometric and dietary data were added from each observation time point. From the total number of observations, all children with at least one out of five observation time points were included in the analysis. TEI and energy intake at each EO were calculated as the mean daily intake (kcal/day) of all valid protocol days. Proportional intakes (%E) at EOs were calculated as the respective intake (kcal) of the EO towards TEI. Data are presented as arithmetic means (standard deviation) for continuous variables and as counts (%) for categorical variables. Compositional data are displayed with geometric means and a variation matrix.

Meal timing was analyzed by compositional data analysis. Compositional data analysis applied to health research is described elsewhere [23–25]. Briefly, the basis of compositional data analysis is that several parts of a total are examined concurrently. In this study, the parts are the proportional intakes at EOs in which parts add to 100% of the mean daily TEI and total CHO, protein, and fat intake, respectively, recorded at a particular age and study visit. Furthermore, the total sum (here: TEI or total CHO, protein, and fat intake, respectively, as 100% or 1) is fixed, which is practical as we do not want to investigate the increase in TEI or nutrient intake on BMI as in usual regression analyses. Instead, we want to investigate the shift in intake at EOs through the day for a given TEI or nutrient intake. Both the fixed sum and the collinearity between parts imply that the compositional data need to be transformed to be used in linear regression analysis. Firstly, the ratio between parts needs to be calculated. This is performed by sequential binary partitioning (SBP), which splits parts of a composition step-by-step and in a hierarchical manner in smaller groups. The SBP for each EO at first rank is displayed in Table 1.

Table 1. Sequential binary partitioning of a four-part composition (breakfast, lunch, supper, and snacks) with each eating occasion at first rank.

Partition	Breakfast	Lunch	Supper	Snacks
1	Breakfast: mean (lunch, supper, snacks)	Lunch: mean (supper, snacks, breakfast)	Supper: mean (snacks, breakfast, lunch)	Snacks: mean (breakfast, lunch, supper)
2	Lunch: mean (supper, snacks)	Supper: mean (snacks, breakfast)	Snacks: mean (breakfast, lunch)	Breakfast: mean (lunch, supper)
3	Supper: Snacks	Snacks: Breakfast	Breakfast: Lunch	Lunch: Supper

Only the first partition includes the relevant information to examine the effect of the EO of interest, however the other partitions are needed to include the co-dependence between parts. Secondly, all partitions are transformed into new variables (coordinates) via isometric log–ratio (ILR) transformations. The rationale and the process of how to perform compositional data analysis are described in detail in the Appendix A.

The ILR coordinates are calculated by means of logarithm. This implies that the not consumed EOs (so-called zero values) of any dietary record have to be either discarded (the complete dietary record) or the EOs with zero values need to be adapted. Most of the zero values were present in snacks, especially during evenings. Therefore, intakes from snacks during the morning, afternoon, and evening were amalgamated to one EO (snacks). For the remaining zero values ($n = 39$) the parametric robust expectation maximisation algorithm was applied as recommended [26]. Zero values were replaced for each observation time point without distorting the ratio between parts.

Linear mixed-effects models were used to estimate the effects of different distributions of energy and macronutrient intakes throughout the day on zBMI. Mixed-effects models enable the estimation of variables with repeated measurements and allow for missing values at observation time points. Individual regressions were estimated for each set of ILR coordinates (exposure) with the zBMI as the outcome. Each regression contained subject-specific random intercepts and random slopes over age with a piecewise linear spline (knot at 6 years). Relevant covariates were chosen by backwards selection and likelihood ratio testing. This resulted in parental BMI, country, TEI, misreporting as recommended [27], and an interaction term between TEI and country. Further covariates were considered (smoking during pregnancy, sex, intervention type, and parental education), but none of those increased the model fit. Two sub-analyses for each EO were carried out. Firstly, analyses were stratified by country to better account for differences in daytime of the meals. Secondly, EOs were recategorized to examine snack intake in a time-dependent way. For this, the intake from snacks were added to the respective intake from meals and the new EOs resulted in morning (breakfast + morning snack), afternoon (lunch + afternoon snack), and evening (supper + evening snack).

The recategorized EOs were used to calculate "eveningness" as termed by Diederichs et al. [28]. "Eveningness" was calculated as the difference between evening and morning intake for energy and energy from nutrients. However, we expressed "eveningness" in percentage points (pp):

"Eveningness" (pp) = intake at evening from total intake (%)—intake at morning from total intake (%)

The analyses were further evaluated by performing sensitivity analyses including only subjects with at least 3 out of 5 observation time points and by the exclusion of subjects who skipped an EO or consumed very little at one EO (EO with 5% of the TEI or less). Residuals and influential observations were checked graphically, and a few influential observations were removed from the analysis ($n = 13$). All analyses were performed using RStudio [29] version 4.04 in addition with the statistical packages "lme4", "robCompositions", and "zCompositions". Results were considered as significant if $p < 0.05$.

3. Results

Data were available for 729 healthy children (53% girls) with 2487 observations (Figure 1). Most children were recruited in Spain (29%), followed by Italy (27%), Poland (17%), Germany (14%), and Belgium (13%). Children participated on average in 3.4 out of 5 follow-up visits, with 32% of children participating in all 5 follow-up visits. The portion of children who participated in the majority of visits (more than 3 visits) was lower if their parents had a low education level. Half of the parents whose children participated in this study have an intermediate level of education. Dietary records were provided for a mean of 2.9 days. In total, 13% of all records were classified as under-reported and 12% of the records as over-reported. The dietary records of overweight children were more often under-reported (37% in overweight children, 9% in normal-weight children) and less often over-reported than the dietary records of normal-weight children (3% in overweight children, 13% in normal-weight children). In 45% of the children, at least one parent was overweight or obese, and in 20% of the children, both parents were overweight or obese. The highest number of overweight children as a percentage of the total number of children in each country were seen in Italy and Spain (18% each), followed by Poland (15%). Smoking during pregnancy was observed in 28% of all children with the highest numbers in Poland and Spain (34% each).

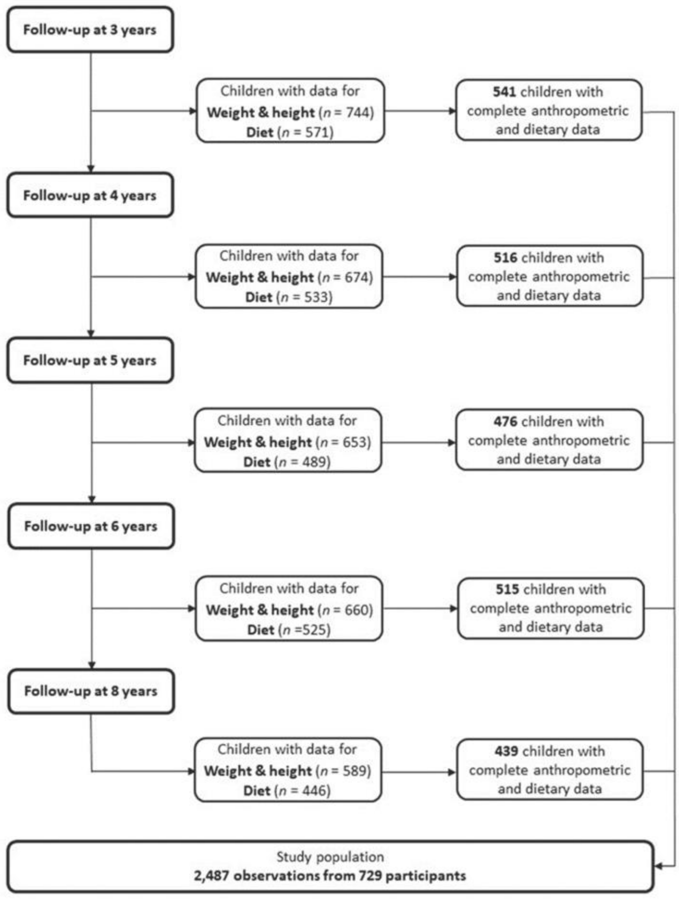

Figure 1. Number of participating children with available data for anthropometric and/or dietary data obtained at 3, 4, 5, 6, and 8 years of age. Children with measurements for both anthropometric and dietary data were added from each observation time point (n = 2487), resulting in 729 children.

Table 2 describes the study population with anthropometric and dietary data by age. The children in this study population were slightly heavier than the WHO reference population (zBMI > 0). Approximately 23% of the children were overweight, and around 6% of the children were obese (highest at 6 years: 6%, lowest at 4 years: 2%) at a given time point. Food intake (kcal/day) increased with age, but food intake in relation to body weight (kcal/kg/day) declined with age. The composition of food intake (CHO, fat, and protein) remained constant with age.

Table 2. Description of study population from age 3 to 8 years.

Age in Years	**3 (*n* = 541)**	**4 (*n* = 516)**	**5 (*n* = 476)**	**6 (*n* = 515)**	**8 (*n* = 439)**	**Overall (*N* = 729) ***
BMI (kg/m^2)	16.0 ± 1.3	15.9 ± 1.4	15.9 ± 1.7	16.0 ± 2.0	16.9 ± 2.7	16.1 ± 1.9
zBMI	0.3 ± 1.0	0.4 ± 1.0	0.4 ± 1.0	0.4 ± 1.2	0.4 ± 1.2	0.4 ± 1.1
Overweight **, N *** (%)	52 (9.6)	62 (12)	68 (14.3)	85 (16.5)	99 (22.6)	366 (14.7)
Energy (kcal/day)	1202 ± 237	1307 ± 235	1374 ± 249	1454 ± 250	1568 ± 285	1374 ± 279
kcal/kg/day	82.5 ± 17.7	78.2 ± 16.0	72.0 ± 15.7	67.2 ± 14.0	56.6 ± 13.5	71.9 ± 17.9
Carbohydrate (%E)	50.4 ± 7.7	50.0 ± 7.0	50.2 ± 7.2	50.3 ± 6.9	49.0 ± 7.0	50.0 ± 7.2
g/day	149.2 ± 44.3	160.1 ± 35.8	171.2 ± 43.5	183.0 ± 43.3	192.2 ± 49.0	170.3 ± 45.8
g/kg/day	10.3 ± 3.4	9.6 ± 2.4	9.0 ± 2.6	8.5 ± 2.3	6.9 ± 2.1	8.9 ± 2.8
Protein (%E)	15.3 ± 3.0	15.0 ± 2.9	14.9 ± 2.9	14.9 ± 2.6	15.2 ± 2.8	15.0 ± 2.8
g/day	44.9 ± 11.6	48.0 ± 12.3	50.5 ± 12.8	54.0 ± 12.5	59.5 ± 14.6	51.1 ± 13.6
g/kg/day	3.1 ± 0.8	2.9 ± 0.8	2.6 ± 0.7	2.5 ± 0.6	2.1 ± 0.6	2.7 ± 0.8
Total fat (%E)	34.3 ± 6.2	34.9 ± 5.8	34.9 ± 5.7	34.8 ± 5.6	35.8 ± 5.8	34.9 ± 5.8
g/day	45.0 ± 12.4	50.1 ± 13.1	52.8 ± 13.4	56.3 ± 13.6	62.7 ± 16.6	53.0 ± 15.0
g/kg/day	3.1 ± 0.9	3.0 ± 0.8	2.8 ± 0.8	2.6 ± 0.7	2.3 ± 0.7	2.8 ± 0.8

Values are presented as arithmetic mean ± standard deviation or as otherwise indicated. * In total, 729 subjects with 2487 observations. ** According to the cut-offs from the International Obesity Task Force [22]. *** Number of observations. Abbreviations: BMI = body mass index; zBMI = body mass index z-score; %E = energy from nutrients in percentage from total energy intake.

The variations at EOs were estimated as a variation matrix, with values close to 0 indicating a high proportionality/co-dependence between ratios. The highest proportionality was observed between lunch and supper, and the lowest proportionality was observed between snacks and supper for energy and energy from nutrients (Supplementary Material—Table S5). The average eating frequency per day (foods and beverages excluding water and unsweetened tea) declined from 5.8 to 5.1, while the average frequency of snacks declined from 3.1 to 2.3 from 3 to 8 years of age. The number of children with an average snack frequency of more than three snacks per day decreased from 21% to 5% in the same age range.

The distribution of energy and nutrient intakes according to EOs averaged by age is shown in Figure 2A. Most energy intakes were consumed at lunch, followed by snacks, supper, and breakfast. Protein and fat intakes were mostly consumed at lunch, followed by supper, whereas most of CHO intakes were consumed with snacks. Overweight children consumed higher intakes of total energy, protein, and fat than normal-weight children and consumed proportionally higher energy intakes at lunch and less at snacks (Figure 2B). Similar differences in the proportional intakes at EOs between overweight and normal-weight children were seen for energy from nutrients.

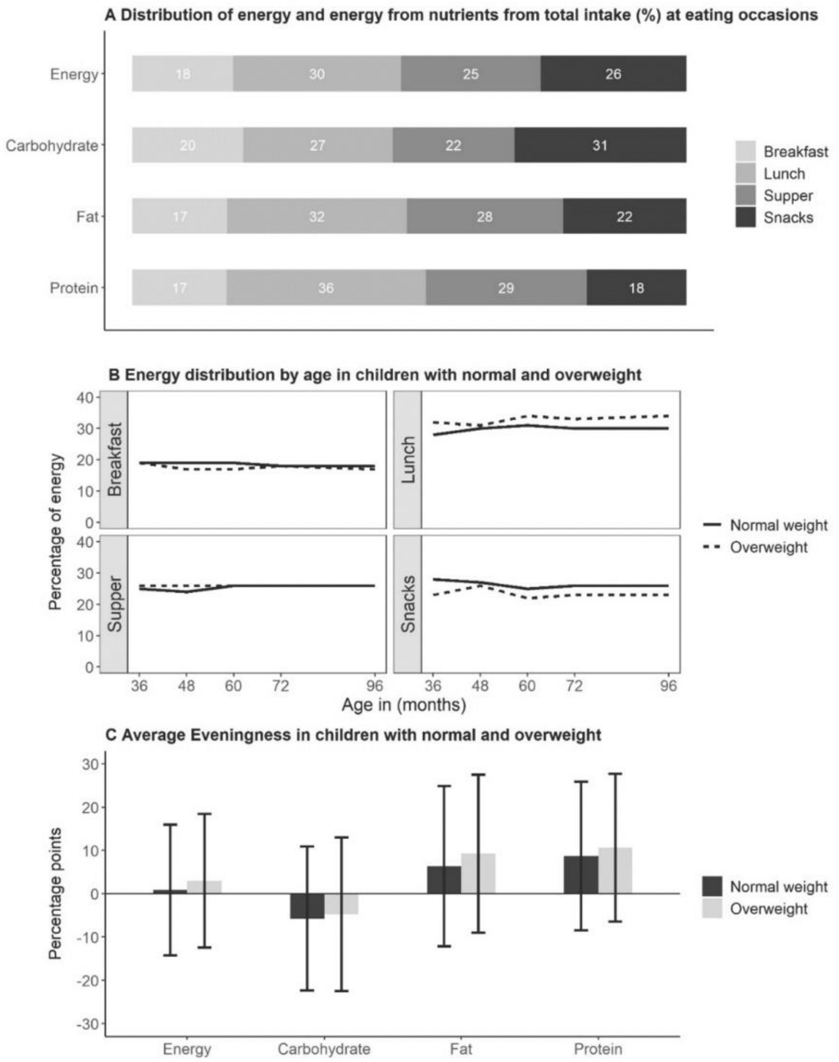

Figure 2. Distribution of energy and macronutrients by eating occasion, evening preference ("eveningness"), time, and overweight status of 729 children. (**A**) Average distribution of energy and nutrient intake as a percentage of the total intake at eating occasions; (**B**) Average energy intake at each eating occasion and age stratified by weight status. (**C**) Average eveningness § in percentage points (pp) stratified by weight status (mean ± standard deviation). § Difference in percentage intake between evening (supper + evening snack) and morning (breakfast + morning snack). Weight status was defined for normal-weight and overweight children according to IOTF. All analyses were performed in children aged 3 to 8 years (overweight children: $n_{observations}$ = 366, normal-weight children: $n_{observations}$ = 2121).

If the intake from snacks were added to the intake from respective meals with the EO categories morning, afternoon, and evening, most foods were consumed in the afternoon (46%E ± 9), followed by the evening (27%E ± 9) and morning (26%E ± 8). The intakes for energy from protein and fat followed a similar distribution, whereas energy intakes for CHO were larger in the morning (29%E) compared to the evening (24%E). A higher "eveningness" (difference between evening and morning intake) was observed for protein and fat intakes, whereas energy intakes were similar, and CHO intakes showed a negative "eveningness". However, the variations in energy and energy from nutrients were large in both weight groups (Figure 2C).

The results of the regression analysis on the effects of meal timing, expressed by the distribution of energy and energy from nutrients over the day in EOs on zBMI, are depicted in Table 3. The interpretation of the β-estimates estimated by compositional data analysis is different from usual regression analyses. The compositional data analysis split the exposure variable into co-dependent parts that are interpreted concurrently, whereas in usual regressions, only one exposure variable is interpreted at a time. Specifically, the ratio between EOs is interpreted. The β-estimates of the ILR coordinates applied to our results are interpreted as follows: For a given TEI, the redistribution of energy intake with an increase in energy at breakfast as compared to the other EOs was not significantly associated with zBMI ($\beta = -0.02$; $p > 0.05$). Similarly, the redistribution of energy with an increase at lunch, supper, or snacks compared to the remaining EOs and a given TEI were not significantly associated with zBMI ($p > 0.05$). Furthermore, results of different distributions of energy from nutrients throughout EOs were not statistically significant, as well as all sensitivity analyses (Supplementary Materials Tables S1 and S2). Stratification by country and reorganizing EOs to examine snacks in a time-dependent manner revealed no significant effects (Supplementary Materials Tables S3 and S4).

Table 3. Regression results of ILR coordinates against body mass index z-score from age 3 to 8 years (N = 729 children).

ILR *	Energy			Carbohydrate			Protein			Fat		
	β	SE	*p*-Value	β	SE	*p*-Value	β	SE	*p*-Value	β	SE	*p*-Value
Breakfast	−0.02	0.02	0.429	−0.01	0.02	0.493	0.00	0.02	0.957	−0.02	0.02	0.192
Lunch	0.00	0.03	0.945	0.01	0.02	0.747	0.00	0.02	0.942	0.00	0.02	0.825
Supper	0.01	0.03	0.616	−0.00	0.02	0.864	−0.01	0.02	0.577	0.03	0.02	0.123
Snacks	0.01	0.02	0.677	0.01	0.02	0.580	0.01	0.02	0.472	−0.01	0.01	0.380

Estimates were based on linear mixed-effects models which contained a subject-specific random intercept and a slope for age. The random slope is estimated by piecewise linear splines with a knot at 6 years. The analysis was adjusted for each set of ILR coordinates, parental BMI, misreporting, country, total energy intake, and interaction between country and total energy intake. * ILR coordinates refer to the mentioned eating occasion in relation to the geometric mean of the remaining eating occasions. Abbreviation: SE—standard error.

4. Discussion

In this large study evaluating the data from 729 healthy children aged 3 to 8 years from five European countries, children who are overweight consumed more energy than children with normal weights and also had a different intake distribution during the day, with higher intakes at lunch and fewer intakes of snacks. Despite these differences between normal-weight and overweight children, no statistically significant differences in weight status were seen for intakes with any EO. Thus, the distribution of energy and macronutrient intake over the day had no significant impact on weight status in children.

We found overweight children to consume proportionally lower intakes with snacks and higher intakes with lunch than normal-weight children. A similar pattern was also seen in French children aged 3–6 years and 7–11 years, but with significant differences in snack and main meal contribution only in older children [12]. A possible explanation for this pattern in overweight children might be that access to snacks is limited and/or consumption of snack foods is restricted due to parental concerns, as reported previously [30]. It has been suggested that healthy snacks, defined as nutrient dense and with a low energy density, during the mid-morning and mid-afternoon promote appetite control and may prevent overeating at the following meal [31,32]. However, shifting more energy or energy from nutrients to snacks and less to meals did not affect zBMI in children, as implied by our regression analyses.

The timing of food intake and its distribution throughout the day have been examined in this study, without appreciable effects of timing of energy or macronutrient intakes on BMI in children. Some studies found associations of meal timing with weight status in children [10,11,13,33], but another study did not [34]. Comparisons with other studies are difficult since we used an entirely different methodological approach. Furthermore, no generally agreed definition exists on how to determine a meal or a snack [7,30,35], which further limits comparisons.

Our study population exhibited a regular eating pattern where the skipping of meals was rarely seen, as previously shown [36]. A systematic review suggested that the skipping of meals, especially breakfast, is negatively associated with being overweight in children [37]. Meal skipping was also negatively associated with children's diet quality [38,39]. Thus, the more regular eating patterns of our study population may have contributed to the absence of an association of EO timing with BMI.

The chronotype of individuals might influence the timing of food intake. A chronotype is defined as an individual's circadian preference/phenotype and is classified as a morning or evening chronotype [8]. Studies in children are limited, but studies in adolescents and adults showed that individuals with an evening chronotype prefer to eat later in the day [40]. Although we have not assessed the chronotype of the study participants, our results showed a similar evening and morning ratio for energy, which might indicate that few children exhibit an evening chronotype. The chronotype in children generally tends to be early and shifts to later times in adolescence [41]. An evening chronotype was associated with more frequent breakfast skipping [40] or less fruit and vegetable consumption [42], both being associated with a higher weight status [43]. Thus, it is possible that the relatively low degree of "eveningness" in our study could have influenced our results.

Randomized controlled clinical trials and observational studies in overweight adults have shown the timing of food intake to modify weight-loss effectiveness. For instance, overweight and obese women have lost significantly more weight if an evening meal was consumed at earlier compared to later times [44] and if lunch was consumed earlier compared to later [45]. Possible mechanisms which may explain the timing of food intake as a risk factor for being overweight or weight-loss effectiveness are circadian rhythms [5,6]. Briefly, the circadian timing system is responsible for daily biological rhythms and synchronizes physiological and behavioural aspects to the outside world. External cues, such as the light–dark cycle or timing of food intake, provide signals to entrain the circadian clocks. It has been suggested that the timing of food intake aligned to the circadian rhythms of metabolic processes may be beneficial for health. For instance, it has been shown that insulin and glucose exhibit a circadian rhythm, with a decrease in insulin sensitivity and glucose tolerance during the day and a nadir in the evening [46,47]. A further factor might be diet-induced thermogenesis, which is not only affected by the nutrient composition of meals but was also found to be higher in the morning than in the evening [48,49]. These factors might contribute to potential untoward effects of higher evening intakes on weight status in adults, whereas no such evidence is available in children.

The present study benefited from the longitudinal study design with five years of follow-up, which allowed for examining between and within differences in a large number of study participants. Furthermore, the inclusion of children from five European countries enhances the generalizability of our study findings and the use of 3-day weighted dietary records allowed for a more accurate description of dietary intake than other methods. Another strength of our study is the applied compositional data analysis by taking into account EOs of an entire day concurrently. In addition, compositional data analysis has the benefit to disentangle the effects of meal timing from TEI, which allowed for a comprehensive isocaloric analysis. These results add to the limited understanding between meal timing and overweight in children.

One of the limitations of this study is that EOs were analyzed in categories (breakfast, lunch, supper, and snacks) which allowed for comparing and analysing EOs between countries. However, the timing of EOs may vary between countries, which could not be considered in the analysis. We tried to counteract the limitation by stratified analyses for each country. Another limitation is that we could not perform a detailed analysis of snack intakes by time of day because many children did not consume snacks, particularly in the evening, but also during mornings. However, we examined snacks in a time-related manner by summarizing meals and snacks combined into morning, afternoon, and evening. A further limitation of this study is a potential social desirability bias during data collection, suggested by the lower snack intake and the lower number of possible under-reporting of TEI in overweight compared to normal-weight children. The social desirability bias describes a behaviour in which an individual reports selected foods or beverages due to societal beliefs or norms to act in favour of the researcher [50]. We tried to minimize this bias by including misreporting as a covariate in all regression analyses.

Further studies examining the relationship between meal timing and obesity might also take the chronotype of each child into consideration. Studies in adults have shown that the chronotype influenced the effect between meal timing and being overweight [51]. The development of consistent and broadly agreed definitions on how to define a meal and a snack would be most helpful to enable comparisons of the respective results from different studies and to strengthen conclusions.

5. Conclusions

This study described the diurnal differences in energy and nutrient intakes in overweight compared to normal-weight children, with proportionally lower reported intakes at snacks and higher intakes at lunch in overweight children. The timing of meals and snacks did not influence the weight status in the total population of children. These data do not provide a basis for approaches to reduce the obesity risk in children based on the shifting distribution of dietary intakes across EOs.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/nu14204356/s1, Table S1: Regression results of ILR coordinates of EOs against body mass index z-score including only subjects with at least three observation time points; Table S2: Regression of ILR coordinates against body mass index z-score excluding subjects with intakes of 5% or less of the total intake at an eating occasion; Table S3: Regression of ILR coordinates against body mass index z-score stratified by country; Table S4: Regression of ILR coordinates for morning (breakfast + morning snack), afternoon (lunch + afternoon snack), evening (supper + night snack), and ratio of evening and morning intake ("eveningness") against body mass index z-score; Table S5: Mean and pair-wise variation of eating occasions for energy and energy from nutrient intake in children aged 3 to 8 years.

Author Contributions: Conceptualization, V.J.; methodology, V.J.; formal Analysis, V.J.; investigation and resources, B.K., V.G., V.L., M.G.-L., D.G., P.S., and E.V.; data curation, V.G.; writing—original draft preparation, V.J.; writing—review & editing, V.G., B.K., V.L., M.G.-L., D.G., P.S., E.V., L.E. and G.V.Z.; visualization, V.J. and V.L.; supervision, V.G. and B.K.; funding acquisition, B.K. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors report no conflict of interest in relation to the content of this article.

Abbreviations

BMI—body mass index; CHO—carbohydrates; EO—eating occasion; ILR—isometric log ratios; TEI—total energy intake; WHO—World Health Organization; zBMI—body mass index z-score.

Appendix A

Compositional Data Analysis and Calculation of ILR-Coordinates

Dietary data are typical examples of compositional data because the contribution of dietary nutrients depends on each other, and they sum to the TEI. Nutrients are not consumed isolated, and a variation in one nutrient likely leads to variations in further nutrients. Likewise, the size of a meal depends on the size of the previous meal and/or if a snack was consumed between meals. This correlation between nutrients or meals induces collinearity between parts when analyzed as independent variables in regression analyses simultaneously. Due to the collinearity between independent variables, it is difficult to measure the influence of individual variables on the outcome which may lead to unstable and inaccurate coefficients [23].

Compositional data is best analyzed by expressing the composition with a set of logarithmized ratios (log ratio) [52]. The log ratio approach is an established method in many scientific disciplines (e.g., geology). Dumuid et al. [24,53], Corrêa Leite et al. [15], and Chastin et al. [25] have described in detail the log ratio approach for use in health research and nutritional epidemiology. Briefly, a composition is a set of parts which sum to a whole. For instance, energy intake at breakfast, lunch, supper, and snacks are a composition of four parts and sum to the TEI. The composition is analyzed as a ratio, which highlights the relative nature of the parts (each part as a proportion of the TEI). Consequently, if one part of a composition increases, the other part(s) need to decrease to keep the sum constant. This dependence between parts and the constant sum assumption imply that the composition is constrained, but regression analyses are typically conducted in unconstrained real space in which each part can vary freely. Thus, the composition needs to be transformed into real space, which is performed using a specific set of log ratios, namely the isometric log ratio (ILR) coordinates. The derivation of the ILR was described elsewhere [15,24].

Compositional data analysis in the present study has the following properties: Firstly, it is possible to analyze all EOs simultaneously without biasing the coefficient due to collinearity. Secondly, the transformed variables of interest are independent from the TEI and enable us to disentangle the effect of meal timing from the effect of the TEI. Thirdly, the generic effect of the TEI on the outcome is considered. Further methods in nutritional epidemiology are available to deal with appropriate considerations of the TEI, such as the residual method [54]. However, to our knowledge, none of these methods include appropriate adjustments of the TEI and additionally deal with perfect multi-collinearity.

The ILR coordinates were calculated based on an orthonormal basis. A widely used approach to generate the basis is sequential binary partitioning (SBP) [55], which splits parts of a composition step-by-step and in a hierarchical manner into smaller groups. An example of a SBP with breakfast at first rank is given in Table A1. In a first step, the composition is split in two groups that will form the parts of the ratio. Parts, which belong to the first group, are coded as 1, and parts which belong to the second group are coded as −1. This leads to the first partition. In a second step, one of the two groups in step one is further split into two new groups. The parts, which belong to the first group, are coded as 1, and parts which belong to the second group are coded as −1. Parts, which do not belong to one group at that order are coded as 0. The second step results in the second partition. This procedure is continued until a subgroup consists only of one part. Formally, a composition D is split in (D-1) parts.

Table A1. Sequential binary partition of a four-part composition with breakfast at first rank.

Partition	Breakfast	Lunch	Supper	Snacks	Ratio
1	1	−1	−1	−1	Breakfast: mean (lunch, supper, snacks)
2	0	1	−1	−1	Lunch: mean (supper, snacks)
3	0	0	1	−1	Supper: Snacks

1: Part is assigned to the first group; −1: part is assigned to the second group; 0: part is not involved in the partition at that order.

The partition is in the next step transformed into an ILR coordinate for each of the (D-1)-dimensional partitions presented using the following formula [52]:

$$\mathrm{ILR}_i = \frac{\sqrt{(r*s)}}{\sqrt{(r+s)}} \ln\left(\frac{y_i}{g(y_j)}\right),\ for\ i = 1,\ \ldots,\ \mathrm{D}-1$$

- r and s, respectively, are the number of parts in the first (coded as 1) and second group (coded as −1) at each order of the partition;
- y_i is the proportional intake (coded as 1);
- $g(y_j)$ is the geometric mean of the components of y, for $j = i + 1, \ldots$, D (geometric mean of the components coded as −1).

We used an SBP in which the first partition group consist of only one (so-called pivot coordinate [56]). The advantage of pivot coordinates is that the first coordinate contains all the relevant information of the first part (here: breakfast) compared to the remaining parts which facilitate interpretation. All remaining coordinates are included in the regression analysis as covariates to consider the co-dependence between EOs. Inference of the other parts of the composition (lunch, supper, and snacks) is received by changing the hierarchical order of the composition. Thus, SBP was conducted four times, with each time a different EO at first rank, and each SBP resulted in three pivot coordinates. A set of pivot coordinates was defined as consisting of one SBP with resulting coordinates.

References

1. Lobstein, T.; Baur, L.; Uauy, R. Obesity in children and young people: A crisis in public health. *Obes. Rev.* **2004**, *5* (Suppl. 1), 4–104. [CrossRef] [PubMed]
2. World Health Organization. *Obesity: Preventing and Managing the Global Epidemic: Report of a WHO Consultation*; WHO Technical Report Series; World Health Organization: Geneva, Switzerland, 2000; Volume 894.
3. Emmett, P.M.; Jones, L.R.; Northstone, K. Dietary patterns in the Avon Longitudinal Study of Parents and Children. *Nutr. Rev.* **2015**, *73* (Suppl. 3), 207–230. [CrossRef] [PubMed]
4. Luque, V.; Escribano, J.; Closa-Monasterolo, R.; Zaragoza-Jordana, M.; Ferré, N.; Grote, V.; Koletzko, B.; Totzauer, M.; Verduci, E.; ReDionigi, A.; et al. Unhealthy Dietary Patterns Established in Infancy Track to Mid-Childhood: The EU Childhood Obesity Project. *J. Nutr.* **2018**, *148*, 752–759. [CrossRef]
5. Flanagan, A.; Bechtold, D.A.; Pot, G.K.; Johnston, J.D. Chrono-nutrition: From molecular and neuronal mechanisms to human epidemiology and timed feeding patterns. *J. Neurochem.* **2021**, *157*, 53–72. [CrossRef] [PubMed]
6. Covassin, N.; Singh, P.; Somers, V.K. Keeping Up With the Clock: Circadian Disruption and Obesity Risk. *Hypertension* **2016**, *68*, 1081–1090. [CrossRef] [PubMed]

7. Almoosawi, S.; Vingeliene, S.; Karagounis, L.G.; Pot, G.K. Chrono-nutrition: A review of current evidence from observational studies on global trends in time-of-day of energy intake and its association with obesity. *Proc. Nutr. Soc.* **2016**, *75*, 487–500. [CrossRef] [PubMed]
8. Almoosawi, S.; Vingeliene, S.; Gachon, F.; Voortman, T.; Palla, L.; Johnston, J.; Van Dam, R.M.; Darimont, C.; Karagounis, L.G. Chronotype: Implications for Epidemiologic Studies on Chrono-Nutrition and Cardiometabolic Health. *Adv. Nutr. Int. Rev. J.* **2019**, *10*, 30–42. [CrossRef] [PubMed]
9. Maffeis, C.; Provera, S.; Filippi, L.; Sidoti, G.; Schena, S.; Pinelli, L.; Tatò, L. Distribution of food intake as a risk factor for childhood obesity. *Int. J. Obes.* **2000**, *24*, 75–80. [CrossRef] [PubMed]
10. Vilela, S.; Oliveira, A.; Severo, M.; Lopes, C. Chrono-Nutrition: The Relationship between Time-of-Day Energy and Macronutrient Intake and Children's Body Weight Status. *J. Biol. Rhythm.* **2019**, *34*, 332–342. [CrossRef] [PubMed]
11. Thompson, O.M.; Ballew, C.; Resnicow, K.; Gillespie, C.; Must, A.; Bandini, L.G.; Cyr, H.; Dietz, W.H. Dietary pattern as a predictor of change in BMI z-score among girls. *Int. J. Obes.* **2006**, *30*, 176–182. [CrossRef] [PubMed]
12. Lioret, S.; Touvier, M.; Lafay, L.; Volatier, J.-L.; Maire, B. Are Eating Occasions and Their Energy Content Related to Child Overweight and Socioeconomic Status? *Obesity* **2008**, *16*, 2518–2523. [CrossRef] [PubMed]
13. Kupek, E.; Lobo, A.S.; Leal, D.B.; Bellisle, F.; de Assis, M.A.A. Dietary patterns associated with overweight and obesity among Brazilian schoolchildren: An approach based on the time-of-day of eating events. *Br. J. Nutr.* **2016**, *116*, 1954–1965. [CrossRef] [PubMed]
14. Diederichs, T.; Roßbach, S.; Herder, C.; Alexy, U.; Buyken, A.E. Relevance of Morning and Evening Energy and Macronutrient Intake during Childhood for Body Composition in Early Adolescence. *Nutrients* **2016**, *8*, 716. [CrossRef] [PubMed]
15. Leite, M.L.C. Applying compositional data methodology to nutritional epidemiology. *Stat. Methods Med Res.* **2016**, *25*, 3057–3065. [CrossRef] [PubMed]
16. Koletzko, B.; von Kries, R.; Closa, R.; Escribano, J.; Scaglioni, S.; Giovannini, M.; Beyer, J.; Demmelmair, H.; Gruszfeld, D.; Dobrzanska, A.; et al. Lower protein in infant formula is associated with lower weight up to age 2 y: A randomized clinical trial. *Am. J. Clin. Nutr.* **2009**, *89*, 1836–1845. [CrossRef] [PubMed]
17. Weber, M.; Luque, V.; Escribano, J.; Closa-Monasterolo, R.; Verduci, E.; ReDionigi, A.; Hoyos, J.; Langhendries, J.-P.; Gruszfeld, D.; Socha, P.; et al. Effect of Lower Versus Higher Protein Content in Infant Formula Through the First Year on Body Composition from 1 to 6 Years: Follow-Up of a Randomized Clinical Trial. *Obesity* **2018**, *26*, 1203–1210. [CrossRef]
18. Gomes, D.; Luque, V.; Xhonneux, A.; Verduci, E.; Socha, P.; Koletzko, B.; Berger, U.; Grote, V. A simple method for identification of misreporting of energy intake from infancy to school age: Results from a longitudinal study. *Clin. Nutr.* **2018**, *37*, 1053–1060. [CrossRef] [PubMed]
19. European Food Safety Authority. Guidance on the EU Menu methodology. *EFSA J.* **2014**, *12*, 3944. [CrossRef]
20. De Onis, M.; Garza, C.; Victora, C.; Onyango, A.W.; Frongillo, E.A.; Martines, J. The who Multicentre Growth Reference Study: Planning, Study Design, and Methodology. *Food Nutr. Bull.* **2004**, *25* (Suppl. 1), S15–S26. [CrossRef]
21. De Onis, M.; Onyango, A.W.; Borghi, E.; Siyam, A.; Nishida, C.; Siekmann, J. Development of a WHO growth reference for school-aged children and adolescents. *Bull. World Health Organ.* **2007**, *85*, 660–667. [CrossRef] [PubMed]
22. Cole, T.J.; Bellizzi, M.C.; Flegal, K.M.; Dietz, W.H. Establishing a standard definition for child overweight and obesity worldwide: International survey. *BMJ* **2000**, *320*, 1240–1243. [CrossRef] [PubMed]
23. Leite, M.L.C. Compositional data analysis as an alternative paradigm for nutritional studies. *Clin. Nutr. ESPEN* **2019**, *33*, 207–212. [CrossRef] [PubMed]
24. Dumuid, D.; Stanford, T.E.; Martín-Fernández, J.A.; Pedišić, Ž.; Maher, C.; Lewis, L.K.; Hron, K.; Katzmarzyk, P.; Chaput, J.-P.; Fogelholm, M.; et al. Compositional data analysis for physical activity, sedentary time and sleep research. *Stat. Methods Med. Res.* **2017**, *27*, 3726–3738. [CrossRef] [PubMed]
25. Chastin, S.F.; Palarea-Albaladejo, J.; Dontje, M.L.; Skelton, D.A. Combined Effects of Time Spent in Physical Activity, Sedentary Behaviors and Sleep on Obesity and Cardio-Metabolic Health Markers: A Novel Compositional Data Analysis Approach. *PLoS ONE* **2015**, *10*, e0139984. [CrossRef]
26. Rasmussen, C.L.; Palarea-Albaladejo, J.; Johansson, M.S.; Crowley, P.; Stevens, M.L.; Gupta, N.; Karstad, K.; Holtermann, A. Zero problems with compositional data of physical behaviors: A comparison of three zero replacement methods. *Int. J. Behav. Nutr. Phys. Act.* **2020**, *17*, 126. [CrossRef]
27. Jessri, M.; Lou, W.Y.; L'Abbé, M.R. Evaluation of different methods to handle misreporting in obesity research: Evidence from the Canadian national nutrition survey. *Br. J. Nutr.* **2016**, *115*, 147–159. [CrossRef]
28. Diederichs, T.; Perrar, I.; Roßbach, S.; Alexy, U.; Buyken, A.E. In adolescence a higher 'eveningness in energy intake' is associated with higher total daily energy intake. *Appetite* **2018**, *128*, 159–166. [CrossRef]
29. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2021; Available online: https://www.R-project.org/ (accessed on 20 February 2021).
30. Blaine, R.E.; Kachurak, A.; Davison, K.K.; Klabunde, R.; Fisher, J.O. Food parenting and child snacking: A systematic review. *Int. J. Behav. Nutr. Phys. Act.* **2017**, *14*, 146. [CrossRef]
31. Njike, V.Y.; Smith, T.M.; Shuval, O.; Shuval, K.; Edshteyn, I.; Kalantari, V.; Yaroch, A.L. Snack food, satiety, and weight. *Adv. Nutr.* **2016**, *7*, 866–878. [CrossRef]

32. Marangoni, F.; Martini, D.; Scaglioni, S.; Sculati, M.; Donini, L.M.; Leonardi, F.; Agostoni, C.; Castelnuovo, G.; Ferrara, N.; Ghiselli, A.; et al. Snacking in nutrition and health. *Int. J. Food Sci. Nutr.* **2019**, *70*, 909–923. [CrossRef]
33. Palla, L.; Almoosawi, S. Diurnal Patterns of Energy Intake Derived via Principal Component Analysis and Their Relationship with Adiposity Measures in Adolescents: Results from the National Diet and Nutrition Survey RP (2008–2012). *Nutrients* **2019**, *11*, 422. [CrossRef] [PubMed]
34. Coulthard, J.D.; Pot, G.K. The timing of the evening meal: How is this associated with weight status in UK children? *Br. J. Nutr.* **2016**, *115*, 1616–1622. [CrossRef] [PubMed]
35. Chiva, M. Cultural aspects of meals and meal frequency. *Br. J. Nutr.* **1997**, *77* (Suppl. 1), S21–S28. [CrossRef]
36. Jaeger, V.; Koletzko, B.; Luque, V.; Ferré, N.; Gruszfeld, D.; Gradowska, K.; Verduci, E.; Zuccotti, G.V.; Xhonneux, A.; Poncelet, P.; et al. Distribution of energy and macronutrient intakes across eating occasions in European children from 3 to 8 years of age: The EU Childhood Obesity Project Study. *Eur. J. Nutr.* **2022**. [CrossRef]
37. SSzajewska, H.; Ruszczyński, M. Systematic Review Demonstrating that Breakfast Consumption Influences Body Weight Outcomes in Children and Adolescents in Europe. *Crit. Rev. Food Sci. Nutr.* **2010**, *50*, 113–119. [CrossRef] [PubMed]
38. Coulthard, J.D.; Palla, L.; Pot, G.K. Breakfast consumption and nutrient intakes in 4–18-year-olds: UK National Diet and Nutrition Survey Rolling Programme (2008–2012). *Br. J. Nutr.* **2017**, *118*, 280–290. [CrossRef] [PubMed]
39. Fayet-Moore, F.; McConnell, A.; Tuck, K.; Petocz, P. Breakfast and Breakfast Cereal Choice and Its Impact on Nutrient and Sugar Intakes and Anthropometric Measures among a Nationally Representative Sample of Australian Children and Adolescents. *Nutrients* **2017**, *9*, 1045. [CrossRef] [PubMed]
40. Roßbach, S.; Diederichs, T.; Nöthlings, U.; Buyken, A.E.; Alexy, U. Relevance of chronotype for eating patterns in adolescents. *Chronobiol. Int.* **2018**, *35*, 336–347. [CrossRef]
41. Roenneberg, T.; Kuehnle, T.; Juda, M.; Kantermann, T.; Allebrandt, K.; Gordijn, M.; Merrow, M. Epidemiology of the human circadian clock. *Sleep Med. Rev.* **2007**, *11*, 429–438. [CrossRef]
42. Patterson, F.; Malone, S.K.; Lozano, A.; Grandner, M.; Hanlon, A.L. Smoking, Screen-Based Sedentary Behavior, and Diet Associated with Habitual Sleep Duration and Chronotype: Data from the UK Biobank. *Ann. Behav. Med.* **2016**, *50*, 715–726. [CrossRef]
43. Poorolajal, J.; Sahraei, F.; Mohamdadi, Y.; Doosti-Irani, A.; Moradi, L. Behavioral factors influencing childhood obesity: A systematic review and meta-analysis. *Obes. Res. Clin. Pract.* **2020**, *14*, 109–118. [CrossRef] [PubMed]
44. Madjd, A.; Taylor, M.A.; Delavari, A.; Malekzadeh, R.; Macdonald, I.A.; Farshchi, H.R. Effects of consuming later evening meal *v.* earlier evening meal on weight loss during a weight loss diet: A randomised clinical trial. *Br. J. Nutr.* **2020**, *126*, 632–640. [CrossRef] [PubMed]
45. Garaulet, M.; Gómez-Abellán, P.; Alburquerque-Béjar, J.J.; Lee, Y.C.; Ordovás, J.M.; Scheer, F.A. Timing of food intake predicts weight loss effectiveness. *Int. J. Obes.* **2013**, *37*, 604–611. [CrossRef] [PubMed]
46. Morgan, L.M.; Shi, J.-W.; Hampton, S.M.; Frost, G. Effect of meal timing and glycaemic index on glucose control and insulin secretion in healthy volunteers. *Br. J. Nutr.* **2012**, *108*, 1286–1291. [CrossRef] [PubMed]
47. Van Cauter, E.; Shapiro, E.T.; Tillil, H.; Polonsky, K.S. Circadian modulation of glucose and insulin responses to meals: Relationship to cortisol rhythm. *Am. J. Physiol. Metab.* **1992**, *262 Pt 1*, E467–E475. [CrossRef] [PubMed]
48. Romon, M.; Edme, J.L.; Boulenguez, C.; Lescroart, J.L.; Frimat, P. Circadian variation of diet-induced thermogenesis. *Am. J. Clin. Nutr.* **1993**, *57*, 476–480. [CrossRef]
49. Morris, C.J.; Garcia, J.I.; Myers, S.; Yang, J.N.; Trienekens, N.; Scheer, F.A. The Human Circadian System Has a Dominating Role in Causing the Morning/Evening Difference in Diet-Induced Thermogenesis. *Obesity* **2015**, *23*, 2053–2058. [CrossRef]
50. Hebert, J.R.; Clemow, L.; Pbert, L.; Ockene, I.S.; Ockene, J.K. Social desirability bias in dietary self-report may compromise the validity of dietary intake measures. *Int. J. Epidemiol.* **1995**, *24*, 389–398. [CrossRef]
51. Xiao, Q.; Garaulet, M.; Scheer, F.A. Meal timing and obesity: Interactions with macronutrient intake and chronotype. *Int. J. Obes.* **2019**, *43*, 1701–1711. [CrossRef]
52. Pawlowsky-Glahn, V.; Egozcue, J.J.; Tolosana-Delgado, R. *Modeling and Analysis of Compositional Data*; John Wiley & Sons: Chichester, UK, 2015.
53. Dumuid, D.; Pedišić, Z.; Palarea-Albaladejo, J.; Martín-Fernández, J.A.; Hron, K.; Olds, T. Compositional Data Analysis in Time-Use Epidemiology: What, Why, How. *Int. J. Environ. Res. Public Health* **2020**, *17*, 2220. [CrossRef]
54. Willett, W.C.; Howe, G.R.; Kushi, L.H. Adjustment for total energy intake in epidemiologic studies. *Am. J. Clin. Nutr.* **1997**, *65* (Suppl. S4), 1220S–1228S, discussion 1229S–1231S. [CrossRef] [PubMed]
55. Egozcue, J.J.; Pawlowsky-Glahn, V. Groups of Parts and Their Balances in Compositional Data Analysis. *Math. Geol.* **2005**, *37*, 795–828. [CrossRef]
56. Hron, K.; Filzmoser, P.; Thompson, K. Linear regression with compositional explanatory variables. *J. Appl. Stat.* **2012**, *39*, 1115–1128. [CrossRef]

6. Publication III

Jaeger, V., Koletzko, B., Luque, V., Gruszfeld, D., Verduci, E., Xhonneux, A., Grote, V. Eating Frequency in European Children from 1 to 96 Months of Age: Results of the Childhood Obesity Project Study. Nutrients, 15, 984 (2023).

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I contributed to this publication by conceptualizing the research question, selecting the methodology, performing the formal analysis and interpreting the results. Moreover, I have prepared the original draft, created the figures and tables, incorporated feedback from my supervisors and co-authors and I was responsible for the submission to Nutrients and the peer-review process.

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MDPI

Article

Eating Frequency in European Children from 1 to 96 Months of Age: Results of the Childhood Obesity Project Study

Vanessa Jaeger [1], Berthold Koletzko [1,*], Veronica Luque [2,3], Dariusz Gruszfeld [4], Elvira Verduci [5], Annick Xhonneux [6] and Veit Grote [1]

1 Division of Metabolism and Nutritional Medicine, Department of Pediatrics, Dr. von Hauner Children's Hospital, LMU University Hospitals, 80337 Munich, Germany
2 Paediatrics Research Unit, Universitat Rovira I Virgili-IISPV, 43201 Reus, Spain
3 Serra Hunter Fellow, Universitat Rovira I Virgili-IISPV, 43201 Reus, Spain
4 Neonatal Department and Neonatal Intensive Care Unit, Children's Memorial Health Institute, 04-730 Warsaw, Poland
5 Department of Paediatrics, Vittore Buzzi Children's Hospital, University of Milan, 20154 Milan, Italy
6 Groupe Santé CHC, Bd. Patience et Beaujonc 2—(B), 4000 Liège, Belgium
* Correspondence: berthold.koletzko@med.uni-muenchen.de

Abstract: We aimed to investigate the eating frequency (EF) in children over age, and examined the influence of country, sex, feeding mode and weight status on EF. We used the dietary data of the Childhood Obesity Project, which comprised European children from five countries. Dietary data of 3-days weighed and estimated records were available monthly from 1 to 9 and at 12-, 24-, 36-, 48-, 60-, 72- and 96-months old. Generalized additive mixed effects models were used to estimate EF trajectories with EF as outcome and applying age splines. Additionally, the models were further adjusted for country, feeding mode, sex or weight status. Data from 1244 children were analysed. EF was highest at 1 month with on average 7.3 ± 1.9 feeds per day, and fell to 5.1 ± 1.1 eating occasions at the age 96 months. Night feeding was similarly often than day feeding at 1 month but declined thereafter. Significant differences in EF were observed between countries ($p < 0.05$), with the highest EF in Poland, and between infant feeding modes, with a higher EF in breastfed than non-breastfed infants ($p < 0.05$). Sex and body weight were not associated with EF. Despite the importance of EF towards total energy intake, no association with weight status was found.

Keywords: eating frequency; feeding frequency; formula feeding; infants; children; eating behavior; overweight; meal; snack; time-of-day

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1. Introduction

Eating behavior typically includes the amount as well as the type of foods and beverages consumed. However, eating frequency (EF) as the third component of eating behavior is less frequently examined [1]. Recently, more studies have examined EF because it seems to affect appetitive, digestive and metabolic processes [2], and thus may influence health and wellbeing.

The eating pattern of adults typically consists of three main meals and one to two snacks per day [3]. In children and infants, such a structured eating behavior is less well established. EF is expected to change throughout childhood, along with marked changes of development and dietary needs: infants receive either breastmilk or formula, along with complementary feeding introduced usually between the 4th and 6th month [4]. At around 12-months-old, children are able to eat similar type of foods to the other family members [5]. With increasing age, infants and children acquire added motor, cognitive, social and language skills which may influence EF. For instance, motor skills enable the child to eat without further major help from the 2nd and 3rd year of life onwards. With increasing age, children are more interested in peers and want to be similar to them, which can affect eating behavior [6].

Few studies have examined the EF in infants and children. The available studies focus on specific eating occasions (EO) such as snacking [7–9]. However, to study regular habits, EFs of the whole day are needed. In addition, many studies in infants and children comprise of short observation periods or are of cross-sectional design [10–12]. Since the eating behavior of young children is likely to track into older ages [13] it is important to examine the longitudinal effects of the EF during the whole day, ideally prospectively from infancy to childhood.

It is not known whether factors such as sex or cultural differences influence EF. Data on associations of EF with weight status in children are inconsistent. A meta-analysis of cross-sectional studies in children and adolescents concluded that further longitudinal studies are needed [14]. It has been suggested that infant feeding mode (breastfed vs. non-breastfed) may influence the eating behavior in infants [15], but data are limited.

In this prospective longitudinal study, we studied the EF of European children during the age from 1-to-96-months-old. We aimed to examine the EF trajectory evolution with age, during the time-of-day, and potential associations with infant feeding mode (breastfeeding vs. non-breastfeeding), sex, country of residence and weight status.

2. Methods

2.1. Study Design and Population

This analysis used dietary data collected as part of the Childhood obesity Project (CHOP) initiated as a double-blinded randomized controlled trial to test the effects of early protein intake on growth and later obesity risk. Some 1678 healthy, singleton infants born at full-term were enrolled at a median age of 2 weeks and no later than the age of 8-weeks-old [16,17]. Infants received isoenergetic study formulas with different protein contents, and isoenergetic follow-on formulas with different protein contents were offered after introduction of complementary feeding until the age of 12 months. In addition to the intervention, an observational group of infants fully breastfed for at least 3 months was included. Infants were recruited from Belgium (Brussels, Liege), Germany (Munich, Nuremberg), Italy (Milano), Poland (Warsaw) and Spain (Tarragona, Reus). Written informed consent was received from all legal guardians prior to enrolment, and additional assent from children at the follow-up at 96 months of age. All time points with dietary records were included for this analysis, namely, monthly nutritional data for the first 9 months of the infant's life, and at 12-, 18-, 24-, 36-, 48-, 60-, 72- and 96-months-old. The trial was conducted in accordance with the Declaration of Helsinki, approved by the ethical committee from each study site and registered at clinical trials (NCT00338689).

2.2. Dietary Assessment

Dietary data for the aforementioned time points were collected using food records for three consecutive days, including one weekend day. The dietary records were completed as proxy reports by parents or legal caretakers. Food records from 1 to 24 months of age were weighed using food scales (Unica 66,006 food scale; Soehnle, Murrhardt, Germany). From 36 months onwards, foods and leftovers were weighed wherever possible. Otherwise, an atlas of food pictures was used to support estimation of portion sizes as described previously [18]. Breastfeeding was entered as an EO with zero calories because quantitative intake data were collected only in a subgroup of Italian children (weighing infants before and after breastfeeding).

Data were entered in a software specifically dedicated for this study, where nutritional data were based on the German food database BLS 2.3 (Bundeslebensmittelschlüssel). If needed, the database was enriched by information from product labels, manufacturers or national food databases of participating countries. Nutritional values of foods were updated for analysis to BLS 3.1. Quality checks were performed for each food record by trained nutritionists following standard operating procedures [19,20].

Misreporting of food intakes was assessed for all time points with weight measurements by comparing individual cut-off values with the ratio of reported energy intakes to estimated energy requirements as described in detail elsewhere [21]. Individual reports were identified as misreporting if the individual ratio was above or below the cut-off value, but were not excluded from the analysis as recommended [22].

2.3. Eating Frequency (EF)

EF was assessed as the total number of EOs (foods and beverages) consumed within a day. Water intake was not considered as an EO because water consumption was typically reported as a summarized intake during the day. Further beverage intakes with an energy density (amount of energy (kcal) per gram of food (g)) less than 0.01 (analogous to [23]), which typically include unsweetened tea, were not considered to avoid potential bias; i.e., some children prefer water as a beverage, and others prefer tea. Food records, in which EF was not assigned to a particular point in time (e.g., stating "during day") were excluded.

EOs were collected either as exact time-of-day (<36 months of age) or were assigned according to pre-defined categories (≥36 months of age). These categories include breakfast, lunch, supper and morning, afternoon and evening snacks as described previously [24]. Additionally, all EOs were seen as one EO when time was less than 30 min for lunch and supper, or less than 15 min for breakfast and snacks. This time differentiation was used to include foods such as dessert as one meal, which are typically eaten at lunch or supper.

2.4. Covariates

Height or length, respectively, as well as weight during the first 2 months of age and at 3, 6, 12, 24, 36, 48, 60, 72 and 96 months were assessed according to the procedures of the World Health Organization (WHO) Growth reference study [25]. Children were categorized as being normal or overweight according to the International Obesity Task force (IOTF) cut-off values, available for children aged 24 months and older [26]. Parental education was collected by questionnaire at baseline and classified according to the International Standard Classification of Education [27] into low, medium and high levels of education. Smoking in pregnancy, maternal age at child's birth, birth weight, week of birth, country and sex were collected by questionnaire. Start of complementary feeding was defined through questionnaires and dietary records.

2.5. Statistical Analysis

All subjects with dietary data from ≥one observation time point were included in the analysis. The data were divided for a main analysis and a sub-analysis. The main analysis included all dietary records except those from breastfed infants, where total energy and nutrient intakes could not be calculated. Data of breastfed infants were added to the sub analysis. EF, total energy (kcal/day) and nutrient (g/day) intakes were averaged over the three-day dietary records. Descriptive data are presented as arithmetic mean (standard deviation) for continuous variables and as counts (%) for categorical variables.

EF trajectories by age were estimated by generalized additive mixed-effects models. Mixed effects model enable the estimation of repeated measurements and allow for missing values at observation time points, whereas the generalized additive mixed model is particular suitable to model non-linear effects via splines such as age over time. Furthermore, trajectory modelling by regression analysis in contrast to crude averages allow to estimate confidence intervals by taking into account the EF correlation of the same child. A regression analysis was estimated with EF as outcome and age in months (penalized cubic spline; "cs") as exposure. Additionally, the model contained a random intercept for each study participant, a random slope over age and an autocorrelation term for age in months ("CorCAR1"). The inclusion of an autocorrelation term was necessary to consider residual serial correlation from repeated measurements of the same child. Additional models were estimated to examine country, feeding mode, sex or weight status differences in EF. These models additionally included country, feeding type (breastfeeding vs. non-breastfeeding), sex or weight status (normal weight vs. overweight) as a covariate, and an additional interaction term was added. This term contained a spline with age in months interacting with either country, feeding mode, sex or weight status. The interaction allowed different shapes between countries, sexes, feeding modes or weight groups, and additionally examined differences over time. Country or sex differences in EF were estimated for all ages, whereas IOTF cutoffs for weight status were available only from 24 months onwards [26]. Differences between feeding mode were estimated during the 1st year of life. Results of the regression analyses were plotted using predicted average values of EF over time and EF over time, country and feeding type, respectively. Models were graphically checked by inspection of residuals.

For the analysis of EF by time-of-day, EF was either examined according to EOs or by time-of-day (in hours) in percentage of total records. The observation time points were further summarized to plot daytime differences between countries and feeding modes.

Several sensitivity analyses were performed. Firstly, a sensitivity analysis was calculated by the exclusion of EOs contributing less than 5% towards total energy intake (TEI), and secondly, a sensitivity analysis was performed by exclusion of study participants in which TEI deviated more than three standard deviations from the average TEI for each time point. Thirdly, a sensitivity analysis was performed to examine the effect of continuous BMI z-score instead of cut-off values. Cut-off values facilitate the visual presentation; on the other hand, they do face some drawbacks such as loss of information and thus loss of statistical power [28]. Lastly, we examined the effect of the intervention on EF by including the intervention type as covariate variable into the model. All analyses were performed in R (R Foundation for Statistical Computing, version 4.2.2., Vienna, Austria) [29] in addition to the packages "mgcv" and "ggplot2". Results were considered as significant at $p < 0.05$.

3. Results

The main analysis of this study included 1244 healthy children from 1 to 96 months of age. About a quarter of the children were in the observational breastfed group. The sub analysis also included all children with breastfeeding, which resulted in 1370 children (Supplementary Online Material—Table S1). In addition to the number of breastfed children, the main and sub-analysis had similar study characteristics, and the characteristics of the main study population are reported in the following. Thirty-seven percent of the children each belonged to the intervention and control group. Children participated on average in 10.9 out of 17 dietary follow-up visits, with 13% of children participating in all of the 17 follow-up visits. Most children were recruited in Spain (27%), followed by Italy (25%), Germany and Poland (17% each), and Belgium (13%). Around half of the parents of participating children had a medium education level, and the average age of the mother at birth of the study child was 30 years (Supplementary Online Material—Table S2).

Dietary intakes by age is shown in Table 1. Average EF decreased from 7.3 to 5.1 from 1 to 96 months of age, whereas TEI and dietary energy density increased. TEI by weight declined from 1 to 96 months of age at time points with weight measurements. Average nutrient (g) intakes increased with age, but the distributions of energy from nutrients changed by age, with an increase of energy from carbohydrates from 44%E to 53%E from 1 to 7 months, to 49%E at 96 months. Similarly, energy from protein increased from 9%E to 17%E in children from 1 to 18 months to 15%E at 96 months. Instead, energy from fats decreased from 44%E to 36%E from 1 to 96 months. Possible misreporting of TEI was highest at 96 months (underreporting: 25%) and 12 months (overreporting: 33%). Children classified as underreporting of TEI had on average a lower EF than children classified as overreporting or children with plausible report of TEI. In contrast, children classified as overreporting had on average a higher EF than children with plausible report or underreport. Around 15% of children aged 2 to 8 years were overweight at a given time point with highest frequency at 8 years (23% of the children) and lowest frequency at 2 years of age (6% of the children).

Table 1. Dietary intake by age in participants from 1 to 96 months of age (n = 1244).

Age in Months	n	Eating Frequency	Energy		Carbohydrates		Protein		Fat		Misreporting **		Energy Density
			Kcal/day	Kcal/kg/day *	%E	g/day	%E	g/day	%E	g/day	Under-Report *	Over-Report *	
1	616	7.3 ± 1.9	526 ± 117	154 ± 42	44 ± 2	57 ± 12	9 ± 2	12 ± 4	47 ± 3	27 ± 6	—	—	0.7 ± 0.3
2	833	7.1 ± 2.2	571 ± 125	—	44 ± 2	62 ± 13	9 ± 2	13 ± 4	47 ± 3	29 ± 6	—	—	0.7 ± 0.3
3	839	6.6 ± 2.1	600 ± 136	99 ± 22	44 ± 2	65 ± 15	9 ± 2	14 ± 4	47 ± 3	31 ± 6	104 (12)	86 (10)	0.7 ± 0.3
4	808	6.4 ± 2.1	640 ± 136	—	46 ± 4	72 ± 17	9 ± 2	15 ± 5	45 ± 5	31 ± 6	—	—	0.7 ± 0.3
5	815	6.4 ± 2.2	685 ± 147	—	49 ± 6	83 ± 21	10 ± 3	18 ± 6	40 ± 6	30 ± 7	—	—	0.7 ± 0.3
6	812	6.3 ± 2.3	731 ± 176	93 ± 21	52 ± 6	94 ± 24	11 ± 3	20 ± 7	36 ± 6	29 ± 8	39 (5)	205 (25)	0.7 ± 0.3
7	784	6.1 ± 2.1	772 ± 172	—	53 ± 6	101 ± 24	13 ± 3	24 ± 8	34 ± 6	29 ± 8	—	—	0.8 ± 0.4
8	778	6.1 ± 2.1	812 ± 183	—	53 ± 6	106 ± 24	13 ± 3	26 ± 8	33 ± 6	30 ± 8	—	—	0.8 ± 0.4
9	783	6.2 ± 2.2	838 ± 200	—	53 ± 6	109 ± 26	13 ± 3	28 ± 9	33 ± 6	30 ± 9	—	—	0.8 ± 0.5
12	829	5.9 ± 1.9	896 ± 208	91 ± 22	52 ± 7	115 ± 28	15 ± 3	33 ± 10	33 ± 6	32 ± 9	47 (6)	273 (33)	1.0 ± 0.7
18	742	5.8 ± 1.7	1042 ± 246	—	50 ± 7	128 ± 32	17 ± 3	42 ± 12	33 ± 6	38 ± 10	—	—	1.2 ± 0.9
24	778	5.9 ± 1.7	1120 ± 283	90 ± 24	49 ± 7	136 ± 33	16 ± 3	45 ± 13	34 ± 6	42 ± 12	61 (8)	171 (22)	1.2 ± 0.9
36	556	5.8 ± 1.6	1234 ± 303	83 ± 22	50 ± 7	150 ± 37	16 ± 3	47 ± 12	34 ± 6	46 ± 13	36 (6)	95 (17)	1.3 ± 1.0
48	528	5.5 ± 1.4	1329 ± 304	78 ± 20	50 ± 7	162 ± 35	15 ± 3	50 ± 13	35 ± 6	51 ± 14	29 (5)	73 (14)	1.4 ± 1.1
60	483	5.3 ± 1.4	1404 ± 325	72 ± 19	50 ± 7	172 ± 40	15 ± 3	52 ± 13	35 ± 6	54 ± 14	48 (10)	57 (12)	1.4 ± 1.0
72	520	5.2 ± 1.2	1481 ± 325	68 ± 17	50 ± 6	183 ± 39	15 ± 3	55 ± 13	35 ± 6	57 ± 14	60 (12)	43 (8)	1.4 ± 1.0
96	444	5.1 ± 1.1	1596 ± 364	57 ± 16	49 ± 7	191 ± 42	15 ± 3	60 ± 15	36 ± 6	63 ± 18	109 (25)	14 (3)	1.5 ± 1.1

Values are presented as mean ± standard deviation or as counts (percentage). * Weight in kg was not collected at months 2, 4, 5, 7, 8, 9 and 18; ** Misreporting was calculated by the ratio of reported energy intakes and estimated energy requirements [21]. Abbreviation: %E = Nutrient intake in percentage of energy intake.

The average predicted EF by age is shown in Figure 1. The difference of the predicted EF records (Figure 1A) compared to the raw average EF (Table 1) were very similar. From the 1st to 3rd months a steep decline in EF was observed; complementary feeding was on average introduced at the end of the 4th month (18.9 weeks). Thereafter, the decrease in EF was decelerated or constant, whereas EF started to increase slightly with 24 to 36 months and declined thereafter. The calculated EFs by country are shown in Fig 1B. Polish children showed the highest average EF due to a regular consumption of tea in the first months. For all countries, the highest EF was seen at 1 months, with a steep decline thereafter, and the lowest EF at 96 months of age. The most pronounced differences in EF between countries were seen during the first months of life, while EF were more similar at later ages. Differences between breastfed and non-breastfed infants during the 1st year of life are shown in Figure 1C. In total, 536 infants were breastfed at a given time point (Supplementary Online Material—Table S1). Infants were grouped as breastfed as long as breastfeeding persisted and otherwise grouped as non-breastfed along with formula-fed infants from the main analysis. Infants showed a higher EF for the duration of breastfeeding than non-breastfed infants, particularly pronounced from around 6 months onwards. From that time point onwards, breastfed infants were fed on average two times more often than non-breastfed infants. Sex and body weight status were not associated with EF ($p > 0.05$).

EF by time of the day in children from 1 to 24 months is shown in Figure 2. At 1 month of age, the feeding distribution is almost equally distributed over 24 h. From 2 months onwards, the frequency of feedings during nighttime decreased steadily, and from around 6 months onwards peaks of feeding/eating appear at typical times during morning, midday and evening (Figure 2). EF by daytime showed earlier peaks of preferred meal times in non-breastfed compared to breastfed infants. Nighttime feeding was prevalent longer in breastfed infants (Figure 3). From around 4 months onwards, breastfed infants were fed twice as often during the night (10 pm to 5 am) than non-breastfed infants. With the introduction of complementary feeding, country differences in daytime EF were observed (Figure 4). The distribution is equally distributed during daytime, especially in Poland, though also in Germany. However, the eating distribution between 12–24 months in Germany is more pronounced during the afternoon and evening. Instead, in Belgium, Italy and Spain, there are noticeable peaks of EF. In Belgium and Italy, the first peak is visible between 7 to 8 am followed by noon, 4 pm and 7 pm. In Spain, all peaks were shifted 1 or 2 h later. Children aged 3 to 8 years showed a regular eating pattern with three meals (90% of the children; 8% of the children consumed more than three meals) and a regular snacking pattern with one, two or more than two snacks (28%, 47% and 24% of the children, respectively). Almost all children consumed at least one snack during the afternoons (95%), 62% of the children consumed at least one snack during the mornings and some during the evenings (18%). Children in Poland and Germany consumed (two or more than two times) a snack during the mornings (Poland: 43% and Germany: 20%) and the afternoons (Poland: 66% and Germany: 42%) more frequently.

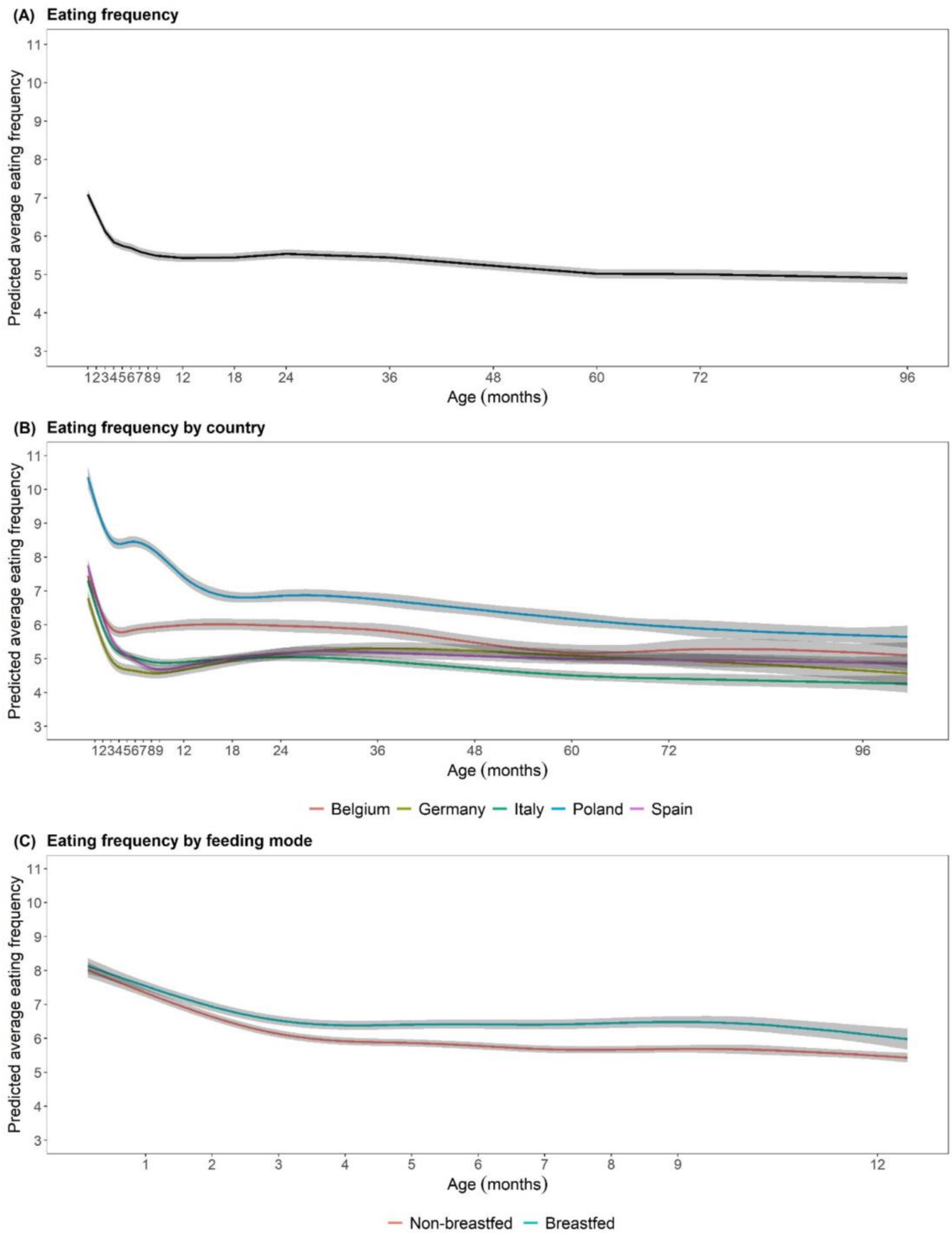

Figure 1. (**A**) Predicted average eating frequency by age (n = 1244). (**B**) Predicted average eating frequency by age and country ($n_{Belgium}$ = 163, $n_{Germany}$ = 214; n_{Italy} = 314; n_{Poland} = 214; n_{Spain} = 339). (**C**) Predicted average eating frequency by feeding mode (n = 1370, whereas 536 infants were breastfed at a given time point). All models are estimated by generalized additive mixed-effects models with EF as outcome and a spline for age in months, a spline for age in months and country and a spline for age in months and feeding mode, respectively, as exposure.

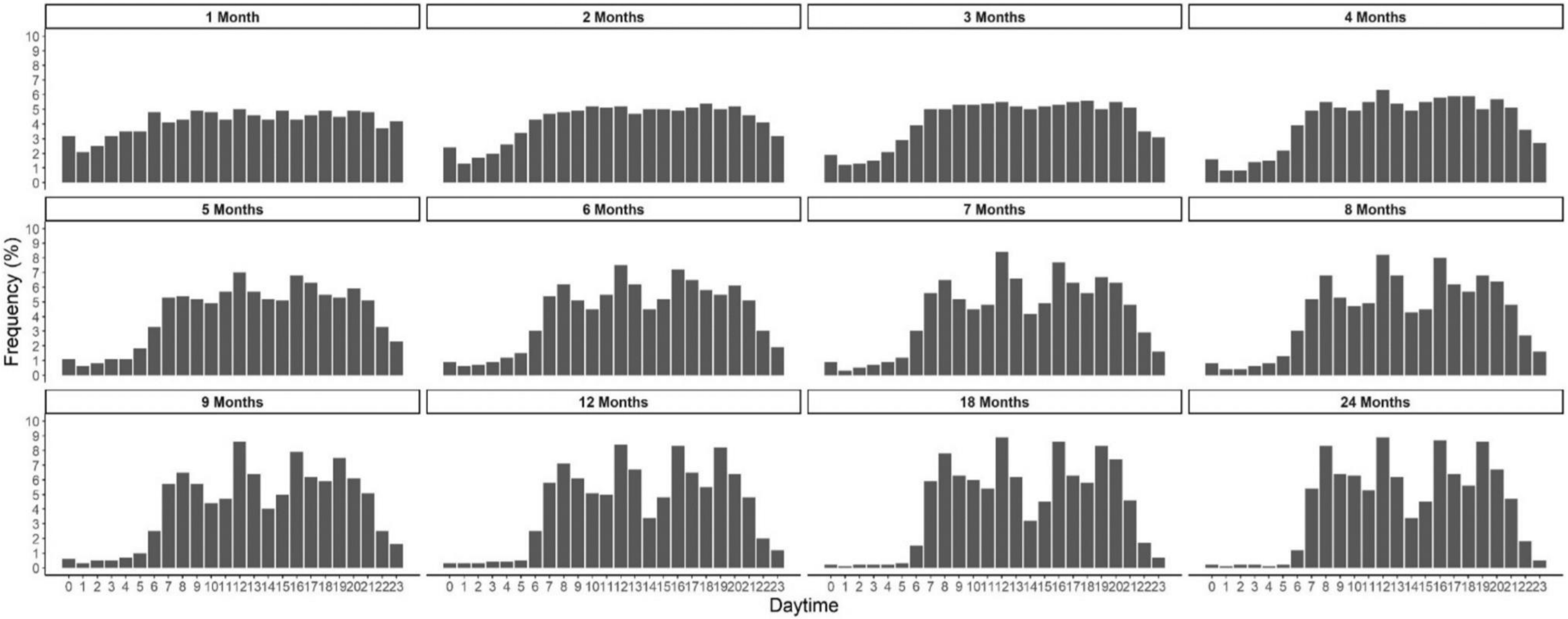

Figure 2. Eating frequency by time-of-day in percentage from total records and by age in months (n = 1244).

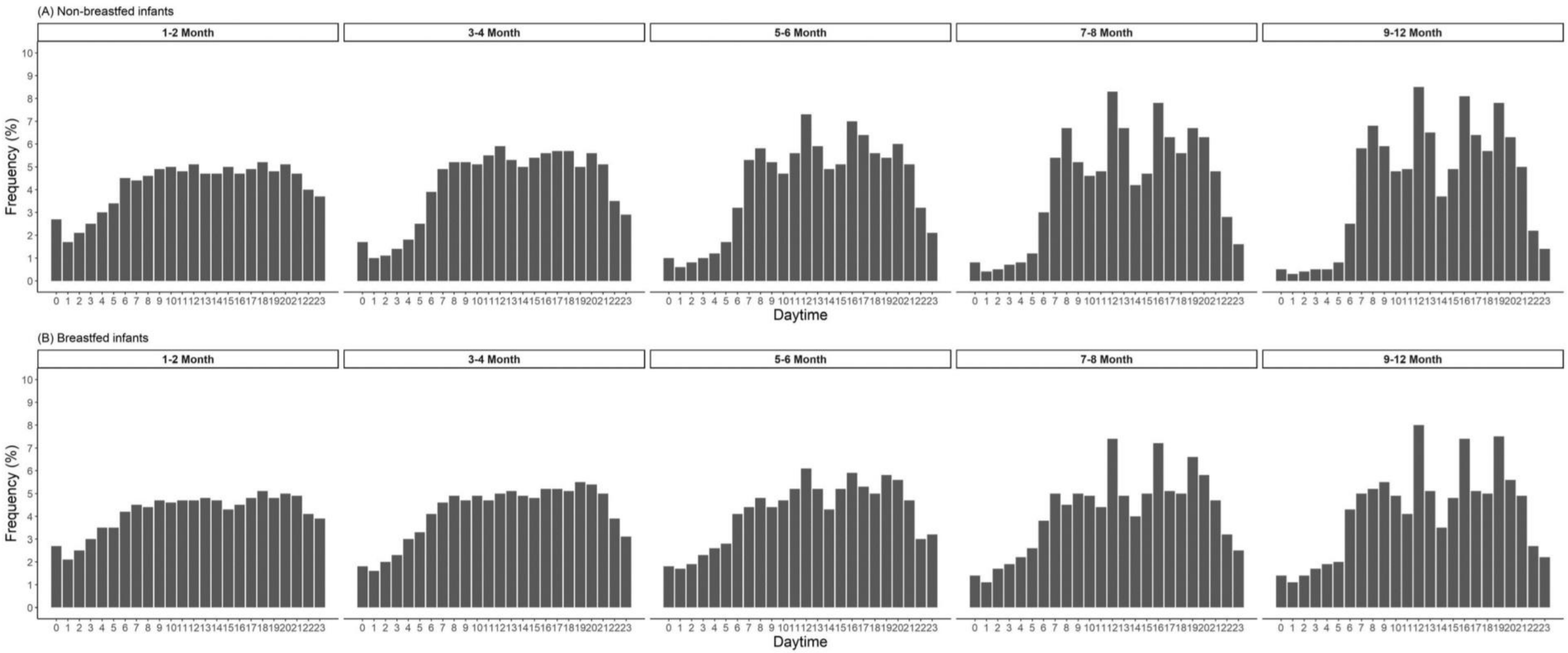

Figure 3. Eating frequency by time-of-day in percentage from total records and by age groups and feeding mode (n = 1370 infants, whereas 536 infants were breastfed at given time point).

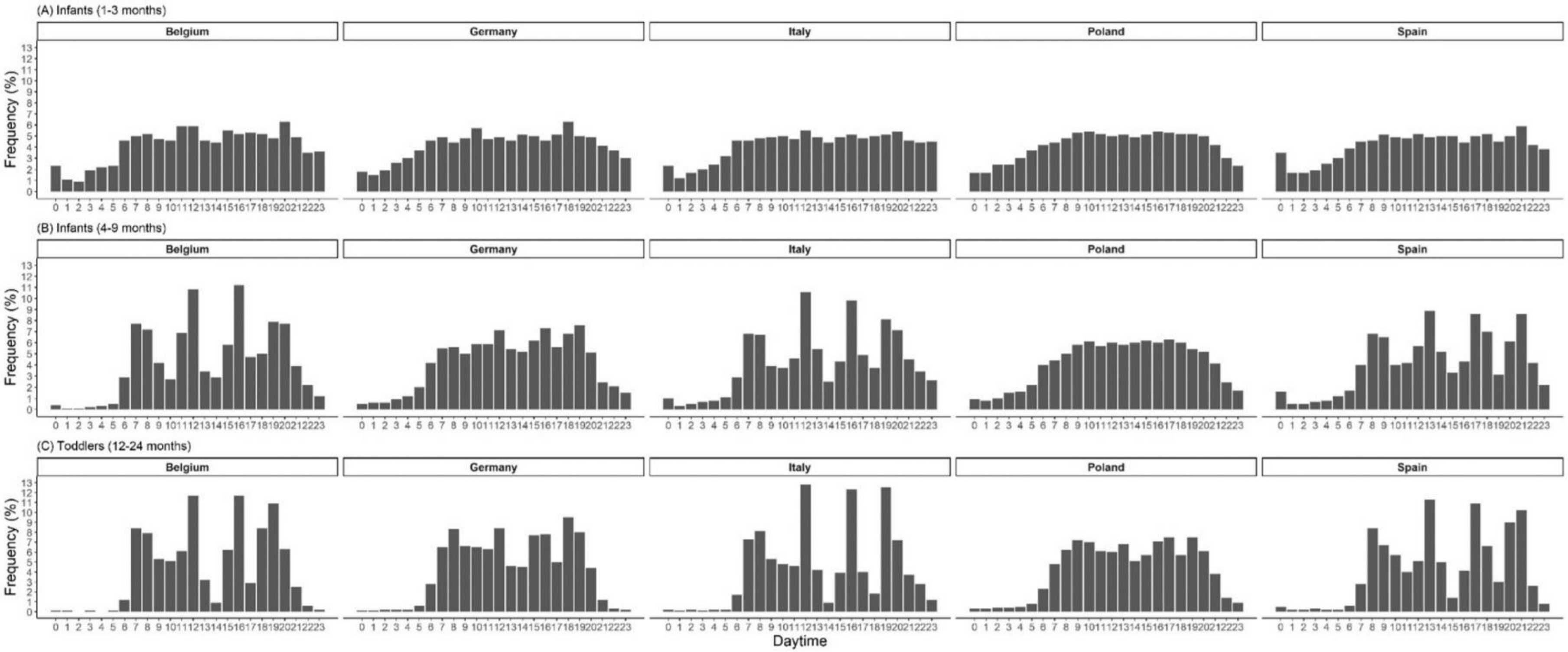

Figure 4. Eating frequency by time-of-day in percentage from total records and by age groups (**A–C**) and country ($n_{Belgium}$ = 163, $n_{Germany}$ = 214; n_{Italy} = 314; n_{Poland} = 214; n_{Spain} = 339).

Several sensitivity analyses were carried out. One sensitivity analysis was performed by exclusion of dietary records deviating at least three standard deviations from the average TEI. The EF by age was similar to the main analysis. The second sensitivity analysis was performed by exclusion of EOs contributing only 5% or less towards TEI. The EF was slightly lower compared to the main analysis but was more similar towards 96 months of age (Supplementary Online Material—Figure S1). As a third sensitivity analysis, we examined BMI as a continuous variable, but found similar results to BMI as cut-off values. Lastly, we examined the effect of the original intervention on EF, but found a statistically significant higher EF over time only in the observational breastfed arm ($p < 0.05$). Particularly, the EF was elevated in the observational arm during the first year of life (Supplementary Online Material—Figure S2).

4. Discussion

This study examined the dietary data of 1244 healthy children from five European countries and from 1 to 96 months of age. We assessed the average EF by age and found the highest EF at 1 month followed by a steep decline until complementary feeding was introduced, and a stable-to-slightly decreasing EF until 96 months with the lowest EF. EF trajectories were shaped by feeding mode, with a higher EF in breastfed than non-breastfed infants, and by country, with the highest EF in Poland. Depiction of EF by daytime revealed a change in EF during the first 9 months of life from a rather uniform distribution at 1 months to peaks of preferred meal times from 4 months onwards. Daytime differences in EF were visible between countries as soon as complementary feeding was introduced. Breastfed infants were more often and longer fed during the night than non-breastfed infants. No differences in EF between children with different weight status or between sexes were found.

The WHO recommends to breastfeed on demand during the first about 6 months of life, which means to breastfeed whenever the infants signals the desire to drink—day or night [30]. Feeding on demand is also applicable for formula-fed infants [31]. In our study population, we found a slightly higher frequency of day compared to night feeding in the first month. The night-feeding frequency dropped from month to month, particularly in non-breastfed infants. A study in breastfed infants found night feeding to be higher before midnight and after 4 am [32], similarly to our study. We found the highest average feeding frequency to be at 1 month. Thereafter, the feeding frequency declined rapidly until 3 months, with a rather constant feeding frequency until 6 months. A similar feeding trajectory was found in breastfed Australian infants [33]. In this study, the authors additionally examined the breastfeeding duration and total 24 h milk intake, and found that the duration of feeding decreased steadily but the total milk intake remained constant. They concluded that breastfeeding becomes more efficient between the 1st and 3rd month of lactation, which may be related to an increasing stomach capacity of the infant. The feeding frequency in Polish infants was noticeable higher than in infants from remaining countries due to regular tea consumption from the 1st month onwards. Some institutions such as the WHO [34] recommend the addition of extra fluids, preferably water, only to non-breastfed children, as formula feeding compared to breastfeeding might not provide enough liquids. The consumption of tea or sugary drinks, however, should be limited due to its low nutrient density [35].

With introduction of complementary feeding, the WHO recommends to practice responsive feeding [5,35]. The principle of responsive feeding is based on reciprocity between the caregiver and the child. In particular, it means to feed children depending on the hunger and satiety cues of the child. The caregiver responds to these cues in an emotionally supportive and age-appropriate way, and the child experiences a predictable response to these signals [36]. Responsive feeding supports the child to self-regulate food intake and reduces overeating [37]. The WHO reported theoretical calculations as a benchmark for an appropriate number of feedings in children aged 6 to 23 months. These are four to five meals daily and one–two snacks if desired for formula-fed children, depending on the energy density and usual amounts consumed [35]. A meal may consist of milk feeds, other foods or a combination of both. In breastfed infants, the number of feeds from breastfeeding needs to be considered and were subtracted by the WHO from the recommended number of meals. Thus, the WHO [5] reported a benchmark of two–three meals at 6–8 months and of three–four meals at 9–11 months, depending on energy density and usual amounts consumed. Additionally, one–two snacks may be consumed if desired. Comparison of our results with the calculations by the WHO are difficult since WHO separated between meals and snacks. Nonetheless, the average energy density and energy requirements on which the required meals are calculated are comparable to the WHO.

In our study population, complementary feeding was on average introduced at the end of the 4th month. Changes in eating behavior were visible in the 24 h-food distribution as peaks of preferred meal timing started to appear. A cross-sectional study in American infants and children examined EF based on a meal and snack pattern instead of times, and found a meal and snack pattern emerging in the age group of 7–8-month-old infants and was established in 9–11-month-olds [38]. When looking in our analysis at the 24-h food distribution stratified by country, we found large country differences starting as early as from the 4th month onwards. The country differences we observed might suggest that the cultural component seems to be more important than purely physiological aspects to determine time and frequency of eating, as previously proposed by Chiva [3].

We found a higher EF in breastfed compared to non-breastfed infants. Complementary feeding was introduced later in breastfed (mean 5th month) compared to formula-fed infants (mean 4th month). However, this does not entirely explain the difference in EF, as the difference in EF was stable until the end of the 1st year of life. The higher EF in breastfed infants was particularly visible during nighttime, and is likely to result in different sleeping patterns between breastfed and non-breastfed infants during the entire lactation period.

Most studies examining EF in children focused on children aged 2 years or older. The average daily EF in Portuguese children aged 3–9 years was 5.7, with on average three meals and three snacks [10]; in New Zealand children aged 2 years, the average EF was 5.5, with most children eating four–seven times per day [39], and in British children aged 4–10 years, the daily EF was 4.9 times per day [11]. The EF of these studies are comparable to our results; however, comparison is hampered due to different definitions of an EO and due to summarized age ranges as EOs decrease with age. Furthermore, EF from above studies were examined in countries not examined in our analysis.

We observed a decrease in average EF throughout the examined age range, as well as a decrease in variation with the smallest variation at 8 years (SD = 1.1). A possible explanation for the observed decrease in EF might be the improved gastric capacity and the decreased requirements for energy and nutrients per kilogram body weight [40,41]. Furthermore, the EF between children becomes more and more similar. A possible reason might be that with increasing age, the day becomes more structured and eating more institutionalized, with fixed times for meals and snacks in day care or school. Additionally, in older children, peers are becoming more important, and the eating behavior of peers might influence the eating behavior of the child to a greater extend [6].

A further major influencing factor of EF might be by the rate of gastrointestinal motility as gastric motility is linked to the sensations of hunger, appetite and satiety [42,43]. Gastric emptying, defined as the rate at which gastric contents are delivered to the duodenum, is regulated, for instance, by the volume or energy density of the ingested foods [43]. Thus, large EOs or EOs with a high energy density may slow down sensations of hunger and affecting EF. In addition, it has been found that gastric emptying is faster in breastfed than formula-fed infants [44,45]. A possible explanation might be the different nutrient composition. For instance, the milk fat content in breastmilk seems to increase throughout a feeding, whereas in infant formula the composition is uniform throughout a feeding [44,46].

We examined how EF is affected by weight status and sex differences, and found neither differences in EF between children with overweight and normal weight nor between boys and girls. A meta-analysis [14] of cross-sectional studies examining EF and overweight in children and adolescences found a beneficial effect of EF (highest vs. lowest EF category) on body weight, which was only significant in boys. However, the results of the meta-analysis are hampered by a high heterogeneity between study results and of the cross-sectional design of the studies included. In contrast, we performed a longitudinal study, which may reduce the risk of chance findings. We used EF as outcome and weight status as exposure variable, which is in reverse to previous studies and further limits comparison. Our results suggest that EF is of minor relevance in the overweight and obesity development. Future studies examining the overweight and obesity risk should focus on other dietary aspects than on the EF in children.

5. Strength and Limitations

Our study benefited from the long prospective observation period from 1 to 96 months of age, which provided a detailed EF trajectory, particularly during the 1st year of life. Furthermore, the longitudinal study design allows not only the capacity to examine between-subject variations, but also within-subject variations, which strengthens evidence. We used 3-days weighed and estimated dietary records, which allowed a more accurate description of dietary data than other methods. Our study population comprised children from five European countries. This increased, on the one hand, the generalizability of our results, and additionally enabled the capacity to study country and cultural differences, respectively. On the other hand, it limits study comparison by age due to the different definitions used in younger and older children. A further limitation is that beverages could not be examined on their own. Beverages may influence metabolic and digestive processes differently than solid foods, and may have a different influence on eating behavior and health outcomes [1]. However, beverages are difficult to collect as beverages are often consumed over longer time periods, and are thus difficult to assign to one particular point in time. Furthermore, it may be easier to forget to report beverages. Due to these reasons, we did not perform a trajectory analysis of beverages only. Future studies need to focus on beverages to explicitly collect beverage consumption. Lastly, the frequency of breastfeeding, but not the volume of breastfeeding, was reported. Due to this, we could not validate the intake in breastfed infants, and we thus analysed breastfeeding only in a sub-analysis. The volume of breastfeeding is usually received by weighing the infant before and after the feeding. This method was not possible to apply in a large study such as ours, and was performed only in a subgroup of Italian children.

6. Conclusions

This study examined EF in European infants and children from 1 to 96 months of age and found a varying EF as children grow as well as between countries, feeding mode and during daytime. These findings, particularly during the first months of life, are relevant for health care professionals in supporting parents and their infants. Although EF is an important contributor towards TEI, it did not affect overweight risk in children. It seems that the cultural differences found in the EF and its distribution throughout the day may be more important than purely physiological factors.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/nu15040984/s1, Table S1: Participant flow of enrolled infants with dietary data at baseline and follow-ups; Table S2: Characteristics of participating children and their parents; Figure S1: Average predicted eating frequency by sensitivity analyses and age. All models are estimated by generalized additive mixed effects models with EF as outcome and a spline for age in months; Figure S2: Average predicted eating frequency by intervention ($n_{control}$ = 463; $n_{experimental}$ = 469; $n_{observational}$ = 438). Statistical significant differences over age only in the observational arm (breastfed infants). No statistical significant difference between experimental and control group. Model is estimated by generalized additive mixed effects models with EF as outcome and a spline for age in months and age in months and intervention group.

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Informed Consent Statement: Parents and children gave their written informed consent.

Data Availability Statement: Data available on request due to ethical restrictions.

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References

1. Dietary Guidelines Advisory Committee. Scientific Report of the 2020 Dietary Guidelines Advisory Committee: Advisory Report to the Secretary of Agriculture and the Secretary of Health and Human Services. U.S. Department of Agriculture, Agricultural Research Service: Washington, DC, USA, 2020.
2. Leidy, H.J.; Campbell, W.W. The effect of eating frequency on appetite control and food intake: Brief synopsis of controlled feeding studies. *J. Nutr.* **2011**, *141*, 154–157. [CrossRef] [PubMed]
3. Chiva, M. Cultural aspects of meals and meal frequency. *Br. J. Nutr.* **1997**, *77* (Suppl. 1), S21–S28. [CrossRef] [PubMed]
4. EFSA Panel on Nutrition Novel Foods Food Allergens. Appropriate age range for introduction of complementary feeding into an infant's diet. *EFSA J.* **2019**, *17*, e05780. [CrossRef]
5. Pan American Health Organization; World Health Organization. *Guiding Principles for Complementary Feeding of the Breastfed Child*; Pan American Health Organization: Washington, DC, USA, 2003.
6. Fisher, J.; Lumeng, J.; Miller, L.; Smethers, A.; Lott, M. *Evidence-Based Recommendations and Best Practices for Promoting Healthy Eating Behaviors in Children 2 to 8 Years*; Healthy Eating Research: Durham, NC, USA, 2021.
7. Vatanparast, H.; Islam, N.; Patil, R.P.; Shafiee, M.; Smith, J.; Whiting, S. Snack Consumption Patterns among Canadians. *Nutrients* **2019**, *11*, 1152. [CrossRef]
8. Xue, H.; Maguire, R.L.; Liu, J.; Kollins, S.H.; Murphy, S.K.; Hoyo, C.; Fuemmeler, B.F. Snacking frequency and dietary intake in toddlers and preschool children. *Appetite* **2019**, *142*, 65–70. [CrossRef]
9. Fayet-Moore, F.; Peters, V.; McConnell, A.; Petocz, P.; Eldridge, A.L. Weekday snacking prevalence, frequency, and energy contribution have increased while foods consumed during snacking have shifted among Australian children and adolescents: 1995, 2007 and 2011–12 National Nutrition Surveys. *Nutr. J.* **2017**, *16*, 65. [CrossRef]

10. Vilela, S.; Correia, D.; Severo, M.; Oliveira, A.; Torres, D.; Lopes, C. Eating frequency and weight status in Portuguese children aged 3–9 years: Results from the cross-sectional National Food, Nutrition and Physical Activity Survey 2015–2016. *Public Health Nutr.* **2019**, *22*, 2793–2802. [CrossRef]
11. Murakami, K.; Livingstone, M.B. Associations of eating frequency with adiposity measures, blood lipid profiles and blood pressure in British children and adolescents. *Br. J. Nutr.* **2014**, *111*, 2176–2183. [CrossRef]
12. Syrad, H.; Llewellyn, C.H.; Johnson, L.; Boniface, D.; Jebb, S.A.; van Jaarsveld, C.H.; Wardle, J. Meal size is a critical driver of weight gain in early childhood. *Sci. Rep.* **2016**, *6*, 28368. [CrossRef]
13. Luque, V.; Escribano, J.; Closa-Monasterolo, R.; Zaragoza-Jordana, M.; Ferré, N.; Grote, V.; Koletzko, B.; Totzauer, M.; Verduci, E.; ReDionigi, A.; et al. Unhealthy Dietary Patterns Established in Infancy Track to Mid-Childhood: The EU Childhood Obesity Project. *J. Nutr.* **2018**, *148*, 752–759. [CrossRef]
14. Kaisari, P.; Yannakoulia, M.; Panagiotakos, D.B. Eating frequency and overweight and obesity in children and adolescents: A meta-analysis. *Pediatrics* **2013**, *131*, 958–967. [CrossRef] [PubMed]
15. Sievers, E.; Oldigs, H.-D.; Santer, R.; Schaub, J. Feeding Patterns in Breast-Fed and Formula-Fed Infants. *Ann. Nutr. Metab.* **2002**, *46*, 243–248. [CrossRef]
16. Koletzko, B.; von Kries, R.; Closa, R.; Escribano, J.; Scaglioni, S.; Giovannini, M.; Beyer, J.; Demmelmair, H.; Gruszfeld, D.; Dobrzanska, A.; et al. Lower protein in infant formula is associated with lower weight up to age 2 y: A randomized clinical trial. *Am. J. Clin. Nutr.* **2009**, *89*, 1836–1845. [CrossRef] [PubMed]
17. Weber, M.; Grote, V.; Closa-Monasterolo, R.; Escribano, J.; Langhendries, J.-P.; Dain, E.; Giovannini, M.; Verduci, E.; Gruszfeld, D.; Socha, P.; et al. Lower protein content in infant formula reduces BMI and obesity risk at school age: Follow-up of a randomized trial. *Am. J. Clin. Nutr.* **2014**, *99*, 1041–1051. [CrossRef]
18. Pawellek, I.; Grote, V.; Theurich, M.; Closa-Monasterolo, R.; Stolarczyk, A.; Verduci, E.; Xhonneux, A.; Koletzko, B. Factors associated with sugar intake and sugar sources in European children from 1 to 8 years of age. *Eur. J. Clin. Nutr.* **2017**, *71*, 25–32. [CrossRef]
19. Luque, V.; Escribano, J.; Mendez-Riera, G.; Schiess, S.; Koletzko, B.; Verduci, E.; Stolarczyk, A.; Martin, F.; Closa-Monasterolo, R. Methodological approaches for dietary intake assessment in formula-fed infants. *J. Pediatr. Gastroenterol. Nutr.* **2013**, *56*, 320–327. [CrossRef] [PubMed]
20. Verwied-Jorky, S.; Schiess, S.; Luque, V.; Grote, V.; Scaglioni, S.; Vecchi, F.; Martin, F.; Stolarczyk, A.; Koletzko, B.; European Childhood Obesity, P. Methodology for longitudinal assessment of nutrient intake and dietary habits in early childhood in a transnational multicenter study. *J. Pediatr. Gastroenterol. Nutr.* **2011**, *52*, 96–102. [CrossRef]
21. Gomes, D.; Luque, V.; Xhonneux, A.; Verduci, E.; Socha, P.; Koletzko, B.; Berger, U.; Grote, V. A simple method for identification of misreporting of energy intake from infancy to school age: Results from a longitudinal study. *Clin. Nutr.* **2018**, *37*, 1053–1060. [CrossRef]
22. European Food Safety Authority. Guidance on the EU Menu methodology. *EFSA J.* **2014**, *12*, 3944. [CrossRef]
23. Appelhans, B.M.; Bleil, M.E.; Waring, M.E.; Schneider, K.L.; Nackers, L.M.; Busch, A.M.; Whited, M.C.; Pagoto, S.L. Beverages contribute extra calories to meals and daily energy intake in overweight and obese women. *Physiol. Behav.* **2013**, *122*, 129–133. [CrossRef]
24. Jaeger, V.; Koletzko, B.; Luque, V.; Ferré, N.; Gruszfeld, D.; Gradowska, K.; Verduci, E.; Zuccotti, G.V.; Xhonneux, A.; Poncelet, P.; et al. Distribution of energy and macronutrient intakes across eating occasions in European children from 3 to 8 years of age: The EU Childhood Obesity Project Study. *Eur. J. Nutr.* **2022**, *62*, 165–174. [CrossRef]
25. de Onis, M.; Onyango, A.W.; Borghi, E.; Siyam, A.; Nishida, C.; Siekmann, J. Development of a WHO growth reference for school-aged children and adolescents. *Bull. World Health Organ.* **2007**, *85*, 660–667. [CrossRef] [PubMed]
26. Cole, T.J.; Lobstein, T. Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. *Pediatr. Obes.* **2012**, *7*, 284–294. [CrossRef] [PubMed]
27. UNESCO United Nations Educational, Scientific and Cultural Organization. *Advances in Cross-National Comparison: A European Working Book for Demographic and Socio-Economic Variables*; International standard classification of education, ISCED 1997; Springer Science & Business Media: Berlin/Heidelberg, Germany, 2003.
28. Altman, D.G.; Royston, P. The cost of dichotomising continuous variables. *BMJ* **2006**, *332*, 1080. [CrossRef] [PubMed]
29. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria; Available online: https://www.R-project.org/ (accessed on 4 November 2022).
30. World Health Organization. Breastfeeding Recommendations. Available online: https://www.who.int/health-topics/breastfeeding#tab=tab_2 (accessed on 22 December 2022).
31. Entwistle, F.M. *The Evidence and Rationale for the UNICEF UK Baby Friendly Initiative Standards*; UNICEF UK: London, UK, 2013.
32. Hörnell, A.; Aarts, C.; Kylberg, E.; Hofvander, Y.; Gebre-Medhin, M. Breastfeeding patterns in exclusively breastfed infants: A longitudinal prospective study in Uppsala, Sweden. *Acta Paediatr.* **1999**, *88*, 203–211. [CrossRef]
33. Kent, J.C.; Hepworth, A.R.; Sherriff, J.L.; Cox, D.B.; Mitoulas, L.R.; Hartmann, P.E. Longitudinal changes in breastfeeding patterns from 1 to 6 months of lactation. *Breastfeed. Med.* **2013**, *8*, 401–407. [CrossRef]
34. National Academies of Sciences, Engineering, and Medicine. *Feeding Infants and Children from Birth to 24 Months: Summarizing Existing Guidance*; The National Academies Press: Washington, DC, USA, 2020. [CrossRef]

35. World Health Organization. *Guiding Principles for Feeding Non-Breastfed Children 6–24 Months of Age*; World Health Organization: Geneva, Switzerland, 2005.
36. Lutter, C.K.; Grummer-Strawn, L.; Rogers, L. Complementary feeding of infants and young children 6 to 23 months of age. *Nutr. Rev.* **2021**, *79*, 825–846. [CrossRef]
37. Redsell, S.A.; Edmonds, B.; Swift, J.A.; Siriwardena, A.N.; Weng, S.; Nathan, D.; Glazebrook, C. Systematic review of randomised controlled trials of interventions that aim to reduce the risk, either directly or indirectly, of overweight and obesity in infancy and early childhood. *Matern. Child Nutr.* **2016**, *12*, 24–38. [CrossRef]
38. Skinner, J.D.; Ziegler, P.; Pac, S.; Barvara, D. Meal and Snack Patterns of Infants and Toddlers. *J. Am. Diet. Assoc.* **2004**, *104*, 56–70. [CrossRef]
39. Taylor, R.W.; Iosua, E.; Heath, A.M.; Gray, A.R.; Taylor, B.J.; Lawrence, J.A.; Hanna, M.; Cameron, S.L.; Sayers, R.; Galland, B. Eating frequency in relation to BMI in very young children: A longitudinal analysis. *Public Health Nutr.* **2017**, *20*, 1372–1379. [CrossRef]
40. EFSA Panel on Dietetic Products Nutrition and Allergies. Scientific Opinion on nutrient requirements and dietary intakes of infants and young children in the European Union. *EFSA J.* **2013**, *11*, 3408. [CrossRef]
41. Wang, Z. High ratio of resting energy expenditure to body mass in childhood and adolescence: A mechanistic model. *Am. J. Hum. Biol.* **2012**, *24*, 460–467. [CrossRef] [PubMed]
42. Gallagher, T.K.; Geoghegan, J.G.; Baird, A.W.; Winter, D.C. Implications of altered gastrointestinal motility in obesity. *Obes. Surg.* **2007**, *17*, 1399–1407. [CrossRef] [PubMed]
43. Janssen, P.; Vanden Berghe, P.; Verschueren, S.; Lehmann, A.; Depoortere, I.; Tack, J. Review article: The role of gastric motility in the control of food intake. *Aliment. Pharmacol. Ther.* **2011**, *33*, 880–894. [CrossRef] [PubMed]
44. Camps, G.; van Eijnatten, E.J.M.; van Lieshout, G.A.A.; Lambers, T.T.; Smeets, P.A.M. Gastric Emptying and Intragastric Behavior of Breast Milk and Infant Formula in Lactating Mothers. *J. Nutr.* **2021**, *151*, 3718–3724. [CrossRef]
45. Van Den Driessche, M.; Peeters, K.; Marien, P.; Ghoos, Y.; Devlieger, H.; Veereman-Wauters, G. Gastric emptying in formula-fed and breast-fed infants measured with the 13C-octanoic acid breath test. *J. Pediatr. Gastroenterol. Nutr.* **1999**, *29*, 46–51. [CrossRef]
46. Forsum, E.; Lonnerdal, B. Variation in the contents of nutrients of breast milk during one feeding. *Nutr. Rep. Int.* **1979**, *19*, 815–820.

References

1. Jebeile H, Kelly AS, O'Malley G, Baur LA (2022) Obesity in children and adolescents: epidemiology, causes, assessment, and management. Lancet Diabetes Endocrinol 10 (5):351-365. http://dx.doi.org/10.1016/s2213-8587(22)00047-x

2. Schienkiewitz A, Brettschneider A-K, Damerow S, Schaffrath Rosario A (2018) Overweight and obesity among children and adolescents in Germany. Results of the cross-sectional KiGGS Wave 2 study and trends. Journal of Health Monitoring 3 (1):15-22. http://dx.doi.org/10.17886/RKI-GBE-2018-022

3. Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128·9 million children, adolescents, and adults (2017). Lancet 390 (10113):2627-2642. http://dx.doi.org/10.1016/s0140-6736(17)32129-3

4. Verduci E, Bronsky J, Embleton N, Gerasimidis K, Indrio F, Köglmeier J, de Koning B, Lapillonne A, Moltu SJ, Norsa L, Domellöf M (2021) Role of Dietary Factors, Food Habits, and Lifestyle in Childhood Obesity Development: A Position Paper From the European Society for Paediatric Gastroenterology, Hepatology and Nutrition Committee on Nutrition. J Pediatr Gastroenterol Nutr 72 (5):769-783. http://dx.doi.org/10.1097/mpg.0000000000003075

5. Beccuti G, Monagheddu C, Evangelista A, Ciccone G, Broglio F, Soldati L, Bo S (2017) Timing of food intake: Sounding the alarm about metabolic impairments? A systematic review. Pharmacol Res 125 (Pt B):132-141. http://dx.doi.org/10.1016/j.phrs.2017.09.005

6. Manoogian ENC, Chaix A, Panda S (2019) When to Eat: The Importance of Eating Patterns in Health and Disease. J Biol Rhythms 34 (6):579-581. http://dx.doi.org/10.1177/0748730419892105

7. Garaulet M, Gómez-Abellán P, Alburquerque-Béjar JJ, Lee YC, Ordovás JM, Scheer FAJL (2013) Timing of food intake predicts weight loss effectiveness. Int J Obes (Lond) 37 (4):604-611. http://dx.doi.org/10.1038/ijo.2012.229

8. Flanagan A, Bechtold DA, Pot GK, Johnston JD (2021) Chrono-nutrition: From molecular and neuronal mechanisms to human epidemiology and timed feeding patterns. J Neurochem 157 (1):53-72. http://dx.doi.org/https://doi.org/10.1111/jnc.15246

9. Covassin N, Singh P, Somers VK (2016) Keeping Up With the Clock: Circadian Disruption and Obesity Risk. Hypertension (Dallas, Tex : 1979) 68 (5):1081-1090. http://dx.doi.org/10.1161/HYPERTENSIONAHA.116.06588

10. Mendoza J (2007) Circadian clocks: setting time by food. J Neuroendocrinol 19 (2):127-137. http://dx.doi.org/10.1111/j.1365-2826.2006.01510.x

11. Morgan LM, Shi JW, Hampton SM, Frost G (2012) Effect of meal timing and glycaemic index on glucose control and insulin secretion in healthy volunteers. Br J Nutr 108 (7):1286-1291. http://dx.doi.org/10.1017/s0007114511006507

12. Van Cauter E, Shapiro ET, Tillil H, Polonsky KS (1992) Circadian modulation of glucose and insulin responses to meals: relationship to cortisol rhythm. Am J Physiol 262 (4 Pt 1):E467-475. http://dx.doi.org/10.1152/ajpendo.1992.262.4.E467

13. McHill AW, Phillips AJ, Czeisler CA, Keating L, Yee K, Barger LK, Garaulet M, Scheer FA, Klerman EB (2017) Later circadian timing of food intake is associated with increased body fat. Am J Clin Nutr 106 (5):1213-1219. http://dx.doi.org/10.3945/ajcn.117.161588

14. Madjd A, Taylor MA, Delavari A, Malekzadeh R, Macdonald IA, Farshchi HR (2020) Effects of consuming later evening meal versus earlier evening meal on weight loss during a Weight Loss Diet: a randomized clinical trial. Br J Nutr:1-25. http://dx.doi.org/10.1017/s0007114520004456

15. Pot GK, Almoosawi S, Stephen AM (2016) Meal irregularity and cardiometabolic consequences: results from observational and intervention studies. Proc Nutr Soc 75 (4):475-486. http://dx.doi.org/10.1017/s0029665116000239

16. Almoosawi S, Vingeliene S, Gachon F, Voortman T, Palla L, Johnston JD, Van Dam RM, Darimont C, Karagounis LG (2019) Chronotype: Implications for Epidemiologic Studies on

Chrono-Nutrition and Cardiometabolic Health. Advances in Nutrition 10 (1):30-42. http://dx.doi.org/10.1093/advances/nmy070

17. Zakrzewski JK, Gillison FB, Cumming S, Church TS, Katzmarzyk PT, Broyles ST, Champagne CM, Chaput JP, Denstel KD, Fogelholm M, Hu G, Kuriyan R, Kurpad A, Lambert EV, Maher C, Maia J, Matsudo V, Mire EF, Olds T, Onywera V, Sarmiento OL, Tremblay MS, Tudor-Locke C, Zhao P, Standage M, Group IR (2015) Associations between breakfast frequency and adiposity indicators in children from 12 countries. Int J Obes Suppl 5 (Suppl 2):S80-S88. http://dx.doi.org/10.1038/ijosup.2015.24

18. Küpers LK, de Pijper JJ, Sauer PJJ, Stolk RP, Corpeleijn E (2014) Skipping breakfast and overweight in 2- and 5-year-old Dutch children—the GECKO Drenthe cohort. Int J Obes 38 (4):569-571. http://dx.doi.org/10.1038/ijo.2013.194

19. Monzani A, Ricotti R, Caputo M, Solito A, Archero F, Bellone S, Prodam F (2019) A Systematic Review of the Association of Skipping Breakfast with Weight and Cardiometabolic Risk Factors in Children and Adolescents. What Should We Better Investigate in the Future? Nutrients 11 (2):387. http://dx.doi.org/10.3390/nu11020387

20. Maffeis C, Provera S, Filippi L, Sidoti G, Schena S, Pinelli L, Tatò L (2000) Distribution of food intake as a risk factor for childhood obesity. Int J Obes 24 (1):75-80. http://dx.doi.org/10.1038/sj.ijo.0801088

21. Vilela S, Oliveira A, Severo M, Lopes C (2019) Chrono-Nutrition: The Relationship between Time-of-Day Energy and Macronutrient Intake and Children's Body Weight Status. J Biol Rhythms 34 (3):332-342. http://dx.doi.org/10.1177/0748730419838908

22. Thompson OM, Ballew C, Resnicow K, Gillespie C, Must A, Bandini LG, Cyr H, Dietz WH (2006) Dietary pattern as a predictor of change in BMI z-score among girls. Int J Obes (Lond) 30 (1):176-182. http://dx.doi.org/10.1038/sj.ijo.0803072

23. Lioret S, Touvier M, Lafay L, Volatier JL, Maire B (2008) Are eating occasions and their energy content related to child overweight and socioeconomic status? Obesity (Silver Spring) 16 (11):2518-2523. http://dx.doi.org/10.1038/oby.2008.404

24. Kupek E, Lobo AS, Leal DB, Bellisle F, de Assis MA (2016) Dietary patterns associated with overweight and obesity among Brazilian schoolchildren: an approach based on the time-of-day of eating events. Br J Nutr 116 (11):1954-1965. http://dx.doi.org/10.1017/s0007114516004128

25. Diederichs T, Roßbach S, Herder C, Alexy U, Buyken AE (2016) Relevance of Morning and Evening Energy and Macronutrient Intake during Childhood for Body Composition in Early Adolescence. Nutrients 8 (11). http://dx.doi.org/10.3390/nu8110716

26. Kaisari P, Yannakoulia M, Panagiotakos DB (2013) Eating frequency and overweight and obesity in children and adolescents: a meta-analysis. Pediatrics 131 (5):958-967. http://dx.doi.org/10.1542/peds.2012-3241

27. Weaver CM, Miller JW (2017) Challenges in conducting clinical nutrition research. Nutr Rev 75 (7):491-499. http://dx.doi.org/10.1093/nutrit/nux026

28. Satija A, Yu E, Willett WC, Hu FB (2015) Understanding nutritional epidemiology and its role in policy. Adv Nutr 6 (1):5-18. http://dx.doi.org/10.3945/an.114.007492

29. Coulthard JD, Palla L, Pot GK (2017) Breakfast consumption and nutrient intakes in 4-18-year-olds: UK National Diet and Nutrition Survey Rolling Programme (2008-2012). Br J Nutr 118 (4):280-290. http://dx.doi.org/10.1017/S0007114517001714

30. Luque V, Escribano J, Closa-Monasterolo R, Zaragoza-Jordana M, Ferré N, Grote V, Koletzko B, Totzauer M, Verduci E, ReDionigi A, Gruszfeld D, Socha P, Rousseaux D, Moretti M, Oddy W, Ambrosini GL (2018) Unhealthy Dietary Patterns Established in Infancy Track to Mid-Childhood: The EU Childhood Obesity Project. The Journal of Nutrition 148 (5):752-759. http://dx.doi.org/10.1093/jn/nxy025

31. Daniels SR (2009) The Use of BMI in the Clinical Setting. Pediatrics 124 (Supplement_1):S35-S41. http://dx.doi.org/10.1542/peds.2008-3586F

32. Pérez-Escamilla R, Segura-Pérez S, Lott M, on behalf of the RWJF HER Expert Panel on Best Practices for Promoting Healthy Nutrition FP, and Weight Status for Infants and Toddlers

from Birth to 24 Months (2017) Feeding Guidelines for Infants and Young Toddlers: A Responsive Parenting Approach. Healthy Eating Research, Durham, NC

33. Fisher J, Lumeng J, Miller L, Smethers A, Lott M (2021) Evidence-Based Recommendations and Best Practices for Promoting Healthy Eating Behaviors in Children 2 to 8 Years. Healthy Eating Research, Durham, NC

34. Chiva M (1997) Cultural aspects of meals and meal frequency. Br J Nutr 77 Suppl 1:S21-28. http://dx.doi.org/10.1079/bjn19970101

35. Van Den Driessche M, Peeters K, Marien P, Ghoos Y, Devlieger H, Veereman-Wauters G (1999) Gastric emptying in formula-fed and breast-fed infants measured with the 13C-octanoic acid breath test. J Pediatr Gastroenterol Nutr 29 (1):46-51. http://dx.doi.org/10.1097/00005176-199907000-00013

36. Koletzko B, von Kries R, Closa R, Escribano J, Scaglioni S, Giovannini M, Beyer J, Demmelmair H, Gruszfeld D, Dobrzanska A, Sengier A, Langhendries JP, Rolland Cachera MF, Grote V (2009) Lower protein in infant formula is associated with lower weight up to age 2 y: a randomized clinical trial. Am J Clin Nutr 89 (6):1836-1845. http://dx.doi.org/10.3945/ajcn.2008.27091

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Affidavit

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Promotionsbüro

Eidesstattliche Versicherung

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Name, Vorname

Ich erkläre hiermit an Eides statt, dass ich die vorliegende Dissertation mit dem Thema

Chrono-Nutrition - The impact of regularity, time-of-day and frequency of energy and energy from macronutrient consumption on body mass index in children. Results of the Childhood Obesity Project Study

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