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Znaczenie czynników prenatalnych, środowiskowych oraz parametrów biochemicznych w kształtowaniu się proporcji i składu ciała oraz tempa rozwoju człowieka na różnych etapach ontogenezy progresywnej

The significance of the prenatal, environmental and biochemical factors in the forming of the body proportion and composition, as well as tempo of human development on the various stages of the progressive ontogenesis

Praca doktorska

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Promotor:

- prof. dr hab. Elżbieta Żądzińska

Kopromotor:

- prof. Niels Morling

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Spis treści.

1. Wykaz artykułów składających się na dysertację.

Prace składające się na rozprawę doktorską pt. „Znaczenie czynników prenatalnych, środowiskowych oraz parametrów biochemicznych w kształtowaniu się proporcji i składu ciała oraz tempa rozwoju człowieka na różnych etapach ontogenezy progresywnej. The significance of the prenatal, environmental and biochemical factors in the forming of the body proportion and composition, as well as tempo of human development on the various stages of the progressive ontogenesis"

Pruszkowska-Przybylska, P., Nieczuja-Dwojacka, J., & Żądzińska, E. (2018). Supplementation of vitamin D after birth affects body size and BMI in Polish children during the first 3.5 years of life-an analysis based on two cohorts measured in the years 1993-1997 and 2004-2008. Anthropologischer Anzeiger, 74, 413-421. https://doi.org/10.1127/anthranz/2018/0823 (IF=0.752, MNiSW=20 (70) pkt., liczba cytowań=2)

Pruszkowska-Przybylska, P., Rosset, I., Sitek, A., & Żądzińska, E. (2019). Familial factors more importantly modify the age of achieving motor developmental milestones than duration of breastfeeding amongst Polish children. Homo: HOMO - Journal of Comparative Human Biology Volume: 70 (4), 297-303. https://doi.org/10.1127/homo/2019/1121 (IF=0,791, MNiSW=25 (70) pkt., liczba cytowań=0)

Pruszkowska-Przybylska, P., Sitek, A., Rosset, I., Sobalska-Kwapis, M., Słomka, M., Strapagiel, D., & Żądzińska, E. (2018). Association of the 2D: 4D digit ratio with body composition among the Polish children aged 6-13 years. Early human development, 124, 26- 32. https://doi.org/10.1016/j.earlhumdev.2018.08.001 (IF=2,455, MNiSW=35 (70) pkt., liczba cytowań=3)

Pruszkowska-Przybylska, P., Sitek, A., Rosset, I., Żądzińska, E., Sobalska-Kwapis, M., Słomka, M., & Strapagiel, D. (2019). The association between socioeconomic status, duration of breastfeeding, parental age and birth parameters with BMI, body fat and muscle mass among prepubertal children in Poland. Anthropologischer Anzeiger, 76(5), 409- 419.https://doi.org/10.1127/anthranz/2019/0955 (IF=0,752, MNiSW=20 (70) pkt., liczba cytowań=2)

Pruszkowska-Przybylska P., Sitek A., Rosset I., Sobalska-Kwapis M., Słomka M., Strapagiel D., Żądzińska E., Morling N. (2020). Association of saliva 25(OH)D concentration with body composition and proportion among pre-pubertal and pubertal Polish children. American Journal of Human Biology. Praca zaakceptowana do druku (23.01.2020). https://doi.org/10.1002/ajhb.23397 (IF=1,834, MNiSW=35 (70) pkt., liczba cytowań=0)

2. Streszczenie w języku polskim.

Znaczenie czynników prenatalnych, środowiskowych oraz parametrów biochemicznych w kształtowaniu się proporcji i składu ciała oraz tempa rozwoju człowieka na różnych etapach ontogenezy progresywnej

Wzrost częstości zaburzeń proporcji wagowo-wzrostowych we współczesnych populacjach ludzkich, w tym istotny wzrost nadmiarów masy ciała u coraz młodszych dzieci sprawia, że kształtowanie się proporcji i składu ciała oraz zmiana tempa rozwoju człowieka w trakcie ontogenezy progresywnej jest wciąż niezwykle ważnym obszarem badawczym. Identyfikacja nowych czynników mogących wyjaśniać dodatkową część zmienności proporcji ciała, składu ciała, tempa osiągania kolejnych etapów dojrzałości morfologicznej w trakcie ontogenezy progresywnej istotnie poszerza wiedzę z zakresu auksologii, pediatrii, medycyny wieku rozwojowego. W efekcie wyniki takich badań mogą zostać wykorzystane w opracowywaniu kompleksowych programów profilaktycznych przeciwdziałających zaburzeniom proporcji wagowo-wzrostowych (otyłość, nadmiary masy ciała, ale również niedobory masy ciała), zaburzeniom składu ciała, czy zaburzeniom tempa rozwoju człowieka na etapie ontogenezy progresywnej. Badania ciągle jednoznacznie wskazują, że zaburzenia w kształtowaniu się proporcji i składu ciała na etapie ontogenezy progresywnej istotnie wiążą się z podobnymi zaburzeniami w wieku dorosłym.

W ramach badań wchodzących w skład pracy doktorskiej dokonano analiz z wykorzystaniem archiwalnych baz danych z lat 1993-1997 i 2004-2008 zbieranych w żłobkach i przychodniach małego dziecka na terenie Łodzi (odpowiednio n=1065 oraz n=865, wiek 3-54 miesięcy) oraz baz danych stworzonych w oparciu o materiał zbierany w latach 2016-2018 w losowo wybranych szkołach podstawowych w Łodzi (n=607, wiek 6-13 lat). Bazy danych zostały stworzone w oparciu o kwestionariusze wypełniane przez rodziców badanych dzieci oraz pomiary przeprowadzane przez pracowników Katedry Antropologii UŁ oraz Pracowni Biobank UŁ. Pytania dotyczące parametrów urodzeniowych oraz wieku ciążowego uzyskano z dokumentacji medycznej dzieci przechowywanej przez rodziców, którą uzupełniał personel medyczny bezpośrednio po porodzie. W przypadku badań przeprowadzanych w latach 1993-1997 i 2004-2008 wykonywane były m.in. następujące pomiary antropometryczne: masa ciała (kg), wysokość ciała (cm), które wykorzystano w analizach. Natomiast w latach 2016-2018 na zebrany materiał składały się: pomiary antropometryczne (masa ciała (kg), wysokość ciała (cm), obwody talii i bioder (mm), długość palca wskazującego (2D) i serdecznego (4D) (mm), skład ciała oceniony przy użyciu metody bioimpedancji elektrycznej (BIA), próbki śliny, które posłużyły do zbadania stężenia witaminy D metodą immunoenzymatyczną (ELISA).

W oparciu o bazy archiwalne analizie poddano związek suplementacji witaminą D w pierwszych miesiącach po urodzeniu z osiąganymi wartościami wysokości i masy ciała oraz proporcji wagowo-wzrostowych (BMI) w pierwszych 3 latach życia badanych dzieci, uwzględniając inne zmienne towarzyszące tj. parametry urodzeniowe (urodzeniowa masa i długość ciała), sezon urodzenia jako konsekwencja sezonu rozwoju prenatalnego oraz rodzaj karmienia w pierwszych miesiącach życia (karmienie naturalne vs. sztuczne). Wykorzystując bazy archiwalne zbadano także związek wieku i wykształcenia rodziców oraz długości karmienia naturalnego w pierwszym roku życia z wiekiem osiągania przez dzieci kolejnych zdolności psychomotorycznych tj.: samodzielne siadanie, stawanie i chodzenie. W analizach uwzględniono również następujące zmienne niezależne: wiek oraz wykształcenie ojca i matki, palenie tytoniu przez matki, urodzeniową masę ciała.

Bazy danych opracowane w latach 2016-2018 uwzględniające materiał dotyczący dzieci w wieku wczesnoszkolnym (6-13 lat) umożliwiły weryfikację, czy proporcja hormonów płciowych w okresie prenatalnym oszacowana na podstawie wskaźnika długości palca drugiego do palca czwartego (2D:4D) jest związana z proporcjami i składem ciała, uwzględniając poziom wykształcenia matki, przybieranie masy ciała w czasie ciąży oraz długość karmienia naturalnego. Kolejnym poruszonym problemem była ocena związku pomiędzy statusem socjoekonomicznym (SES), długością karmienia naturalnego, wiekiem matki i ojca oraz parametrami urodzeniowymi, a BMI i składem ciała. Ostatnim etapem badań była ocena związku stężenia witaminy D (25(OH)D) z proporcjami i składem ciała z uwzględnieniem następujących zmiennych towarzyszących: rodzaj karmienia w pierwszych miesiącach życia (karmienie naturalne vs. sztuczne), poziom wykształcenia matki i ojca oraz sezon przeprowadzanych badań.

W badaniach opublikowanych w artykule pt. "Supplementation of vitamin D after birth affects body size and BMI in Polish children during the first 3.5 years of life-an analysis based on two cohorts measured in the years 1993-1997 and 2004-2008" wykazano, że suplementowanie witaminą D w pierwszych miesiącach życia istotnie wpływa na masę i proporcje ciała w późniejszych etapach ontogenezy. Celem tego badania była ocena, czy czas suplementacji witaminą D w ciągu pierwszych miesięcy po urodzeniu, sezon urodzenia i typ karmienia (karmienie naturalne vs. pokarm sztuczny) wpływają na masę ciała, wysokość ciała i wskaźnik proporcji wagowo-wzrostowej Queteleta II (BMI) u dzieci w wieku 3–56 miesięcy. Dodatkowo przeanalizowano, czy urodzeniowa masa ciała i urodzeniowa długość ciała są skorelowane z BMI oraz masą i wysokością ciała dzieci w chwili badania. Materiał badawczy uwzględniony w analizach obejmował 849 dzieci z dwóch kohort: 1993–1997 oaz 2004–2008 w wieku 3–56 miesięcy. Aby znaleźć grupę najważniejszych zmiennych wyjaśniających aktualną masę ciała, wysokość ciała i BMI zastosowano model regresji wielorakiej krokowej oraz uogólnione modele liniowe dla interakcji dwukierunkowych analizowanych zmiennych objaśniających.

Wykazano, że sezon urodzenia (jesień vs. pozostałe pory roku) oraz rozpoczęcie suplementacji witaminą D od 4 miesiąca życia lub później (vs. wcześniej) są ujemnie związane z aktualną masą ciała badanych dzieci. Natomiast urodzeniowa masa ciała i urodzeniowa długość ciała są związane z wyższymi wartościami aktualnej masy ciała dziecka na przestrzeni badanego fragmentu ontogenezy (3-56 miesięcy). Wysokość ciała jest związana wprost proporcjonalnie z urodzeniową długością ciała i jest większa u dzieci urodzonych wiosną (vs. pozostałe pory roku). Zatem wyższe wartości BMI w chwili badania dzieci - konsekwencja masy i wysokości ciała, są częściowo prawdopodobnie związane z większą urodzeniową masą i mniejszą długością ciała.

Ponadto zaobserwowano również interakcje między czasem suplementacji witaminą D, a sezonem urodzenia, a także między czasem suplementacji witaminą D, a rodzajem karmienia w pierwszych miesiącach życia dziecka, wykazujące związek z masą ciała i BMI w pierwszych latach życia.

Dzieci, które urodziły się latem, i którym podano witaminę D między 2. a 3. miesiącem po urodzeniu (Beta=10,9; $p<0{,}0014$) były cięższe niż dzieci, które otrzymywały witaminę D wcześniej niż od 2. miesiąca życia (Beta=10,8; $p<0{,}0024$). Ponadto wykazano, że dzieci, które nie były karmione piersią i którym podawano witaminę D między 2. a 3. miesiącem po urodzeniu (Beta=151,6; $p<0{,}001$) miały wyższą wartość BMI niż te, które otrzymywały witaminę D wcześniej niż od drugiego miesiąca życia (Beta=146,6; $p<0{,}001$).

Suplementacja witaminą D była inna w dwóch analizowanych kohortach. Dzieci przebadane w latach 2004-2008 otrzymywały witaminę D wcześniej i częściej niż dzieci pochodzące z kohorty 1993-1997. Ponadto dzieci przebadane w latach 1993-1997 były bardziej podatne na niedobór witaminy D, a sezon urodzenia i rodzaj karmienia istotnie różnicował ich masę i wysokość ciała oraz BMI. Urodzeniowa masa i długość ciała były istotnymi niezależnymi zmiennymi parametrów masy i wysokości ciała oraz BMI. Badania wykazały, że dzieci, które nie były karmione piersią i które urodziły się latem, były bardziej podatne na niedobór witaminy D, co spowodowało większą masę ciała i wyższe BMI w dalszym życiu.

Wyniki opisanych analiz podkreślają znaczenie suplementacji witaminą D, sezonu urodzenia oraz urodzeniowej masy i długości ciała w osiąganych wartościach masy i wysokości ciała oraz BMI w pierwszych 3,5 latach życia.

Wyniki kolejnej pracy pt. "Familial factors more importantly modify the age of achieving motor developmental milestones than duration of breastfeeding amongst Polish children" potwierdziły, że wiek i wykształcenie rodziców są czynnikami mogącymi znacząco modyfikować tempo rozwoju psychomotorycznego potomstwa, natomiast długość karmienia naturalnego jest czynnikiem, który w analizowanej grupie dzieci polskich nie różnicuje istotnie ich wieku rozpoczęcia siadania, stawania i chodzić. Materiał badawczy obejmował 460 dzieci, w tym 252 chłopców i 208 dziewcząt w wieku od 9 do 56 miesięcy, urodzonych w terminie (37–42 tygodniu życia) przebadanych w latach 1993–1997. Badanymi zmiennymi zależnymi był wiek (miesiąc) rozpoczęcia samodzielnego: siadania, stawania i chodzenia. Zmienne niezależne podzielono na: zmienną objaśniającą – długość trwania karmienia naturalnego oraz zmienne towarzyszące - urodzeniową masę ciała, wiek ojca i wiek matki w chwili porodu, palenie tytoniu przez matkę podczas ciąży i po jej zakończeniu, a także poziom edukacji rodziców. Wyniki modeli regresji po usunięciu czynników prenatalnych i rodzinnych nie wykazały istotnego związku między długością karmienia piersią, a wiekiem w którym dziecko rozpoczyna samodzielnie siedzieć (F=0,03; p=0,8569), samodzielnie stawać (F=0,79; p=0,3774) i samodzielnie chodzić (F=0,20, p=0,6568). Wykazano, że wcześniejszy czas rozpoczęcia samodzielnego siadania był związany z wyższym wykształceniem ojców (Beta=-0,133; p=0,038) i niższym wykształceniem matek (wyższe vs. podstawowe lub zawodowe: Beta=0,172; p=0,014 oraz średnie vs. podstawowe lub zawodowe: Beta=0,113; p=0,030). Natomiast wcześniejszy czas rozpoczęcia stawania dotyczył dzieci, których ojcowie byli młodsi (Beta=0.155; p=0,025), a matki starsze (Beta=-0.137; p=0,048). Ponadto dzieci młodszych ojców (Beta=0.171; p=0,013) o wykształceniu wyższym (Beta=-0.157; p=0,020) lub średnim (Beta=-0.105; p=0,043) (vs. podstawowym lub zawodowym) wcześniej rozpoczynały chodzić.

Wyniki pokazały, że wiek i wykształcenie rodziców modyfikują ogólny rozwój motoryczny dzieci, chociaż rozmiary efektów są małe, a kierunki wpływu są różne u ojców i matek. Badanie podkreśla znaczenie czynników rodzinnych dla wieku osiągania tzw. kamieni milowych rozwoju biologicznego człowieka.

W pracy powstałej w oparciu o materiał zebrany w latach 2016-2018, zatytułowanej "Association of the 2D: 4D digit ratio with body composition among the Polish children aged 6-13 years" celem było ustalenie, czy wskaźnik palcowy 2D:4D może być indykatorem masy tłuszczu i masy mięśniowej oraz proporcji ciała u dzieci w wieku 6-13 lat. Współczynnik palcowy 2D:4D kształtuje się głównie pod wpływem prenatalnej ekspozycji na hormony płciowe. Im wyższa wartość wskaźnika, tym wyższe stężenie prenatalne estrogenu, im niższa wartość wskaźnika tym wyższa ekspozycja na testosteron. Powszechnie wiadomo, że testosteron stymuluje rozwój tkanki mięśniowej, natomiast estrogen oddziałuje na adipogenezę. Analizowana kohorta objęła 420 dzieci (221 dziewcząt i 199 chłopców) w wieku 6–13 lat. Korelacje Pearsona i Spearmana posłużyły do oceny, czy wskaźnik 2D:4D był istotnie skorelowany z pomiarami składu ciała. Zastosowano modele regresji wielorakiej krokowej w celu wyboru zmiennych niezależnych istotnie związanych z masą tłuszczu (%) i masą mięśniową (%), a także z BMI i wskaźnikiem proporcji obwodu pasa do obwodu bioder (WHR). Badanie wykazało, że współczynnik palcowy 2D:4D jest ujemnie skorelowany z masą mięśniową (MM%) wśród dziewcząt (Beta=-0,09; p=0,002). Podobnej zależności nie zaobserwowano w grupie chłopców. Modele regresji wskazały na znaczącą rolę w objaśnieniu składu ciała i proporcji ciała dziewcząt czynników matczynych, takich jak: poziom wykształcenia matki, przyrost masy ciała podczas ciąży, ale także długość karmienia piersią. Córki matek z wykształceniem wyższym charakteryzowały się przeciętnie mniejszą zawartością tkanki tłuszczowej (Beta=-0,09; p<0,001), niższym BMI (Beta=-0,17; p<0,001) oraz większą zawartością tkanki mięśniowej (Beta=0,10; p=0,002). Ponadto liczba przybranych przez kobiety kilogramów podczas ciąży korelowała dodatnio z zawartością tkanki tłuszczowej u córek (Beta=0,05; p<0,037). Współczynnik palcowy 2D:4D wydaje się być dobrym wskaźnikiem rozwoju masy mięśniowej wśród dziewcząt w wieku 6-13 lat.

W kolejnych badaniach opisany w artykule pt. "The association between socioeconomic status, duration of breastfeeding, parental age and birth parameters with BMI, body fat and muscle mass among prepubertal children in Poland" starano się wskazać grupę czynników regulujących i modyfikujących nieprawidłowy skład i proporcje ciała u dzieci, takich jak: status społeczno-ekonomiczny rodziny (SES), rodzaj karmienia (karmienie naturalne vs. karmienie sztuczne), wiek matki i ojca i parametry urodzeniowe dziecka. Ostatecznie baza danych do przetestowania postawionego celu obejmowała 469 dzieci w wieku 6-13 lat (247 dziewcząt i 222 chłopców). Analizy regresji wielorakiej krokowej wykazały, że wyższa masa tłuszczu (FM%) była związana z krótszym czasem karmienia piersią (<2 miesiące) (Beta=0,1283; p=0.005) i niższym SES rodziny (Beta=-0,1798; p<0,001). Większa masa mięśniowa (MM%) była powiązana z wyższym SES rodziny (Beta=0,1682; p <0,001) i niższą masą urodzeniową (Beta=-0,1049; p <0,022). Wyższy wskaźnik BMI związany był z wyższą masą urodzeniową (Beta=0,1851; p =0,002), krótszym czasem karmienia piersią (<2 miesiące) (Beta=0,1368; p=0,002) i niższym SES (Beta=-0,2280; p <0,001). Interakcje zaobserwowano w przypadku FM% (karmienie piersią x SES; karmienie piersią x wiek rodziców) i BMI (karmienie piersią x wiek ojca).

Zaobserwowano związek między dłuższym czasem karmienia piersią (2-6 miesięcy vs. < 2 miesiące), a niższym FM% u dzieci z wysokim i niskim SES. Dłuższy czas karmienia piersią (2-6 miesięcy vs. < 2 miesiące) był związany z niższym FM% również u dzieci najstarszych i najmłodszych matek. Wykazano także, że karmienie piersią trwające dłużej niż 6 miesięcy może zwiększyć FM%. Wysokie BMI obserwowano u dzieci karmionych piersią krócej niż 2 miesiące w porównaniu z dziećmi karmionymi piersią dłużej niż 6 miesięcy niezależnie od wieku ich ojców.

Badania wykazały, że skład ciała można powiązać z czasem trwania karmienia piersią, SES, wiekiem rodzicielskim oraz z masą urodzeniową. Krótki czas karmienia piersią (<2 miesiące) związany był z podwyższonym FM% i BMI u dzieci w wieku 6–13 lat. Wysoka masa urodzeniowa była związana z niskim MM% i wysokim BMI. Wysoki status społeczno-ekonomiczny związany był z wysokim MM%, niskim FM% i niskim BMI.

Ze względu na rosnący problem związany z otyłością i niedoborem witaminy D wśród dzieci, przeprowadzono badania, w których analizowano związek składu i proporcji ciała z poziomem witaminy D. Wyniki badań przedstawiono w pracy pt. "Association of saliva 25(OH)D concentration with body composition and proportion among pre-pubertal and pubertal Polish children". 182 losowo wybranych dzieci w wieku 6-13 lat podzielono na dwie grupy wiekowe: przedpokwitaniową (dziewczynki w wieku poniżej 10 lat i chłopcy w wieku poniżej 11 lat) oraz wczesnopokwinaniową (dziewczynki w wieku 10 lat i powyżej oraz chłopcy w wieku 11 lat lub powyżej).

Wykazano, że stężenie 25(OH)D wśród wszystkich badanych dzieci było wyższe późną wiosną (czerwiec) niż jesienią (listopad-grudzień). Korelacja Spearmana wskazała, że poziom 25(OH)D był dodatnio skorelowany z masą komórkową ciała (BCM (%) wśród wszystkich badanych (wczesnopokwitaniowa: R=0,20; p=0,044; przedpokwitaniowa: R= 0,23; p=0,041) i odwrotnie proporcjonalnie skorelowany z WHR wśród dzieci z grupy wczesnopokwitaniowej (R=-0,25; p=0,031).

Analiza regresji wielorakiej krokowej wykazała, że sezon badania - wiosna (czerwiec) i karmienie piersią (vs. brak karmienia naturalnego) wiązało się z: przeciętnie większą masą mięśniową (MM%) (Beta=0,253; p=0,003 i Beta=0,225; p=0,005), wyższym poziomem całkowitej ilości wody w organizmie (TBW% (Beta=0,276; p=0,004 i Beta=0,246; p= 0,011), niższymi wartościami BMI (Beta=-0,222; p=0,024 i Beta=-0,269; p=0,009) oraz niższymi wartościami FM% (Beta=-0,288; p=0,003 i Beta=-0,266; p=0,005).

Wyniki badań wskazują, że sezon pobierania próbek śliny i karmienie piersią były silniej związane ze składem ciała, BMI i WHR niż stężenie 25(OH)D.

Biorąc pod uwagę wszystkie wyniki przeprowadzonych badań wnioski dotyczące składu i proporcji ciała są następujące:

- Zawartość tkanki tłuszczowej jest większa u dzieci w sezonie zimowym, które nie były karmione piersią lub były karmione naturalnie krócej niż przez 2 pierwsze miesiące po urodzeniu, których matki charakteryzowały się niższym wykształceniem i większym przyrostem masy ciała podczas ciąży, pochodzących z rodzin o niższym SES.
- Zawartość tkanki mięśniowej jest większa u dzieci w sezonie wiosennym, które charakteryzowały się mniejszą masą urodzeniową, były karmione piersią dłużej niż 2

miesiące, pochodzących z rodzin o wyższym SES, a matki charakteryzowały się wyższym wykształceniem. Tylko w przypadku dziewcząt zawartość tkanki mięśniowej była wyższa, u tych z niższymi wartościami wskaźnika palcowego 2D:4D (wyższym poziomem testosteronu w czasie rozwoju prenatalnego).

- Masa komórkowa (BCM%) była większa u dzieci o wyższym stężeniu witaminy D w ślinie.
- Całkowita zawartość wody (TBW%) w organizmie była wyższa u dzieci karmionych piersią (vs. karmione tylko sztucznie).
- Masa ciała była większa u dzieci o wyższych parametrach urodzeniowych (długości i masie ciała) oraz tych urodzonych wiosną, a niższa u tych suplementowanych witaminą D od 4 miesiąca życia lub później (vs. wcześniej niż od 4 miesiąca).
- Wysokość ciała była większa u dzieci o większej urodzeniowej długości ciała i tych urodzonych wiosną.
- BMI zarówno w kohorcie 1993-2004 jak i 2016-2018 dzieci o większej urodzeniowej masie ciała miały większe BMI. Należy dodać, że większe wartości BMI były związane z mniejszą urodzeniową długością ciała, brakiem karmienia piersią lub okresem karmienia krótszym niż 2 miesiące, sezonem zimowym, wyższym SES i niższym wykształceniem matek.
- Drugi ze wskaźników proporcji ciała WHR był większy u dzieci o niższym stężeniu witaminy D w ślinie.

Natomiast zmienne niezależne wyjaśniające czas rozpoczynania siadania, stawania i chodzenia to wykształcenie i wiek rodziców. Wykazano, że:

- Wcześniejszy czas rozpoczęcia siadania był związany z wyższym wykształceniem ojców i niższym wykształceniem matek.
- Wcześniejszy czas rozpoczęcia stawania dotyczył dzieci, których ojcowie byli młodsi, a matki starsze.
- W przypadku czasu rozpoczęcia stawania młodszy wiek i wyższe bądź średnie wykształcenie ojców było związane z wcześniejszym rozpoczęciem chodzenia przez potomstwo.

Otrzymane wyniki poszerzają możliwość wyjaśnienia zmienności składu i proporcji ciała dzieci w wieku 3-56 miesięcy i 6-13 lat. Dzięki nim możliwe jest wzbogacenie programów profilaktycznych przeciwdziałających zaburzeniom proporcji wagowo-wzrostowych i nadwagi oraz otyłości wśród dzieci. Nowe programy profilaktyczne mogłyby uwzględniać jednocześnie wszystkie czynniki, które okazały się istotnie różnicować skład i proporcje ciała. Biorąc pod uwagę wyniki badań, czynnikami ryzyka prowadzącymi do nadmiarów masy ciała mogą być: podwyższona urodzeniowa masa ciała, zbyt krótki okres karmienia naturalnego lub jego brak w pierwszych miesiącach życia, zbyt duży przyrost masy ciała podczas ciąży matek, niski status socjoekonomiczny rodziny, niskie wykształcenie rodziców, podwyższony wskaźnik palcowy 2D:4D, sezon zimowy, wiosna jako sezon urodzenia.

W przypadku rozwoju psychosomatycznego wydaje się, że programy profilaktyczne powinny skupiać się na podkreślaniu czynników rodzinnych takich jak: wiek rodziców oraz poziom ich wykształcenia.

Wyniki prowadzonych badań wydają się mieć zatem charakter aplikacyjny, zarówno w kontekście otyłości i nadwagi wieku dziecięcego jak i rozwoju psychomotorycznego.

3. Streszczenie w języku angielskim.

The significance of the prenatal, environmental and biochemical factors in the forming of the body proportion and composition, as well as tempo of human development on the various stages of the progressive ontogenesis

The increase in the frequency of body proportion disturbances in modern human populations, including a significant increase in body weight excess in younger and younger children, causes that the proportion and body composition and change in the rate of human development during progressive ontogenesis is still an extremely important research area. Identification of new factors that may explain an additional part of the variability of body proportions, body composition, tempo of achieving subsequent stages of morphological maturity during progressive ontogenesis significantly broadens knowledge in the fields of auxology, pediatrics, and developmental medicine.

Results of this type of investigation can be used in the development of comprehensive preventive programs to counteract body proportions disorders (obesity, overweight, but also deficiencies in body weight), body composition abnormalities or disorders of the human development rate at the progressive ontogenesis. Studies still clearly show that disorders in the formation of proportion and body composition at the stage of progressive ontogenesis are significantly associated with similar disorders in adulthood.

As part of the research included in the doctoral dissertation, analyzes were made using archival databases from 1993-1997 and 2004-2008 collected in nurseries and outpatient clinics of a small child in Lodz (respectively, n=1065 and n=865, age 3-54 months) and databases created using the material collected in 2016-2018 in randomly selected primary schools in Lodz (n = 607, age 6-13). The databases were created based on questionnaires filled out by the parents of the investigated children and measurements carried out by the employees of the Department of Anthropology of the University of Lodz and the Laboratory of Biobank of the University of Lodz. Questions regarding birth parameters and gestational age were obtained from children's medical records kept by parents, which were provided by medical staff immediately after delivery. In the case of research carried out in 1993-1997 and 2004-2008, the following anthropometric measurements which were used in the analysis were performed: body weight (kg), body height (cm). Whereas in the years 2016-2018 the collected material consisted of: anthropometric measurements (body weight (kg), body height (cm), waist and hip circumferences (mm), length of index finger (2D) and ring finger (4D) (mm), body composition assessed using the electrical bioimpedance method (BIA), saliva samples that were used to study vitamin D concentration by enzyme-linked immunosorbent method (ELISA).

Based on archival databases, the relationship between vitamin D supplementation in the first months after birth and height and weight values as well as body mass index (BMI) in the first 3 years of life of the examined children were analyzed, taking into account other accompanying variables, i.e. birth parameters (birth weight and body length), birth season as a consequence of the prenatal development season, and feeding type in the first months of life (natural vs. formula feeding). Using archival databases, the relationship between parents' age and education as well as the duration of breastfeeding in the first year of life and the age of achieving subsequent unattended psychomotor skills by the children, i.e. sitting, standing and walking, was also examined. The following independent variables were also included in the analyzes: paternal and maternal age and education, maternal smoking, birth weight.

Databases designed in the years 2016-2018, including material about children in early school age (6-13 years), allowed to verify whether the proportion of sex hormones in the prenatal period estimated on the basis of the index of the length of the second to fourth finger (2D:4D) is related to the proportions and body composition, taking into account the maternal level of education, weight gain during pregnancy, and duration of breastfeeding. Additionally, there was assessed the relationship between socioeconomic status (SES), duration of breastfeeding, age of mother and father as well as birth parameters, and BMI and body composition. The final stage of the study was to assess the association between vitamin D (25(OH) D) concentration and body composition, taking into account the following accompanying variables: type of feeding in the first months of life (breastfeeding vs. formula feeding), level of education of the mother and father and the season of the tests.

In research published in the article entitled "Supplementation of vitamin D after birth affects body size and BMI in Polish children during the first 3.5 years of life-an analysis based on two cohorts measured in the years 1993-1997 and 2004-2008" it was shown that vitamin D supplementation in the first months of life significantly affects body mass and proportions in the later stages of ontogenesis. The purpose of this study was to assess whether time of vitamin D supplementation in the first months after birth, season of birth, and feeding type (breastfeeding vs. formula food) affect body weight, body height, and Quetelet II (BMI) among children aged 3-56 months. Additionally, it was analyzed whether birth weight and birth length are correlated with BMI, and body weight and height at the time of the study. The research material consisted of 849 children from two cohorts: 1993–1997 and 2004–2008 aged of 3-56 months. To find a group of the most important variables explaining current body weight, body height and BMI, the multiple stepwise regression models and generalized linear models for two way interactions were applied.

It has been shown that the season of birth (autumn vs. other seasons) and the starting of vitamin D supplementation from 4 months of age or later (vs. earlier) are negatively associated with the current body weight of the examined children. On the other hand, birth weight and birth length are associated with higher values of the current body weight over the examined fragment of ontogenesis (3-56 months) among investigated children. Body height is directly related to body length and is greater in children born in spring (vs. other seasons). Thus, higher BMI values at the time of testing children - a consequence of body weight and height, are partly likely to be associated with higher birth weight and shorter body length.

In addition, interactions were also observed between the time of vitamin D supplementation and the birth season, as well as between the time of vitamin D supplementation and the type of feeding in the first months of the child's life, showing a relationship with body weight and BMI in the first years of life.

Children who were born in the summer and given vitamin D between second and third months after birth (Beta=10.9; $p < 0.0014$) were heavier than children who received vitamin D earlier than 2 months of age (Beta=10.8; $p < 0.0024$). It was shown that children who were not breastfed and given vitamin D between 2 and 3 months after birth (Beta=151.6; $p < 0.001$) had a higher BMI than those who received vitamin D earlier than second month of life (Beta=146.6; $p < 0.001$).

Vitamin D supplementation was different in the two analyzed cohorts. Children examined in 2004-2008 received vitamin D earlier and more often than children from the 1993-1997 cohort. In addition, children examined in the years 1993–1997 were more susceptible to vitamin D deficiency, and the birth season and type of feeding significantly differentiated their weight, body height and BMI. Birth weight and body length were significant independent variables of body weight, height and BMI. Studies have shown that children who were not breastfed and who were born in the summer were more prone to vitamin D deficiency, which resulted in higher body weight and higher BMI later in life.

The results of the described the importance of vitamin D supplementation, birth season and birth weight and body length in body weight and height as well as BMI in the first 3.5 years of life.

The results of the next work entitled "Familial factors more importantly modify the age of achieving motor developmental milestones than duration of breastfeeding amongst Polish children" confirmed that the age and education of parents are factors that can significantly modify the tempo of psychomotor development of the offspring, while the duration of breastfeeding is a factor that in the analyzed group of Polish children do not significantly differentiate their age of starting to sit, stand and walk unattended. The research material included 460 children (252 boys and 208 girls) aged 9-56 months, born on term (37-42 weeks of age) examined in the years 1993–1997. The examined dependent variables were the age (month) of starting: sitting, standing and walking. Independent variables were divided into: explanatory variable - duration of breastfeeding and accompanying variables - birth weight, father's age and mother's age at delivery, mother's smoking during pregnancy and after pregnancy, as well as the level of parents' education. The results of regression models after removal of prenatal and familial factors did not show a significant relationship between the length of breastfeeding and the age at which the child begins to sit ($F=0.03$; $p=0.8569$), stand ($F=0.79$; $p=0.3774$) and walk ($F=0.20$, $p=0.6568$). It was shown that the earlier time of starting unattended sitting was related to the higher paternal education level (Beta=-0.133; $p=0.038$) and lower maternal education level (higher vs. primary or vocational: Beta=0.172; $p=0.014$ and secondary vs. primary or vocational: Beta=0.113; $p=0.030$). In contrast, the earlier time of standing was for children whose fathers were younger (Beta=0.155; $p=0.025$) and mothers were older (Beta=-0.137; $p=0.048$). In addition, children of younger fathers (Beta=0.171; $p=0.013$) with higher education (Beta=-0.157; $p=0.020$) or secondary (Beta=-0.105; $p=0.043$) (vs. primary or vocational) started to walk earlier.

The results showed that parental age and education modify the motor development of children, although the effects are small and the directions of influence are different for fathers and mothers. The study emphasizes the importance of family factors for the age of reaching the milestones of human biological development.

In the work based on material collected in 2016-2018, entitled "Association of the 2D:4D digit ratio with body composition among the Polish children aged 6-13 years", the aim was to determine whether the 2D:4D finger index can be an indicator of fat and muscle mass and body proportions in children aged 6-13 years. The 2D:4D finger ratio is mainly influenced by prenatal exposure to sex hormones. The higher the indicator value, the higher the prenatal concentration of estrogen, the lower the indicator value, the higher the testosterone exposure. It is well known that testosterone stimulates the development of muscle tissue, while estrogen affects adipogenesis. The analyzed cohort included 420 children (221 girls and 199 boys) aged 6–13 years. Pearson and Spearman correlations were used to assess if the 2D:4D index was significantly correlated with body composition measurements. Multiple stepwise regression models were used to select independent variables significantly related to fat mass (%) and muscle mass (%), as well as BMI and the ratio of waist circumference to hip circumference (WHR). The study showed that the 2D:4D digit ratio is negatively correlated with muscle mass (MM%) among girls (Beta=-0.09; $p=0.002$). A similar relationship was not observed in the boys group. Regression models showed a significant role of maternal factors in explaining the body composition and body proportions among girls, such as: the level of maternal education, weight gain during pregnancy, but also the duration of breastfeeding. Daughters of mothers with higher education level were characterized by lower body fat (Beta=-0.09; $p<0.001$), lower BMI (Beta=-0.17; $p <0.001$) and higher muscle mass (Beta=0.10 ; $p=0.002$). In addition, the weight gain by women during pregnancy was positively correlated with the fat mass of their daughters (Beta=0.05; $p<0.037$). The 2D:4D finger ratio seems to be a good indicator of muscle mass development among girls aged 6-13 years.

In subsequent studies described in the article entitled "The association between socioeconomic status, duration of breastfeeding, parental age and birth parameters with BMI, body fat and muscle mass among prepubertal children in Poland" the purpose of the study was to identify a group of factors regulating and modifying abnormal body composition and proportions in children, such as: family socioeconomic status (SES), type of feeding (breastfeeding vs. formula feeding), mother's and father's age and child's birth parameters. Ultimately, the database included 469 children aged 6-13 years (247 girls and 222 boys). Multiple stepwise regression analyzes showed that higher fat mass (FM%) was associated with shorter breastfeeding duration (<2 months) (Beta=0.1283; $p=0.005$) and lower family SES (Beta=-0.1798; $p <0.001$). Higher muscle mass (MM%) was associated with a higher family SES (Beta=0.1682; $p<0.001$) and lower birth weight (Beta=-0.1049; $p <0.022$). Higher BMI was associated with higher birth weight (Beta=0.1851; $p=0.002$), shorter breastfeeding duration (<2 months) (Beta=0.1368; $p=0.002$) and lower SES (Beta=-0, 2280; $p<0.001$). Interactions were observed for FM% (breastfeeding x SES; breastfeeding x parental age) and BMI (breastfeeding x paternal age).

A relationship was observed between longer breastfeeding duration (2-6 months vs. <2 months) and lower FM% in children with high and low SES. Longer breastfeeding (2-6 months vs. <2 months) were also associated with lower FM% also in children of the oldest and youngest mothers. It has also been shown that breastfeeding for more than 6 months can increase FM%. High BMI was observed among individuals breastfed less than 2 months compared to ones breastfed longer than 6 months, regardless of their fathers age.

Studies have shown that body composition can be linked to the duration of breastfeeding, SES, parental age, and birth weight. Short breastfeeding time (<2 months) was associated with increased FM% and BMI in children aged 6–13 years. High birth weight was associated with low MM% and high BMI. High socioeconomic status was associated with high MM%, low FM% and low BMI.

Due to the growing problem of obesity and vitamin D deficiency among children, there were conducted studies in which the relationship between body composition and proportion with vitamin D levels was analyzed. The results of the study are presented in the paper entitled "Association of saliva 25 (OH) D concentration with body composition and proportion among pre-pubertal and pubertal Polish children". 182 randomly selected children aged 6-13 years were divided into two age groups: pre-pubertal (girls under 10 years old and boys under 11 years old) and pubertal (girls 10 years old and above and boys 11 years old and above).

It was shown that the concentration of 25(OH)D among all examined children was higher in late spring (June) than in autumn (November-December). Spearman's correlation indicated that the 25 (OH) D level was positively correlated with body cell mass (BCM%) among all subjects (pubertal: $R=0.20$; $p=0.044$; pre-pubertal: $R=0.23$; $p=0.041$) and inversely correlated with WHR among children from the pubertal group ($R=-0.25$; $p=0.031$).

Multiple stepwise regression analysis showed that the test season - spring (June) and breastfeeding (vs. formula feeding) were associated with: greater muscle mass (MM%) (Beta=0.253; $p=0.003$ and Beta=0.225; $p=0.005$), a higher level of total body water (TBW%) (Beta=0.276; $p=0.004$ and Beta=0.246; $p=0.011$), lower BMI (Beta=-0.222; $p=0.024$ and Beta=-0.269; $p= 0.009$) and lower fat mass (FM%) (Beta=-0.288; $p=0.003$ and Beta=-0.266; $p=0.005$).

Study results indicate that the saliva sampling season and breastfeeding were more strongly linked with body composition, BMI and WHR than the 25(OH)D level.

Considering all the results of the conducted research, the conclusions regarding body composition and proportions are the following:

• Fat mass is higher in children measured in the winter season who have not been breastfed or have been fed naturally less than the first 2 months after birth, whose mothers had lower education and greater weight gain during pregnancy, from families with lower SES.

• Muscle tissue is higher in children measured in the spring season, who had a lower birth weight, were breastfed for more than 2 months, coming from families with a higher SES, and mothers had a higher education. Only in the case of girls, muscle tissue was greater in those with lower values of 2D:4D (higher testosterone levels during prenatal development).

• Body cell mass (BCM%) was higher in children with higher levels of vitamin D in saliva.

• Total body water (TBW%) was higher in breastfed (vs. formula fed) children.

• Body weight was higher in children with higher birth parameters (body length and weight) and those born in spring, and lower in those supplemented with vitamin D from 4 months of age or later (vs. earlier than from 4 months).

• Body height was greater in children with greater birth length and those born in spring.

• BMI in both the 1993-2004 cohort and 2016-2018 children with higher birth weight was higher. It should be added that higher BMI values were associated with lower birth length, no breastfeeding or feeding less than 2 months, winter season of measurement, higher SES and lower maternal education.

• WHR ratio was higher in children with lower vitamin D levels in saliva.

Additionally, independent variables explaining the time of starting to: sit, stand and walk are the education and age of the parents. It has been shown that:

• Earlier sitting time was related to higher education of fathers and lower education of mothers.

• The earlier sitting time was for children whose fathers were younger and mothers older.

• In the case of the starting time of walking, younger and better educated fathers had offspring who started to walk earlier.

The obtained results broaden the possibility of explaining the variability of body composition and proportions of children aged 3-56 months and 6-13 years. Thanks to them, it is possible to enrich preventive programs counteracting body proportions and overweight as well as obesity among children. The new preventive programs could simultaneously take into account all factors that have proven to significantly differentiate body composition and proportions. According to the results, the risk factors leading to excess body weight may include: increased birth weight, insufficient duration or lack of breastfeeding period in the first months of life, excessive weight gain during pregnancy of mothers, low socioeconomic status of the family, low parental education, increased 2D:4D index, winter season of measurement, spring as the birth season.

In the case of psychosomatic development it seems that preventive programs should focus on emphasizing family factors such as the age of parents and their level of education.

Therefore, the results of the conducted research seem to be of an application uses, both in the context of childhood obesity and overweight as well as psychomotor development.

4. Treści artykułów składających się na dysertację wraz z oświadczeniami współautorów.

- Załącznik 1 - "Supplementation of vitamin D after birth affects body size and BMI in Polish children during the first 3.5 years of life-an analysis based on two cohorts measured in the years 1993-1997 and 2004-2008"
- Załącznik 2 - "Familial factors more importantly modify the age of achieving motor developmental milestones than duration of breastfeeding amongst Polish children"
- Załącznik 3 - "Association of the 2D: 4D digit ratio with body composition among the Polish children aged 6-13 years"
- Załącznik 4 - "The association between socioeconomic status, duration of breastfeeding, parental age and birth parameters with BMI, body fat and muscle mass among prepubertal children in Poland"
- Załącznik 5 - "Association of saliva 25(OH)D concentration with body composition and proportion among pre-pubertal and pubertal Polish children"

Załącznik 1 - "Supplementation of vitamin D after birth affects body size and BMI in Polish children during the first 3.5 years of life-an analysis based on two cohorts measured in the years 1993-1997 and 2004-2008"

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Supplementation of vitamin D after birth affects body size and BMI in Polish children during the first 3.5 years of life – an analysis based on two cohorts measured in the years 1993–1997 and 2004–2008

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With 2 figures and 5 tables

Abstract: *Objectives*: The aim of this study was to assess whether the time of vitamin D supplementation after birth, season of birth and the type of feeding affected current body weight, body height and BMI among children aged 3–56 months from two cohorts. Additionally, it was analysed whether birth weight and birth length correlated with current BMI, body height and body weight in both cohorts of children. *Methods*: The study material included 1930 children from the two cohorts, measured in two following periods: 1993–1997 and 2004–2008. Finally 849 healthy individuals aged 3–56 months were included in the analysis. Multiple stepwise regression model was applied to find the group of the most important variables explaining current body weight, body height and BMI. Moreover generalized linear models for two way interactions were used. *Results*: The season of birth, time of supplementation of vitamin D, but also birth weight and length might affect current body weight. Individuals' height could be associated with birth length and the season of birth. BMI value was probably regulated by birth length and weight. Furthermore, interactions between the time of vitamin D supplementation and season of birth and also between the time of vitamin D supplementation and type of feeding resulting in variation of body weight and BMI in the first years of life were also observed. *Conclusions*: The study underlines the importance of a supplementation of vitamin D, season of birth and birth weight and length in current weight, height and body proportion in the first 3.5 years of life.

Keywords: birth length; birth height; birth weight; BMI; season of birth; type of feeding; vitamin D supplementation

Introduction

Nowadays, the importance of vitamin D for human organisms is especially emphasised. Deficiency of vitamin D is primarily connected with rickets among children and osteoporosis among adults. Moreover, vitamin D has an impact on fat metabolism, and its deficiency may affect overweight (Lee et al. 2013a; Lee et al. 2013b; Lourenço et al. 2014, Cunha et al. 2015). Some studies show that an appropriate level of vitamin D protects against mental diseases, such as depression (Gloth et al. 1999) or schizophrenia (McGrath et al. 2002). Vitamin D_3 deficiency is also associated with autoimmune diseases, such as diabetes type 1 (Zipitis & Akobeng 2008) or Crohn's Syndrome (Koutkia et al. 2001). The whole pool of vitamin D_3 stems from exogenous and endogenous sources. The exogenous part comes from food – mostly dairy products and fish, such as cod or herring – as well as from additional artificial supplementation. Endogenous vitamin D_3 is dependent on sunlight exposure. The precursor form of vitamin D_3, 7-dehydroxycholesterol, is converted into vitamin D_3 under sunlight and body temperature (Walicka et al. 2008; Lehman 2005). From the human skin, vitamin D_3 is transported to the liver, where it is hydroxylated to 25-hydroxyvitamin D_3, and finally, in the

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kidneys, it is converted into its most active form, 1–25-dihydroxyvitamin D_3 (Lehman 2005). While stressing the importance of sunlight radiation in the activation of vitamin D_3, it is especially important in the northern hemisphere, where exposure to sunlight varies during the year. Lippi et al. (2015) proved that the season of birth had an influence on vitamin D concentration in the analysed group of adults. Regarding the significant impact of vitamin D on, among others, skeletal composition and fat distribution, the month of birth is a complex factor in human development. Due to seasonal variation of sunlight, the month of birth influences many morphological and physiological features such as body height and weight, but also enamel thickness in deciduous teeth (Shephard et al. 1979; Henneberg & Louw 1990; Henneberg & Louw 1993; Weber et al. 1998; Waldie et al. 2000; Banegas et al. 2001; Kościński et al. 2004; Puch & Kozłowska-Rajewicz 2004; McGrath et al. 2005; Puch et al. 2008; Krenz-Niedbała et al. 2011; Żądzińska et al. 2013; Jensen et al. 2015; Rosset et al. 2017).

The aim of this study was to assess whether the time of vitamin D supplementation after birth, but also the season of birth and the type of feeding (breastfeeding vs. formula feeding after birth) affected current body weight, body height and BMI among children aged 3–56 months measured in the two anthropological surveys in the years 1993–1997 (cohort I) and 2004–2008 (cohort II). Additionally, it was analysed whether birth weight and birth length correlated with current BMI, body height and body weight in both cohorts of children.

Material and methods

The study material consisted of information and measurements of about 1930 children from two different cohorts from 31 nurseries and randomly selected "Healthy Child Clinics" in Łódź (central Poland). The first cohort was examined in the years 1993–1997 (593 boys and 472 girls), and the second group was analysed in the years 2004–2008 (437 boys and 428 girls). The analysis was part of a research programme monitoring the development of infants and pre-school children in Łódź. All the obtained information excluding current height and weight measurements came from questionnaires filled in by the children's mothers. The data about birth parameters came from the children's health records. Anthropometric measurements were carried out by the qualified staff of the Department of Anthropology of the University of Łódź, according to the standard procedure of Martin (Knussmann 1988). An electronic scale was used to measure current body weight with an accuracy of 0.1 kg. Current body height was measured using an anthropometer with an accuracy of 0.001 m. The body mass index (BMI) was calculated as the quotient of body mass (kg) and the square of height (m^2). The calendar age of each examined child was calculated from the difference between the examination date and the date of birth and expressed in decimal years (with an accuracy of 0.01 year).

The calendar age of the examined children, expressed in the decimal format of years with an accuracy of 0.01 year, was determined as the difference between the date of measurement and the date of birth.

All the children were divided into separate groups with the same calendar age and sex. Subsequently, for each group, z-score values for current body weight and height were calculated. Additionally, groups with the same gestational age (months) and sex were created, and for each group, the z-scores for birth length and weight were calculated. In each case, the following equation was used: z-score = $(x - \mu)/\sigma$, where: x – the individual's value for the analysed parameter; μ – the mean of the analysed group; σ – the standard deviation of the created group.

Due to a lack of response, the final data set contained 849 healthy individuals (384 girls and 465 boys) aged 3–56 months in the years 1993–1997 and 2004–2008. The percentage of missing data was the following: height 0.05%, weight 0.05%, breastfeeding 32.90%, time of vitamin D supplementation 43.42%, gestational age 19.84%, birth weight 3.26% and birth length 3.37%.

In the study, 4 categories of the time of beginning vitamin D supplementation were defined: 1 – before the 2nd month after birth, 2 – before the 3rd month, 3 – before the 4th month, and the last one in the 4th month or later or without vitamin D supplementation. There were no differences in the height z-score (cm) ($Z = 0.6628$, $p = 0.5074$), the weight z-score (kg) ($Z = -0.9891$, $p = 0.2991$) and the BMI z-score ($Z = -0.4864$, $p = 0.6278$) between the children who were not given vitamin D and those who were given vitamin D from the 4th month after birth or later, thus a fourth category was defined. The category of the season of birth was created respectively: winter – December, January, February; spring – March, April, May; summer – June, July, August; autumn – September, October, November. The category of the type of feeding after birth included two groups of infants: those breastfed and those not breastfed (formula-fed) after birth.

Statistical analysis

The Shapiro-Wilk test was employed in order to examine the population's distribution of: BMI (z-score), weight (z-score), height (z-score) and the birth parameters: weight (z-score) and length (z-score) among the two cohorts. Except for the birth z-score, weight in the two cohorts and also birth length (z-score) in the cohort 1993–1997, the remaining measurements were not normally distributed. Hence, nonparametric tests were used in order to estimate their statistical significance. The U Mann-Whitney test, the Student t-test and the Kruskal-Wallis test were used in order to check whether there were any differences between the two cohorts in all the analysed dependent variables, including the time of vitamin D supplementation, the season of birth and the type of feeding

Table 1. Descriptive statistics for all continues variables (z-score height, z-score weight, z-score BMI, and birth parameters: z-score birth weight and z-score birth length) among two investigated cohorts 1993–1997, 2002–2004.

Variable	Cohort 1993–1997 N = 565										Cohort 2004–2008 N = 284										Z/t; *p*	
	Mean		Median		Q1		Q3		SD		Mean		Median		Q1		Q3		SD			
	z-score	(kg) or (cm)	z-score	(kg) or (cm)	z-score	(kg) or (cm)	z-score	(kg) or (cm)	z-score	(kg) or (cm)	z-score	(kg) or (cm)	z-score	(kg) or (cm)	z-score	(kg) or (cm)	z-score	(kg) or (cm)	z-score	(kg) or (cm)		
Height (cm)	0.013	89.090	–0.088	89.750	–0.657	80.250	0.702	97.500	1.008	10.686	–0.002	82.873	–0.063	83.000	–0.525	78.000	0.641	88.000	0.995	7.811	–0.190	0.8490[b]
Weight (kg)	0.036	13.098	–0.073	13.000	–0.659	11.000	0.665	15.000	0.995	2.877	–0.060	11.638	–0.188	11.500	–0.707	10.000	0.511	13.000	0.987	2.153	1.244	0.2135[b]
BMI (kg/m^2)	0.012	16.472	–0.057	16.344	–0.555	15.278	0.625	17.667	0.961	1.955	–0.042	16.918	–0.134	16.766	–0.741	15.590	0.517	18.108	1.066	2.026	1.335	0.1819[b]
Birth weight (kg)	–0.029	3.196	–0.111	3.200	–0.764	2.900	0.694	3.550	1.006	0.535	0.059	3.265	–0.002	3.300	–0.575	3.000	0.601	3.550	0.948	0.507	–1.224	0.2573[a]
Birth length (cm)	0.021	53.926	0.093	54.000	–0.688	52.000	0.650	56.000	0.977	3.325	–0.042	53.905	–0.015	54.000	–0.688	52.000	0.650	56.000	1.010	3.293	0.534	0.5934[b]

Differences were assessed using Student t-test[a] or Mann-Whitney U- test[b] after birth. The Chi² test was applied to examine the differences between the groups in the case of categorical variables.

Multiple regression was used in order to determine the group of the most influential variables for current (z-score) weight, height and BMI, including the following independent variables: the time of vitamin D supplementation, the season of birth, the type of feeding, birth weight and length. Afterwards, forward stepwise multiple regression was applied in order to select the most important group of factors.

Due to the lack of normal distribution of the dependent variables, generalised linear models (GLZ) for two-way interactions were used in order to measure the simultaneous effect of an independent variable on current body weight, body height and BMI.

The whole analysis was performed in Statistica ver. 13.0 software.

Results

There were no differences in birth weight and length and BMI between the cohort 1993–1997 and the cohort 2004–2008 ($p > 0.05$) (Table 1). The structure of sex was homogeneous in the two analysed cohorts ($\chi^2 = 0.0044$; $p = 0.9473$) (Table 2). In the cohort 1993–1997, the individuals were most frequently born in summer (28.50%), but in the cohort 2004–2008 in spring (30.28%) ($\chi^2 = 7.8241$; $p = 0.0498$). After one decade, the percentage of breastfed infants went up from 77.17% to 85.53% ($\chi^2 = 8.318057$; $p = 0.0039$). Besides, a change in the supplementation of vitamin D was observed. In the cohort collected in the years 2004–2008, 58.45% individuals were given vitamin D earlier than from the second month after birth, and it was less frequent in the earlier cohort: 49.73% (13.0490; $p = 0.0045$). Moreover, in the analysed groups, after approximately 10 years, the number of the individuals who were not given vitamin D or who started to be given vitamin D from the 4th month after birth or later decreased from 18.58% to 11.27% ($\chi^2 = 13.0490$; $p = 0.0045$) (Table 2).

The Kruskal-Wallis test showed that statistically significant differences occurred in the season of birth in the case of body height (H = 42.96, $p < 0.0001$) and weight (H = 31.97; $p < 0.0001$), but BMI was not statistically significant (H = 1.57; $p = 0.6672$). The U Mann-Whitney test revealed that the type of feeding did not significantly stratify body weight (Z = 0.7149; $p = 0.4306$), height (Z = 1.7149; $p = 0.0863$) and BMI (Z = –0.7955; $p = 0.4263$). The four groups of children who were characterised by a different time of beginning vitamin D supplementation had a statistically significant different body weight (H = 7.97; $p = 0.0466$), but not height (H = 2.18; $p = 0.5358$) or BMI (H = 3.48; $p = 0.3234$).

Multiple regression models for all the dependent variables (current weight, height and BMI) were designed. The model for current weight among the individuals from the cohort 1993–1997 showed that higher values of birth weight (Beta = 0.2463; $p < 0.001$) and birth length (Beta = 0.1458; $p = 0.0047$) corresponded to a higher value of cur-

Table 2. Table of frequencies in categorical variables among two investigated cohorts 1993–1997, 2004–2008.

	Cohort 1993–1997	Cohort 2004–2008	Total	χ^2, p
Sex				
Girls	256 (45.31)	128 (45.07)	384 (45.23)	0.0044 $p = 0.9473$
Boys	309 (54.69)	156 (54.93)	465 (54.77)	
Season of birth				
Spring	136 (24.07)	86 (30.28)	222 (26.15)	7.8241 $p = 0.0498$
Summer	161 (28.50)	61 (21.48)	223 (26.15)	
Autumn	122 (21.59)	71 (25.00)	193 (22.73)	
Winter	146 (25.84)	66 (23.24)	212 (24.93)	
Type of feeding				
Formula feeding	129 (22.83)	41 (14.44)	170 (20.02)	8.3181 $p = 0.0039$
Breastfeeding	436 (77.17)	243 (85.53)	679 (79.98)	
Vitamin D categories				
before 2nd month after birth	281 (49.73)	166 (58.45)	447 (52.65)	13.0489 $p = 0.0045$
before 3rd month after birth	89 (15.75)	54 (19.01)	143 (16.84)	
before 4th month after birth	90 (15.93)	32 (11.27)	122 (14.37)	
since 4th month or later or without vit. D supplementation	105 (18.58)	32 (11.27)	137 (16.14)	
Total	565 (66.55)	284 (33.45)	849	

rent weight. Additionally, the weight value decreased if the individuals were born in autumn in contrast to the other seasons (Beta = –0.1403; p = 0.0038). After applying the stepwise regression model procedure, also birth weight (Beta = 0.2527; $p < 0.001$) and birth length (Beta = 0.1428; p = 0.0053) correlated positively with current weight, and the weight values were higher if the individuals were born in autumn in contrast to the other seasons (Beta = –0.1198; p = 0.0043). Besides, if vitamin D was given to children from the 4th month after birth or later, current weight was lower (Beta = –0.0842; p = 0.0359). All the variables explain (adjusted R^2) 16.12% of weight variability. In the case of the cohort 2004–2008 in the first regression model, only the season of birth was a significant variable. The children who were born in autumn were lighter (Beta = –0.2720; p = 0.0001) than the children who were born in the other seasons. Multiple stepwise regression selected all the significant variables: the season of birth and birth weight. The individuals who were born in autumn (Beta = –0.2640; $p < 0.0001$) and in winter (Beta = –0.1309; p = 0.0321) were lighter than those who were born in spring and in summer. Additionally, a higher value of birth weight (Beta = 0.1328; p = 0.0219) corresponded to a higher current weight. Adjusted R^2 showed that 7.64% of weight variability was explained by all the variables included in the model.

A multiple regression model for current height was created, and it showed that higher values of birth length (Beta = 0.2753; $p < 0.0001$) coincided with a higher value of current height. Furthermore, the children who were born in autumn (Beta = –0.1872; p = 0.0001), in winter (Beta = –0.1618; p = 0.0011) and in summer (Beta = –0.1284; $p < 0.0098$) were shorter than those who were born in spring in the cohort 1993–1997. After applying the stepwise regression model, the above-mentioned significant variables remained the same: birth length (Beta = 0.2786; $p < 0.0001$), the season of birth: autumn (Beta = –0.1901; p = 0.0001), winter (Beta = –0.1625; p = 0.0010) and summer (Beta = –0.1306; $p < 0.0084$), and they explained 13.9% of height variability. The next regression model included current height as a dependent variable among the individuals from the cohort 2004–2008, and it revealed that a lower value of current height characterised the children who were born in autumn (Beta = –0.3064; $p < 0.0001$) and in winter (Beta = –0.1921; p = 0.0058) in contrast to the other seasons. After applying stepwise regression, the significant variables remained the same: autumn (Beta = –0.3116; $p < 0.0001$) and winter (Beta = –0.2034; p = 0.0025), and the final model explained 7.8% of height variability.

The model including current BMI as a dependent variable in the cohort 1993–1997 showed that the BMI value was higher among children with a higher birth weight (Beta = 0.2527; $p < 0.0001$) and lower in the case of children with a higher birth length (Beta = –0.1269; p = 0.0213). After applying stepwise forward regression, the significant variables remained identical: birth weight (Beta = 0.2548; $p < 0.0001$) and birth length (Beta = –0.1294; p = 0.0181). Adjusted R^2 explained 4.3% of BMI variability. In the case of the cohort 2004–2008, the regression model and the stepwise regression model for current BMI were not significant ($p > 0.05$).

The next step of the analysis was to assess the significance of the interactions between vitamin D supplementation, breastfeeding and the season of birth for the dependent variables. Generalised linear models were constructed for all the continuous dependent variables: height (z-score), weight (z-score) and BMI (z-score) grouped by the cohort. Table 3 shows the results for the log-normal generalised linear models (GLZ) for significant interaction effects of the independent variables adjusted for the main effects. We obtained results proving that significant interaction effects occurred in the case of weight (z-score) and BMI (z-score) among the individuals from the cohort 1993–1997. Table 4 shows that the individuals who were born in summer and

Table 3. The log-normal models (GLZ) for important interaction effects independent variables adjusted on main effects. The dependent variables: height z-score, weight z-score, BMI z-score.

Dependent variables	Independent variables	*p*-value	
		1993–1997	2004–2008
Height z-score	Breastfeeding[a] × Season of birth[b]	0.6622	< 0.001
	vit. D supplementation[c] × Season of birth[b]	0.0226	< 0.001
	Breastfeeding[a] × vit. D supplementation[c]	0.0303	–
Weight z-score	Breastfeeding[a] × Season of birth[b]	0.0635	< 0.001
	vit. D supplementation[c] × Season of birth[b]	< 0.001	< 0.001
	Breastfeeding[a] × vit. D supplementation	< 0.001	–
BMI z-score	vit. D supplementation[c] × Season of birth[b]	0.0736	–
	Breastfeeding[a] × vit. D supplementation[c]	< 0.001	–

[a] Yes vs No; [b] summer, autumn, winter vs spring; [c] before 2 month after birth, before 3 months, before 4 months, since 4 or later or without 4 vit. D supplementation; Tests of all effects, p-value for Wald statistics.

Table 4. Evaluations of parameters in the of the independent variables statistically important for weight and BMI.

Dependent variables	Independent variables	Level of effects	β [a]	Sth.Err.	–95% CI	+95% CI	*p*-value [a]
Weight z-score Cohort 1993–1997	vit. D supplementation × season of birth	summer × before 2nd months	10.8	3.5468	3.8082	17.7114	0.0024
		summer × before 3rd months	10.9	3.4050	4.2017	17.5493	0.0014
BMI z-score Cohort 1993–1997	Breastfeeding[a] × vit. D supplementation	No × before 2nd months	146.6	13.4748	120.171	172.992	< 0.001
		No × before 3rd months	151.6	2.3945	146.916	156.302	< 0.001

[a] beta coefficient from the generalized linear model for the variable indicated, which is the log of the mean differences in weight and BMI and *p*-value for beta.

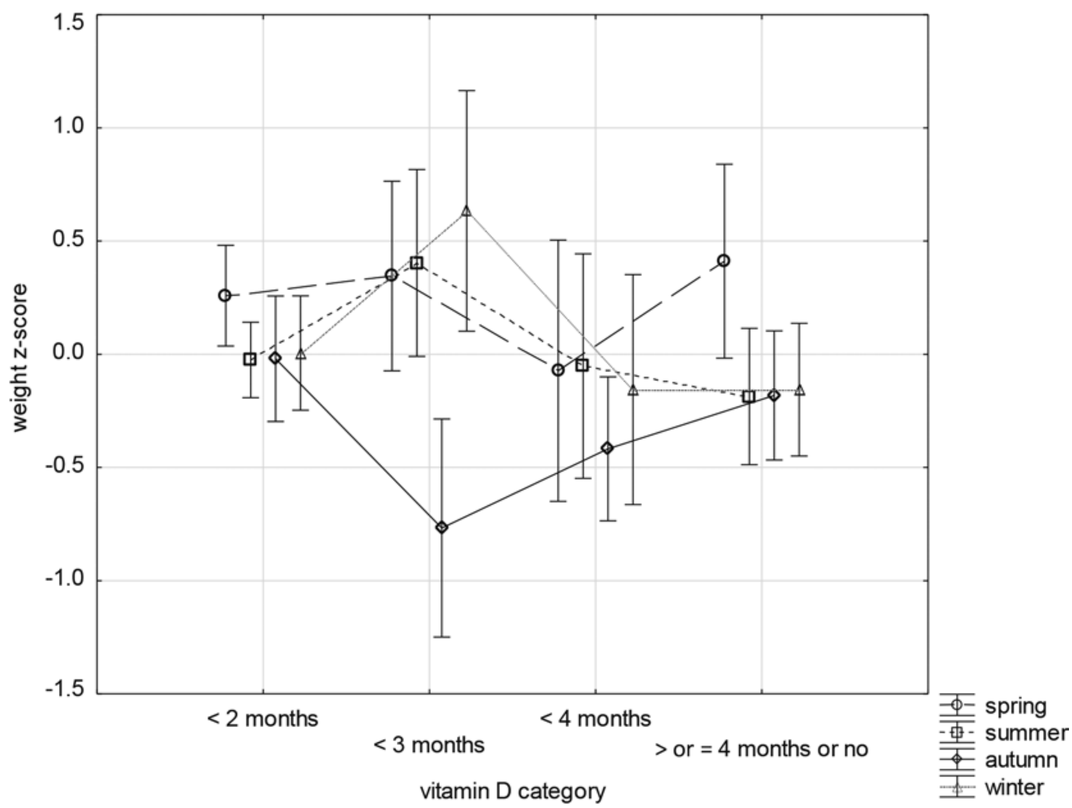

Fig. 1. Interaction between vitamin D supplementation and season of birth among cohort from 1993–1997 years. Statistically important effect: summer and supplementation vitamin D between the 2nd and the 3rd month after birth (Beta = 10.9; $p < 0.0014$) × summer and supplementation vitamin D earlier than from the 2nd month of life (Beta = 10.8; $p < 0.0024$).

who were given vitamin D between the 2nd and the 3rd month after birth (Beta = 10.9; $p < 0.0014$) were heavier than the individuals who were given vitamin D earlier than from the 2nd month of life (Beta = 10.8; $p < 0.0024$) (Fig. 1). In addition, we calculated that the individuals who were not breastfed and who were given vitamin D between the 2nd and the 3rd month after birth (Beta = 151.6; $p < 0.001$) had a higher BMI value than the individuals who were given vitamin D earlier than from the 2nd month of life (Beta = 146.6; $p < 0.001$) (Fig. 2).

Discussion

We observed that after one decade, the awareness of the benefits of breastfeeding rose – the percentage of breast-fed children increased from 77.17% to 85.53%. This may result from the increasing general knowledge about the care of newborns. Moreover, it is positive that the percentage of children who were not vitamin D supplemented or who were supplemented from the 4th month of life or later decreased from 18.58% to 11.27%. This result is also a confirmation of the increasing awareness of the necessity of vitamin D

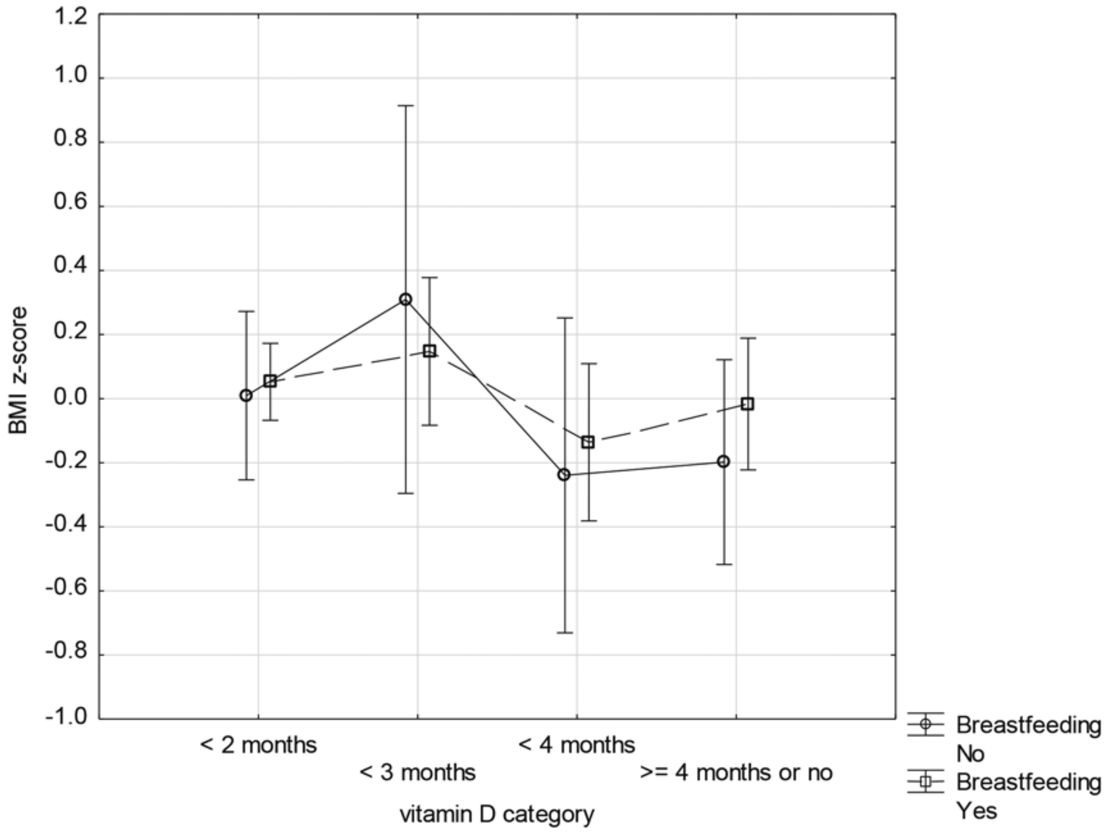

Fig. 2. Interaction between vitamin D supplementation and type of feeding among cohort from 1993–1997 years. Statistically important effect: not breastfed and vitamin D supplementation between the 2nd and the 3rd month after birth (Beta = 151.6; $p < 0.001$) × not breastfed and supplementation vitamin D earlier than from the 2nd month of life (Beta = 146.6; $p < 0.001$).

supplementation. However, some authors assume that deficiency of vitamin D is still observed (Holick 2017; Hassan-Smith et al. 2017). We obtained different results for the two cohorts, which may result from the different conditions of the children's development in Poland in the 1990's – a period of political transformation – and one decade after the political changes; this was analysed by Żądzińska et al. (2012) in the case of weight variability among children and adolescents.

Lagunova et al. (2009) claimed that a lower 25 (OH)D_3 and 1,25 (OH)D_3 serum level increased the BMI value. We did not find any direct association between BMI and the time of vitamin D supplementation, although we observed an interaction between the type of feeding and the time of vitamin D supplementation. The individuals who were not breastfed and who were given vitamin D later than from the 2nd month after birth, but not later than from the 3rd month, had a higher BMI value than the individuals who were given vitamin D earlier than from the 2nd month after birth. We may thus conclude that children who were not breastfed may be more vulnerable to vitamin D deficiency, especially until the 3rd month after birth, than breastfed children, in whose case this effect was not observed. It is noteworthy that the individuals who were born in summer and who were given vitamin D between the 2nd and the 3rd month after birth were heavier than the individuals who were given vitamin D earlier than 2 months after birth. It is probable that a lack of breastfeeding and birth in summer may increase the vulnerability to vitamin D deficiency, which may affect body weight and BMI.

Some authors demonstrated that children who were born in winter had a lower level of vitamin D than those who were born in spring (Selvin Janerich 1971; Lagunova et al. 2009; Lippi et al. 2015). The above-mentioned relationship leads to the conclusion that this is the cause of the higher height value among the children who were born in spring in contrast to the other seasons, because they had an appropriate quantity of sunlight to activate vitamin D, which influences the development of bones.

Among all the facts connecting the season of birth with the level of vitamin D, Zostautiene et al. (2016) demonstrated that there was no association between the season of birth and vitamin D concentration in adults in a northern Norwegian population, but it should be considered that geographical location might affect the variability of the level of vitamin D, and thus it might be different from the Polish population.

Table 5. Descriptive statistics for statistically important dependent variables.

Dependent variables	Independent variables	Level of factor		N	Mean	SD	SE	–95% CI	+95% CI
		Season of birth	Period of vit. D supplementation						
Weight z-score 1993–1997	vit. D supplementation × season of birth	spring	before 2nd month	64	0.2592	0.8915	0.1114	0.0365	0.4819
		spring	before 3rd month	28	0.3457	1.0809	0.2043	–0.0735	0.7648
		spring	before 4th month	22	–0.0725	1.3015	0.2775	–0.6496	0.5046
		spring	since 4th month or later or no	22	0.4112	0.9659	0.2059	–0.0171	0.8394
		summer	before 2nd month	87	–0.0248	0.7827	0.0839	–0.1917	0.1420
		summer	before 3rd month	24	0.4037	0.9772	0.1995	–0.0089	0.8163
		summer	before 4th month	18	–0.0520	0.9977	0.2352	–0.5481	0.4442
		summer	since 4th month or later or no	32	–0.1863	0.8355	0.1477	–0.4875	0.1150
		autumn	before 2nd month	57	–0.0197	1.0445	0.1384	–0.2968	0.2575
		autumn	before 3rd month	9	–0.7674	0.6265	0.2088	–1.2489	–0.2858
		autumn	before 4th month	29	–0.4176	0.8354	0.1551	–0.7353	–0.0998
		autumn	since 4th month or later or no	27	–0.1817	0.7212	0.1388	–0.4670	0.1036
		winter	before 2nd month	73	0.0054	1.0826	0.1267	–0.2472	0.2580
		winter	before 3rd month	28	0.6332	1.3705	0.2590	0.1017	1.1646
		winter	before 4th month	21	–0.1560	1.1162	0.2436	–0.6641	0.3521
		winter	since 4th month or later or no	24	–0.1561	0.6937	0.1416	–0.4490	0.1368
BMI z-score 1993–1997	Breastfeeding[a] × vit. D supplementation	No	before 2nd month	49	0.0092	0.9154	0.1308	–0.2537	0.2721
		No	before 3rd month	22	0.3093	1.3641	0.2908	–0.2956	0.9141
		No	before 4th month	25	–0.2395	1.1903	0.2381	–0.7309	0.2518
		No	since 4th month or later or no	32	–0.1982	0.8862	0.1567	–0.5177	0.1213
		Yes	before 2nd month	232	0.0523	0.9267	0.0608	–0.0675	0.1722
		Yes	before 3rd month	67	0.1471	0.9442	0.1154	–0.0833	0.3774
		Yes	before 4th month	65	–0.1363	0.9897	0.1228	–0.3815	0.1089
		Yes	since 4th month or later or no	72	–0.0170	0.8740	0.1030	–0.2224	0.1884

Conclusion

Supplementation of vitamin D was different in the two analysed cohorts. The children who were born after one decade were given vitamin D earlier and more often.

Cohort I was more vulnerable to vitamin D deficiency and depended more on the season of birth and on the type of feeding.

Birth weight and length were significant independent variables for weight, height and BMI parameters.

Our study revealed that children who were not breastfed and who were born in summer were more vulnerable to vitamin D deficiency, which resulted in a different body weight and BMI in further life.

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Article

Familial factors more importantly modify the age of achieving motor developmental milestones than duration of breastfeeding amongst Polish children

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With 4 tables

Abstract: Breastfeeding in the first year of life is an important factor that may modify the child's later development.The aim of this study was to examine the association between the duration of breastfeeding and the time of achieving motor development of Polish children taking into account their familial background. The study material comprised 460 individuals including 252 boys and 208 girls aged 9 to 56 months who were born at term (37–42 weeks) selected in years 1993–1997. The investigated dependent variables were: first attempts of sitting, standing and walking (months). The independent variables were divided into: explanatory variable – the duration of breastfeeding, and other covariates – birth weight, the paternal and maternal age at the time of childbirth, maternal tobacco smoking during and after pregnancy as well as the parental level of education. The results of regression models after removing the prenatal and familial factors did not indicate a significant relationship between the duration of breastfeeding and sitting up ($F = 0.03$, $p = 0.8569$), standing up ($F = 0.79$, $p = 0.3741$) and walking variability ($F = 0.20$, $p = 0.6568$) in studied group of children. The results showed that parental age and education may affect the offspring's gross motor development, though effect sizes are small and directions of influence vary between fathers and mothers. The study underlines the importance of familial factors over the breastfeeding duration impact on time of developmental milestones.

Keywords: child development; familial factors; breastfeeding; motor development; Poland

Introduction

The type of infant feeding in the first year of life is an important factor that significantly modifies the child's later development. The type of feeding in the first year influences, among others, weight and height, cognitive skills, the occurrence of asthma and allergic diseases, immunity, as well as lower-body explosive strength, flexibility, balance and motor development into later years (Lanting et al. 1994; Scholtens et al. 2009; Oddy et al. 2011; Nyaradi et al. 2015; Grace, et al. 2017; Pruszkowska-Przybylska et al. 2018; Pruszkowska-Przybylska et al. 2019).

It follows from existing studies that what is important is not only the nutritional values of maternal milk, but also the physical contact between the mother and the infant during feeding, which has a strong influence on the formation of the offspring's cognitive skills and also modulates the response of the hypothalamic-pituitary-adrenal (HPA) axis, resulting in the child's lower susceptibility to stress in later life, regulates the correct circadian cycle of waking and sleeping and also strengthens functioning in a social group, with better social assimilation in later development (Liu et al. 1997; Feldman et al. 2014.)

What is significant for the shaping of the child's psychomotor development is also the mother's state of health, as women who undertake breastfeeding achieve better and more rapid return to physical and mental wellbeing after the postpartum period (American Academy of Pediatrics 2012). Studies also indicate that among women who have decided on breastfeeding, the likelihood of ovarian and breast cancer, of osteoporosis and of type 2 diabetes is reduced (Li et al. 2008; Victora et al. 2016).

The critical moments in a child's physical and mental development have been termed "developmental milestones"

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(Gerber et al. 2010). Motor skills have been divided into two groups: so-called gross motor skills, including independent sitting up, standing up, walking; and so-called fine motor skills, including motor skills of the fingers and of the hands, and thus manual skills (Piek et al. 2008; Gerber et al. 2010). The first attempts to sit up, to stand up and to walk are significant developmental milestones in motor functioning, which influence the later physiology and psychology of adults (Vereijken et al. 2009).

The age at which children achieve successive developmental milestones is modified by many factors. Delayed milestones are associated with the child's exposure to parental violence and psychological distress (Gutierrez-Galve et al. 2015), with parental psychosis (Keskinen et al. 2015), with a lower social status of parents (Flensborg-Madsen & Mortensen 2015), with early childhood malnutrition assessed by smaller length for age (Groos 1991), with low or very low birth weight (Villegas et al. 2009), or with intrauterine exposure to low-level methyl mercury (Prpić et al. 2017), and delayed age at first tooth emergence (Żądzińska et al. 2016). According to Flensborg-Madsen & Mortensen (2017), gestational age, birth weight, breastfeeding, having lived in a full-time institution, and weight and height increase in the first year were significantly associated with milestone achievement in the first year of life. On the other hand, prenatal factors such as in utero exposure to polychlorinated biphenyls (PCBs), dichlorodiphenyldichloroethylene (DDE) or maternal serum cotinine do not seem to be related to delayed motor development milestones in children (Høyer et al. 2015; Christensen et al. 2016). The benefits of breastfeeding for children's motor development may be modified, however, by a range of individual, parental and environmental factors.

The aim of this study was to examine the association between the duration of breastfeeding and the age of achieving motor development of Polish children including the effect of the prenatal and environmental variables.

Material and methods

There were investigated 765 healthy individuals randomly selected attending nurseries. However, due to the lack of response, the final sample was narrowed. The percentage of missing data was the following: maternal age 2.74%, paternal age 7.58%, maternal education 3.01%, paternal education 5.88%, breastfeeding 2.22%, maternal smoking 6.01%, gestational age 19.84%. There were no statistically significant differences in achieving all analysed developmental milestones by children between groups with all information and with missing data. The final sample comprised anthropometric and questionnaire data collected on 460 healthy children (252 boys and 208 girls) attending nurseries, aged 9 to 56 months, born at term (37–42 weeks) in the years 1993–1997 in the industrial city of Łódź (central Poland). The questionnaire filled in by the children's mothers included items concerning the age at which the children began to sit (months), the age at which the children began to stand unaided (months), the age at which the children began to walk (months), as well as items concerning prenatal, maternal and socio-economic factors, such as birth weight, gestational age, the mother's age and the father's age at the time of birth, maternal smoking during and after pregnancy, the level of education of both parents, as well as the type of feeding in the first year of life, and – in the case of breastfed children – information about the duration of breastfeeding (months). All data were obtained voluntarily with approval of surveyed individuals.

The birth weight of each examined child was standardised (z-score) for gestational age (weeks) and for sex according to the L, M and S traits for the weight of newborns born in Łódź at term, which were calculated according to Cole's method (Rosset 2009).

The mothers' and the fathers' level of education was categorised as: (1) basic vocational education (2) secondary education and (3) higher education.

The study was retrospectively registered by Ethical Commission of University of Łódź (NR21/KBBN-UŁ/I/2018).

Statistical analysis

Mann-Whitney's U test was applied to present characteristics of the research group separately for boys and girls in terms of motor development, duration of breastfeeding and the age of parents at the time of delivery. Non-parametric correlation (Spearman's "rho") was used to find correlations between the duration of breastfeeding and prenatal and familial factors.

In order to exclude the influence of interrelated prenatal and familial factors on the variability of analysed developmental milestones, linear regression analysis was used. Standard regression and progressive stepwise regression models were performed. The best explanatory models, according to adjusted R^2 values were selected for the variability of the age of independent sitting, standing and walking (dependent variables) including in each model the parental age and education, maternal smoking and the birth weight standardised on sex and gestational age (independent variable). Designed models let us to obtain the residues that were considered to be a part of the total variability of motor development. For each developmental milestone as a dependent variable residues obtained were used to evaluate the relationship of motor development with the duration of breastfeeding removing the influence of familial factors.

All calculations were performed using STATISTICA 12.0 software (StatSoft Poland).

Results

In Tables 1–2 were presented the basic statistical characteristics of the studied group of children with respect to the anal-

Table 1. Characteristics of the research group by sex in terms of motor development, duration of breastfeeding and the age of parents at the time of delivery.

Variables	Total		Boys		Girls		Z	*p*-value
	N = 460		N = 252		N = 208			
	Median	Q_1–Q_3	Median	Q_1–Q_3	Median	Q_1–Q_3		
Sitting up (months)	6	5–6	6	5–6	6	5–6	0.96	0.3347
Standing up (months)	8	7–9	8	7–9	8	7.5–9	–1.33	0.1823
Walking (months)	11	10–12	11	10–12	11	10–12	0.42	0.6726
Duration of breastfeeding (months)	3	1–7	3	1–7	3	1–6	0.42	0.6763
Paternal age (years)	26	23–30	26	23–31	26	23–29.5	0.78	0.4325
Maternal age (years)	24	22–28	23.5	22–28	24	22–27	0.18	0.8568

N sample size, Q_1 lower quartile, Q_3 upper quartile, Z- and *p*-value U Mann-Whitney test

Table 2. Correlations between the duration of breastfeeding and prenatal and family factors.

Variable	& other variables	R	*p*-value
Duration of breastfeeding (months)	Paternal age (years)	0.044	0.3416
	Maternal age (years)	0.020	0.6663
	Paternal education (categories)[a]	0.214	< 0.0001
	Maternal education (categories)[b]	0.271	< 0.0001
	Maternal smoking (categories)[c]	–0.170	0.0002
	Birth weight (z-score)	0.004	0.9374
Paternal age (years)	Maternal age (years)	0.784	< 0.0001
	Paternal education (categories)[a]	0.241	< 0.0001
	Maternal education (categories)[b]	0.172	0.0002
	Maternal smoking (categories)[c]	0.029	0.5412
	Birth weight (z-score)	0.087	0.0614
Maternal age (years)	Paternal education (categories)[a]	0.254	< 0.0001
	Maternal education (categories)[b]	0.280	< 0.0001
	Maternal smoking (categories)[c]	0.001	0.9899
	Birth weight (z-score)	0.103	0.0266
Paternal education (categories)[a]	Maternal education (categories)[b]	0.606	< 0.0001
	Maternal smoking (categories)[c]	–0.208	< 0.0001
	birth weight (z-score)	0.035	0.4603
Maternal education (categories)[b]	Maternal smoking (categories)[c]	–0.281	< 0.0001
	Birth weight (z-score)	0.056	0.2294
Maternal smoking (categories)[c]	Birth weight (z-score)	–0.116	0.0130

R- and *p*-value non-parametric correlation (Spearman's "rho").
[a] Paternal education: Basic n = 249 (54.13%), Secondary n = 134 (29.13%), Higher n = 77 (16.74%); [b] Maternal education: Basic n = 147 (31.95%), Secondary n = 247 (53.70%), Higher n = 66 (14.35%); [c] Maternal smoking: No n = 218 (47.39%) vs Yes n = 242 (52.61%).

ysed traits. For 50% of the investigated children the average age of sitting was between 5 and 6 months, standing 7–9 months, walking 10–12 months and the average duration of breastfeeding was 1–7 months. There were no significant differences in motor development and duration of breastfeeding between boys and girls (Table 1).

The duration of breastfeeding was positively correlated with the level of parental education (respectively for fathers: R = 0.214, $p < 0.0001$, and mothers: R = 0.271, $p < 0.0001$) and negatively correlated with maternal smoking (R = –0.170, $p = 0.0002$).

The percentage of parents with higher education in the examined group of children fluctuated between 14 and 17%, respectively for fathers – 16.74% and mothers – 14.35% (Table 2). More than half of mothers (52.61%) smoked tobacco after pregnancy or/and during pregnancy.

The increasing level of parental education positively correlated with their age (R = 0.241, $p < 0.0001$, R = 0.280, $p < 0.0001$) and negatively with maternal smoking (respectively: R = –0.208, $p < 0.0001$; R = –0.281, $p < 0.0001$). Beside, maternal smoking negatively correlated with the birth weight standardized on sex and gestational age of the offspring (R = –0.116, $p = 0.0130$). Birth weight (z-score) was positively correlated with maternal age (R = 0.103, $p = 0.0266$).

Standard regression and progressive stepwise regression models were performed for each dependent variable to check influence of familial and prenatal factors. The variables positively associated with sitting up were: higher maternal education (vs. basic) (Beta = 0.172; $p = 0.0141$) and secondary maternal education (vs. basic) (Beta = 0.113; $p = 0.0304$) and negatively associated with sitting up was paternal education (vs. basic) (Beta = –0.133; $p = 0.0378$). The variable positively associated with standing up was paternal age (Beta = 0.155; $p = 0.0254$) and negatively associated with standing up was maternal age (Beta = –0.137; $p = 0.0478$). The variable positively associated with walking was paternal age (Beta = 0.171; $p = 0.0134$) and negatively associated with walking were paternal education higher (vs. basic) (Beta = –0.151; $p = 0.0262$) and paternal education secondary (vs. basic) (Beta = –0.106; $p = 0.0423$) (Table 3).

Obtained residues for each developmental milestone as dependent variables in linear regression models were used to evaluate the relationship of motor development with the duration of breastfeeding removing the influence of familial factors. The results of regression analyses did not indicate a significant relationship between the duration of breastfeeding and time of sitting up (F = 0.03, $p = 0.8569$), standing up (F = 0.79, $p = 0.3741$) and walking (F = 0.20, $p = 0.6568$) in studied group of children (Table 4).

Table 3. Linear regression models to explain the variability of motor development milestones dependent on prenatal and familial factors.

Dependent variables	Independent variables	Beta	SE	t	*p*-value	Adjusted R^2	F	*p*-value
Sitting up (months)	Paternal age (years)	–	–	–	–	0.016	2.83	0.0244
	Maternal age (years)	–	–	–	–			
	Paternal education higher[a]	–0.133	0.064	–2.08	0.0378			
	Paternal education secondary[a]	–	–	–	–			
	Maternal education higher[a]	0.172	0.070	2.46	0.0141			
	Maternal education secondary[a]	0.113	0.052	2.17	0.0304			
	Maternal smoking -Yes[b]	–	–	–	–			
	Birth weight (z-score)	–0.082	0.046	–1.76	0.0792			
Standing up (months)	Paternal age (years)	0.155	0.069	2.24	0.0254	0.007	2.63	0.0430
	Maternal age (years)	–0.137	0.069	–1.98	0.0478			
	Paternal education higher[a]	–	–	–	–			
	Paternal education secondary[a]	–	–	–	–			
	Maternal education higher[a]	–	–	–	–			
	Maternal education secondary[a]	–	–	–	–			
	Maternal smoking -Yes[b]	–	–	–	–			
	Birth weight (z-score)	–	–	–	–			
Walking (months)	Paternal age (years)	0.171	0.069	2.48	0.0134	0.048	5.59	< 0.0001
	Maternal age (years)	–	–	–	–			
	Paternal education higher[a]	–0.157	0.067	–2.33	0.0202			
	Paternal education secondary[a]	–0.105	0.052	–2.03	0.0428			
	Maternal education higher[a]	0.133	0.071	1.87	0.0615			
	Maternal education secondary[a]	0.073	0.055	1.31	0.1902			
	Maternal smoking -Yes[b]	–	–	–	–			
	Birth weight (z-score)	–	–	–	–			

Beta standardized regression coefficient, SE standard error of standardized regression coefficient, t t-Student test value, R^2 coefficient of determination, F- and *p*-value for model.
Reference categories: [a] Basic vocational education; [b] No.

Table 4. Linear regression models to explain the variability of motor development milestones independent of prenatal and familial factors by duration of breastfeeding (months).

Dependent variables[a]	Independent variables	Beta	SE	t	*p*-value	Adjusted R^2	F	*p*-value
Sitting up (residuals)	Duration of breastfeeding (months)	0.008	0.047	0.18	0.8569	< 0.001	0.03	0.8569
Standing up (residuals)	Duration of breastfeeding (months)	0.042	0.047	0.89	0.3741	< 0.001	0.79	0.3741
Walking (residuals)	Duration of breastfeeding (months)	0.021	0.047	0.44	0.6568	< 0.001	0.20	0.6568

Beta standardized regression coefficient, SE standard error of standardized regression coefficient, t t-Student test value, R^2 coefficient of determination, F- and *p*-value for model.
[a] Dependent variables: part of the total variability of motor development milestones independent of prenatal and familial factors (received residues from regression models).

Discussion

Our results demonstrated that duration of breastfeeding did not differentiate the time of gross motor development among the investigated children. We presumed that times of developmental milestones were more interestingly modified by familial factors. Similar results were obtained by Stelmach et al. (2019); they also found that duration of breastfeeding was not statistically importantly associated with time of psychomotor development among Polish children, when confounding familial and prenatal factors were included in the analysis. The lack of statistically significant relationship between breastfeeding duration and gross motor development was presented by Siregar et al. (2018) among Indonesian children and also by Khan et al. (2019) among Pakistani children. On the other hand, Jardí et al. (2018) showed that Spanish infants breastfeed for at least four months had better psychomotor development during the first year of life. Moreover, Grace et al. (2017) showed that among Australian children breastfeeding ≥ 6 months had positive influence on their motor development.

Our findings that the offspring of older fathers start standing and walking later were confirmed by the results of studies which showed that the father's age is a significant factor modifying the neurodevelopmental and social functioning of children, which may be connected with motor development (Janecka et al. 2017).

On the other hand we also demonstrated that the older the women the earlier their offspring standing up is. Some authors explained this phenomenon in an indirect way by lower incomes, by a weak educational background and by a lack of health care and adequate support of the newborns among younger parents (Moffitt et al. 2002; Sutcliffe et al. 2012). To support our statement, we additionally obtained positive correlation between maternal age and maternal education - older mothers are better educated.

We have also observed that parental education is significantly associated with the time of sitting up and walking. Higher educated fathers had offspring sitting up and walking earlier. In the case of higher educated mothers the results were inverse and their children started standing up later. Probably better educated mothers have more absorbing work and have to return to work sooner after delivery causing developmental delay of their child (Acharya & Khanal 2015; Herich et al. 2017).

We argued that familial factors may be important moderating factors in gross motor development. Moreover, despite lack of statistical significance of breastfeeding duration more studies in this area is needed. Additionally, the most appropriate duration of breastfeeding is not known yet. There are simultaneously studies which underline that too short and too long duration of breastfeeding can cause a developmental delay. Also Dee et al. (2007) indicate that too long duration (≥ 9 months) of breastfeeding may slow down children's motor development.

We concluded that the duration of breastfeeding is a factor which does not influence the time of sitting and standing and walking among Polish children. Moreover, we presumed that parental age and education are important factors affecting the offspring's gross motor development.

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Association of the 2D:4D digit ratio with body composition among the Polish children aged 6–13 years

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ABSTRACT

The 2D:4D digit ratio is formed as a result of genetic factors but also prenatal exposure to sex hormones. The higher index value the higher concentration of the prenatal oestrogen. It is commonly known that testosterone is a hormone connected with muscle mass growth and that oestrogen affects adipogenesis. The aim of this study was to find if the digit ratio can be an informative indicator of the fat mass and muscle mass and body proportions in prepubertal children. Material and methods The analysed cohort included 420 children (221 girls and 199 boys) aged 6–13 years. Pearson's and Spearman's tests were conducted to assess whether 2D:4D was significantly correlated with the body composition measurements. Multiple regression models and stepwise forward regression were applied to select the most important independent variables affecting fat mass (%) and muscle mass (%) as well as the BMI and the WHR. Results The study shows that the digit ratio is negatively correlated with muscle mass (MM%) among girls ($p < 0.05$).There was no similar relationship in the group of boys. The regression models showed a significant role in determining the body composition and body proportions played by maternal factors such as: maternal level of education and weight gain during pregnancy. Conclusions The 2D:4D digit ratio seems to be an informative indicator of the muscle mass development since girls' early childhood. Moreover, maternal environment is also important in forming the offspring's body composition and proportions.

1. Introduction

The first study dealing with the proportion of the second finger (2D) to the fourth finger (4D) as an indicator of the sex-dependent biological condition was published in the 1950s, and it confirmed that men are characterised by a relatively longer fourth finger than the second finger (a lower 2D:4D digit ratio) [1]. Seeking the cause of this phenomenon, Manning et al. [2] published an article that put forward the hypothesis that the discussed proportion is caused by the effects of concentration of foetal sex hormones in early prenatal development.

A lower value of the 2D:4D digit ratio is connected with a higher prenatal concentration of testosterone and a lower prenatal concentration of oestrogen [3–5]. This is why the 2D:4D digit ratio can be used as a marker of prenatal exposure to androgens and oestrogens [3–7]. It has been shown that a high level of foetal testosterone with simultaneous low concentrations of oestrogen during prenatal life may decrease the 2D:4D digit ratio in the female offspring, resulting in the "male type", whereas a high oestrogen level in relation to testosterone can cause a higher value of the 2D:4D digit ratio, resulting in the "female type" in male offspring [4]. However, despite evidence for high testosterone and low oestradiol association with a low 2D:4D ratio, Lutchmaya et al. [4] reported only one significant correlation between the ratio of testosterone to oestradiol and right hand 2D:4D. They did not find significant correlation for left 2D:4D and neither were the correlations for the individual hormones.

Increased concentrations of testosterone or oestrogen can probably raise the asymmetry of physical features, also in the case of finger length [8]. The evidence of this phenomenon may be reaching different values of the digit ratio 2D:4D depending on the side of the body. These results indicate that prenatal hormone management is significantly associated with developmental homeostasis and that, for this reason, it may be used as a measurement of the biological condition [8,9].

According to literature, the environment during prenatal development affects the biological condition in future life [10]. Moreover, prenatal life has an impact on body proportions in the offspring's subsequent steps of ontogenesis [11].

The sex hormone distribution in prenatal period is significantly correlated with the incidence of various mental disorders (such as autism, dyslexia, migraine, stammering) but also immune dysfunction [12], myocardial infarction [13], breast and prostate cancer [14,15],

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facial attractiveness [16] or hand grip strength [17]. Therefore the 2D:4D digit ratio may serve as an indicator of the risk of certain diseases, attractiveness [18] or of the general biological condition [15,18].

There are some studies which link the digit ratio (2D:4D) with muscle body content [19] and strength [20]. Probably higher prenatal testosterone level may affect higher content of the muscle mass, resulting in greater strength among adults [20] but also among children [3]. The 2D:4D digit ratio is significantly lower among physically active people, what can be associated with higher content of the muscle mass [21]. In the case of fat mass Muller et al. (2013) [22] did not obtain strong negative connection with the digit ratio (2D:4D). The body proportion (BMI) may also be associated with digit ratio, among adults especially among man [20]. Ranson et al. [3] reported that higher value of digit ratio is associated with large body size.

Women with low WHR values (low waist and high hip measurements) are characterised by lower levels of male and higher levels of female sex hormones. The concentration of these hormones in the mother's blood is associated with foetal development resulting in phenomena that maternal WHR is negatively correlated with the 2D:4D digit ratio in children [23]. In turn, in the case of men, the value of the 2D:4D digit ratio is positively correlated with the WHR value determined by the testosterone level [24].

There are some other factors which are connecting with body composition and proportion among children. The important group of factors is these which are connected with prenatal, perinatal and neonatal environment. The examples of this kind of factors are: maternal educational level [25] maternal weight gain during pregnancy [26] or breastfeeding duration [27].

In the case of the significance of the 2D:4D digit ratio in the indication of body composition and body proportion, there are still few studies on groups of children, thus our study is an important contribution to obesity research. Children with obesity with high probability become obese adults in the future [28].

The aim of the study was to evaluate the relationship between the 2D:4D digit ratio and body composition during the chosen step of progressive ontogenesis- among children aged 6 to 13 years. There is few studies which tackle the problem of the fat and muscle mass in relation to the digit ratio (2D:4D) [3,29], thus conducted study seems to be an important contribution to knowledge about the digit ratio (2D:4D).

2. Material and methods

The study included 509 children from primary schools located in Łódź (population 780,000) in the central Poland. It consisted of two main parts: a survey and anthropological measurements. The survey was filled in by the children's parents that contained information about: maternal education, duration of breastfeeding, maternal weight gain during pregnancy. The second part including the following anthropological measurements: body weight (with an accuracy of 0.1 kg) using a scale, height (with an accuracy of 0.001 m) using an anthropometer, waist and hip circumference (with an accuracy of 0.001 m) using an anthropometric tape, the length of the second and the fourth digits in both hands (with an accuracy of 0.001 m) using directly by hands a sliding calliper (Vernier calliper), and also body composition measurements – fat (FM%) and muscle mass (MM%) using the bioelectrical impedance vector analysis (BIA-101 ASE, Akern, Italy).

The BIA method is based on measuring the electrical impedance in various body tissues that is the sum of geometric resistance (active resistance) and reactance (passive resistance) [30].

Plethysmograph with 4 electrodes covered with foil, placed in the midline of the dorsal surface of the hands and feet, was used for the examination. The measurements were carried out in the supine position of the patient, which helps to stabilize and balance the level of body fluids in the body. The measurements with the bioelectrical impedance analysis are easy to obtain, highly repeatable, and noninvasive [31].

All measurements were performed by qualified staff of the Department of Anthropology and the Biobank Laboratory of the University of Łódź [32]. Some of the measurements were used to calculate the following indexes: the 2D:4D digit ratio (the quotient of the length of the second digit and the fourth digit (mm)), the BMI – the body mass index (the quotient of body mass (kg) and the square of height (m^2)) and the WHR – the waist-to-hip ratio (the quotient of the waist and the hip circumference (mm)).

Due to a lack of some data that were not provided in the questionnaires (about maternal educational level, weight gain during pregnancy, gestational age and duration of breastfeeding) 89 cases were excluded. The final data base contained 420 individuals (199 boys and 221 girls) aged 6–13 years, born in term (37th–42nd gestational week).

The measurements of the digits from the right hand were chosen to analyses due to reported in literature greater sex difference than in left hand [33].

In order to analyse the type and the duration of feeding after birth, 3 categories were used. The first group included children who were formula-fed or breastfed for a period shorter than 3 months, the second category included children who were breastfed for a period of 3 to 6 months, and the third category included children who were breastfed for a period longer than 6 months.

The level of education among the children's mothers was categorised as: (1) basic/vocational education (8 years at obligatory primary school plus 3 years at vocational school); (2) secondary education (4–5 years at secondary school, confirmed by the General Certificate of Secondary Education (A-level)) or bachelor degree; and (3) higher education (full university degree).

Maternal weight gain during pregnancy was a continuous variable expressed in kilograms (kg).

The study was accepted by the Ethical Commission at the University of Lodz (nr 19/KBBN-UŁ/II/2016).

In order to conduct a statistical analysis, dependent variables (MM %, FM%, BMI, WHR) were standardised for calendar age within the analysed group of children. The following equation was used to determine the z-score value for (MM%, FM%, BMI, WHR) z-score = (x–mean) / SD, where: x – the individual's value for the analysed parameter; mean – the mean of the analysed age group; SD – the standard deviation of the created group. In order to lack of changing in 2D:4D index along with the investigated individuals' age it was not standardised on age.

2.1. Statistical analysis

Depending on the type of the analysed data to check the sex differences the following tests were used: the Chi^2 test in the case of quantitative variables (the duration of breastfeeding and the maternal level of education), Student's *t*-test in the case of continuous variables with normal distribution (the 2D:4D digit ratio and the MM% z-score) or Mann-Whitney *U* test for continuous variables which were not normal distributed (the maternal weight gain during pregnancy, FM% z-score, the WHR z-score, and BMI z-score).

In order to assess the connection between the 2D:4D digit ratio and the percentage composition of the MM%, FM% and the WHR and BMI among the boys and the girls, the Pearson or the Spearman correlation was applied depending on the normality of distribution of the analysed variables. Multiple regression and stepwise multiple regression were used to select the most important factors that explained the variability of the analysed dependent variables.

All analyses were conducted using the STATISTICA PL.12.0. software.

3. Results

In the analysed sample, important sexually dimorphic differences were observed in the case of the 2D:4D digit ratio in the right hand, the

Table 1
Characteristics of the children of the cohort in terms of analysed features.

Variable		Total N = 420	Boys N = 199	Girls N = 221	Sex differences p-value*
Breastfeeding (months) n (%)	< 3	95 (22.62)	51 (25.63)	44 (19.91)	0.375[a]
	3–6	106 (25.24)	48 (24.12)	58 (26.24)	
	> 6	219 (52.14)	100 (50.25)	119 (53.85)	
Maternal education n (%)	Basic or vocational	26 (6.19)	14 (7.04)	12 (5.43)	0.199[a]
	Secondary or post-secondary	114 (27.14)	61 (30.65)	53 (23.98)	
	Higher	280 (66.67)	156 (70.59)	156 (70.59)	
Maternal weight gain during pregnancy (kg)	Mean	15.37	15.82	14.97	0.069[c]
	SD	6.512	5.98	6.95	
	Median	15.00	15.00	14.00	
	Q1	20.00	20.00	18.00	
	Q3	10.00	11.00	10.00	
2D/4D digit ratio	Mean	0.97	0.97	0.98	0.001[b]
	SD	0.03	0.03	0.03	
	Median	0.97	0.97	0.98	
	Q1	0.99	0.99	1.00	
	Q3	0.95	0.95	0.96	
MM (%) z-score	Mean	−0.01	0.08	−0.10	0.062[b]
	SD	0.95	1.00	0.91	
	Median	−0.06	0.13	−0.15	
	Q1	0.63	0.82	0.46	
	Q3	−0.70	−0.69	−0.76	
FM (%) z-score	Mean	0.01	−0.06	0.06	0.125[c]
	SD	0.99	1.05	0.93	
	Median	−0.10	−0.17	0.01	
	Q1	0.64	0.66	0.64	
	Q3	−0.71	−0.84	−0.63	
BMI z-score	Mean	0.01	0.14	−0.12	0.011[c]
	SD	0.99	1.05	0.92	
	Median	−0.25	−0.14	−0.34	
	Q1	0.48	0.70	0.31	
	Q3	−0.7	−0.63	−0.73	
WHR z-score	Mean	0.01	0.33	−0.29	< 0.001[c]
	SD	0.99	0.92	0.96	
	Median	−0.13	0.16	−0.43	
	Q1	0.66	0.97	0.32	
	Q3	−0.69	−0.34	−0.99	

* Sex differences were assessed using the Pearson's Chi-square[a], Student's t-test[b] or Mann-Whitney U test[c].

BMI and the WHR. The digit ratio values were higher among the girls (mean = 0.98; SD = 0.03) than among the boys (mean = 0.97; SD = 0.03). The girls were characterised by a lower BMI and WHR respectively (Me = −0.34; Me = −0.43) than the boys (Me = −0.14; Me = 0.16) (Table 1).

The next step of the analysis was to assess the correlation between the 2D:4D digit ratio and the FM% and also the MM%. The Spearman correlation showed that in the group of girls and in the group of boys, the correlation between the FM%, BMI and WHR and the digit ratio was not statistically significant (respectively: boys R = 0.11, p = 0.12; R = 0.04, p = 0.61; R = 0.03, p = 0.68 and girls R = 0.04; p = 0.60; R = −0.02, p = 0.74; R = 0.05, p = 0.42). However, the positive value of R shows the tendency that greater values of the digit ratio are correlated with greater values of the fat mass, especially in the group of boys. In the case of the correlation between the 2D:4D digit ratio and the MM%, the Pearson test showed that in both groups (boys and girls), a negative association was found between digit ratio values and muscle mass. The lower were the values of the digit ratio, the higher was the observed value of the MM% both in the group of boys (r = −0.14; p = 0.04) and in the group of girls (r = −0.13; p = 0.05).

3.1. Regression models

Multiple regression was used to select the most significant predictors that explained the analysed dependent variables (FM%, MM%, BMI, WHR). All dependent variables were standardised for children's age (z-score value). The predictors that were included in the statistical models were: the maternal level of education, the maternal weight gain during pregnancy, the type of feeding and the duration of breastfeeding in the first years after birth, the value of the 2D:4D digit ratio and, respectively, the MM%, the FM%, the BMI, the WHR, excluding those variables that were treated as dependent variables in the model. The idea of including the dependent variables as an independent variables stem from interrelation between them (FM% × MM% r = −0.90; p < 0.05; FM% × BMI r = 0.77; p < 0.05; FM% × WHR r = 0.40; p < 0.05; MM% × BMI r = −0.56; p < 0.05; MM% × WHR r = −0.34; p < 0.05; BMI × WHR r = 0.46; p < 0.05).

For all dependent variables multiple regression models separately for both sexes were done. Results for these models are included in supplementary data (Tables S1–S2). Afterwards, stepwise forward multiple regression was applied to select the group of the most important variables for each dependent variable.

The first model of stepwise forward multiple regression included muscle mass (MM%) as a dependent variable among girls showed that, four independent variables remained in the model: the BMI (Beta = 0.29; p < 0.001) and the maternal level of education (Beta = 0.10; p < 0.001), which were positively connected with MM% and the 2D:4D digit ratio (Beta = −0.10; p < 0.001), the FM (Beta = −1.07; p < 0.001), which was negatively connected with the MM%. Despite lack of the statistically significance of maternal weight gain during pregnancy and duration of breastfeeding, these independent variables stayed in model due to booster the model. The corrected coefficient of determination was 0.8 which means that the predictors in the model explained the variability of muscle mass approximately in 80% (Table 2). The highest value of the individual coefficient of determination was obtained respectively for the BMI, the

Table 2
A forward stepwise multiple regression model containing all the most important independent variables explaining FM (%) z-score, MM (%) z-score, BMI z-score, WHR z-score (standardised for calendar age) variation among girls.

Variable	Independent variables	Beta	SE Beta	b	SE b	t	p	Partial R^2
MM (%) z-score	Intercept	–	–	2.48	0.87	2.83	0.005	–
	FM (%) z-score	−1.07	0.05	−1.04	0.05	−22.46	< 0.001	0.59
	BMI z-score	0.29	0.04	0.28	0.05	5.92	< 0.001	0.62
	Maternal education (higher)[a]	0.10	0.03	0.20	0.06	3.19	0.002	0.09
	2D/4D digit ratio	−0.09	0.03	−2.78	0.89	−3.09	0.002	0.03
	Maternal weight gain during pregnancy (kg)	0.04	0.03	0.01	0.00	1.38	0.170	0.04
	Breastfeeding 3–6 (months)[b]	0.03	0.03	0.07	0.06	1.10	0.272	0.04
FM (%) z-score	Intercept	–	–	1.25	0.71	1.76	0.081	–
	MM (%) z-score	−0.65	0.03	−0.67	0.03	−21.95	< 0.001	0.35
	BMI z-score	0.41	0.03	0.42	0.03	13.68	< 0.001	0.38
	Maternal education (higher)[a]	−0.09	0.03	−0.19	0.05	3.57	< 0.001	0.13
	Maternal weight gain during pregnancy (kg)	0.05	0.02	0.01	0.00	2.10	0.037	0.04
	2D/4D digit ratio	−0.05	0.02	−1.43	0.73	−1.95	0.052	0.05
	Breastfeeding > 6 (months)[b]	−0.03	0.03	−0.06	0.05	−1.22	0.225	0.08
	WHR z-score	0.03	0.03	0.03	0.03	1.17	0.242	0.20
BMI z-score	Intercept	–	–	0.27	0.10	2.48	0.014	–
	FM (%) z-score	1.13	0.08	1.11	0.08	13.88	< 0.001	0.77
	MM (%) z-score	0.48	0.08	0.49	0.08	6.02	< 0.001	0.76
	Maternal education (higher)[a]	−0.17	0.04	−0.33	0.08	−4.08	< 0.001	0.07
	Breastfeeding 3–6 (months)[b]	−0.08	0.04	−0.16	0.08	−1.94	0.054	0.02
	WHR z-score	0.07	0.04	0.07	0.04	1.69	0.093	0.19
	Maternal weight gain during pregnancy (kg)	−0.06	0.04	−0.09	0.01	−1.43	0.152	0.03
WHR z-score	Intercept	–	–	0.12	0.18	0.66	0.510	–
	FM (%) z-score	0.26	0.10	0.27	0.10	2.61	0.008	0.15
	Maternal education (higher)[a]	−0.09	0.07	−0.20	0.14	−1.48	0.140	0.13
	BMI z-score	0.16	0.09	0.17	0.10	1.67	0.096	0.61
	Breastfeeding > 6 (months)[b]	−0.09	0.06	−0.17	0.12	−1.42	0.158	0.08
	Maternal weight gain during pregnancy (kg)	−0.08	0.06	−0.01	0.10	−1.30	0.194	0.03

Reference values: [a]basic or vocational; [b] < 3 (months).
FM (%) z-score: R = 0.94; R^2 = 0.88; Adjusted R^2 = 0.87; F = 224.94; $p < 0.001$; Standard error of estimation: 0.33; MM (%) z-score: R = 0.89; R^2 = 0.80; Adjusted R^2 = 0.79; F = 144.81; $p < 0.001$; Standard error of estimation: 0.41; BMI z-score: R = 0.82 R^2 = 0.67; Adjusted R^2 = 0.66; F = 73.71; $p < 0.001$; Standard error of estimation: 0.53; WHR z-score: R = 0.45; R^2 = 0.20; Adjusted R^2 = 0.18; F = 10.94; $p < 0.001$; Standard error of estimation: 0.87.
Categories for: breastfeeding (months): < 3; 3–6; > 6 and maternal education: basic or vocational; secondary or post-secondary; higher.

FM%, the maternal level of education and the 2D:4D digit ratio.

In the case of the boys, forward stepwise regression showed that the most suitable variables in the model were the BMI (Beta = 0.36; $p < 0.001$) and the FM% (Beta = −1.19; $p < 0.001$) with presence of duration of breastfeeding in the model (Table 3). All predictors explained 86% of the variability of the MM% in boys.

The forward stepwise regression model constructed to assess the variability of the FM% showed that in the group of girls, statistically significant independent variables were: the BMI (Beta = 0.41; $p < 0.001$) the maternal weight gain during pregnancy (Beta = 0.05; p = 0.037) with a positive effect and the MM% (Beta = −0.65; $p < 0.001$) and level of education (Beta = −0.09; $p < 0.001$) with a

Table 3
A forward stepwise multiple regression model containing all the most important independent variables explaining FM (%) z-score, MM (%) z-score, BMI z-score, WHR z-score (standardised for calendar age) variation among boys.

Variable	Independent variables	Beta	SE Beta	b	SE b	t	p	Partial R^2
MM (%) z-score	Intercept	–	–	0.93	0.84	1.11	0.267	–
	FM (%) z-score	−1.19	0.05	−1.13	0.04	−25.99	< 0.001	0.65
	BMI z-score	0.36	0.05	0.34	0.04	7.92	< 0.001	0.65
	2D/4D digit ratio	−0.03	0.03	−1.00	0.86	−1.16	0.246	0.02
FM (%) z-score	Intercept	–	–	−0.05	0.03	−2.14	0.034	–
	MM (%) z-score	−0.65	0.03	−0.69	0.03	−26.22	< 0.001	0.35
	BMI z-score	0.41	0.03	0.41	0.03	16.52	< 0.001	0.36
	Breastfeeding 3–6 (months)[a]	−0.02	0.02	−0.06	0.05	−1.10	0.272	0.01
BMI z-score	Intercept	–	–	−0.03	0.11	−0.28	0.782	–
	FM (%) z-score	1.31	0.09	1.31	0.09	15.16	< 0.001	0.83
	MM (%)z-score	0.64	0.08	0.67	0.09	7.65	< 0.001	0.81
	WHR z-score	0.14	0.04	0.16	0.05	3.49	< 0.001	0.20
	Maternal weight gain during pregnancy (kg)	0.05	0.04	0.00	0.01	1.45	0.148	0.03
WHR z-score	Intercept	–	–	0.39	0.08	4.74	< 0.001	–
	BMI z-score	0.42	0.08	0.37	0.07	5.46	< 0.001	0.35
	Breastfeeding > 6 (months)[a]	−0.11	0.06	−0.21	0.11	−1.82	0.070	0.01
	MM (%) z-score	−0.11	0.08	−0.10	0.07	−1.44	0.150	0.36

Reference values: [a] < 3 (months).
FM (%) z-score: R = 0.96; R^2 = 0.92; Adjusted R^2 = 0.92; F = 763.30; $p < 0.001$; Standard error of estimation: 0.30; MM (%) z-score: R = 0.93; R^2 = 0.86; Adjusted R^2 = 0.86; F = 390.26; $p < 0.001$; Standard error of estimation: 0.38; BMI z-score: R = 0.87; R^2 = 0.75; Adjusted R^2 = 0.75; F = 145.70; $p < 0.001$; Standard error of estimation: 0.53; WHR z-score: R = 0.51; R^2 = 0.26; Adjusted R^2 = 0.25; F = 23.26; $p < 0.001$; Standard error of estimation: 0.80.
Categories for: breastfeeding (months): < 3; 3–6; > 6.

negative effect with control of 2D/4D digit ratio, duration of breastfeeding and WHR (Table 2). The constructed model explained 87% of the variability of fat mass among the girls.

In the group of boys, the predictor with a positive association with the FM% was, similarly as in the group of girls, the BMI (Beta = 0.41; $p < 0.001$). Moreover, the MM% was negatively associated with the BMI (Beta = −0.65; $p < 0.001$) including duration of breastfeeding in model (Table 3). The regression model explained in total 92% of the variability of fat tissue.

The next stepwise multiple regression model was constructed to explain the variability of the BMI. In the group of girls, the parameters that was positively associated with the BMI were the FM% (Beta = 1.13; $p < 0.001$) and the MM% (Beta = 0.48; $p < 0.001$), and the parameter that is negatively associated with the BMI was the maternal higher education (Beta = −0.17; $p < 0.001$) with the control of duration of breastfeeding, WHR and maternal weight gain during pregnancy (Table 2). The above-mentioned indicators explained the variability of the BMI in 66%.

In the group of boys, the independent variables that were positively associated with the BMI were, FM% (Beta = 1.31; $p < 0.001$), the MM % (Beta = 0.64; $p < 0.001$) and the WHR (Beta = 0.14; $p < 0.001$) with maternal weight gain during pregnancy in model. All predictors included in the model explained 75% of the variability of the BMI among boys (Table 3). The last stepwise forward regression models were prepared for the WHR separately for both sexes (Tables 2–3). The model for girls showed that the independent variable that was positively connected with the WHR was the FM% (Beta = 0.26; $p = 0.008$) with maternal education, BMI z-score, breastfeeding duration and maternal weight gain during pregnancy in model. All variable included in the model explained 18% of the variability of the dependent variable (Table 2).

In the case of boys, the variable which was positively associated with the WHR was the BMI (Beta = 0.42; $p < 0.001$), with duration of breastfeeding and MM% in the model. All factors in model explained 25% of the variability of the WHR (Table 3).

4. Discussion

The importance of factors which could be a marker of obesity has been rising still in order to worldwide problem of obesity among children [34]. For these reasons the results of our studies which include the significance of digit ratio 2D:4D as a marker of muscle tissue content especially among girls aged 6–13 years poses an important knowledge.

Moreover in the examined group of children, there are statistically significant differences in the finger formula (2D:4D) between the girls and the boys. The direction of the differences was to be expected – boys are characterised by lower values of the 2D:4D digit ratio of the right hand, i.e. a proportionally longer fourth finger. This result is confirmed by previous studies conducted on groups representing various human populations [4–7], also on groups of children [3]. This phenomenon is related to differences in exposure to testosterone and oestrogens during prenatal development [4,7]. According to researchers, oestrogen stimulates the multiplication of fat cells [35], while testosterone is associated with the production of muscle tissue [36]. It may therefore be expected that a higher prenatal oestrogen level in relation to testosterone, phenotypically visible in the form of a proportionately longer second than the fourth finger [2] may be correlated with metabolic mechanisms resulting in a larger number of fat cells, also at further stages of a child's development. There are numerous studies of the relationship between the 2D:4D digit ratio and many phenotypic and behavioural features in adult men and women [23]. However, there are still few studies linking prenatal hormone levels using the 2D:4D digit ratio with body composition disorders and weight increase in children. According to Crocker et al. [37], a higher oestrogen concentration in relation to testosterone in the prenatal period affects increased adipogenesis, as well as increased insulin resistance in adolescents. Other researchers [38] stated that obese boys were characterised by higher oestrogen concentrations than the control group. In addition, the volume of their testes was smaller than among healthy boys. The above-mentioned study is evidence of a lower distribution of testosterone in obese boys.

In our study, we found a significant tendency to increased muscle mass content among girls with a lower, "male" type 2D:4D digit ratio. It was probably caused by a reduced exposure of these girls to female sex hormones (stimulating adipogenesis) in the prenatal period, but an increased exposure to testosterone, which resulted in higher muscle mass content in their subsequent stages of growth and development. We observed a similar effect in boys but only it did not remain significant once when covariates had been controlled for in the analysis.

An important point is the relation between fat tissue and muscle tissue. The amount of fat tissue probably depends on the amount of muscle tissue, because it is metabolically more active, thus it requires more energy for proper functioning, having a significant impact on the basic metabolism [39]. Therefore, probably the amount of adipose tissue may be adjusted by the distribution of muscle tissue.

In a study of a group of 15-year-old children, Hou et al. [40] showed that glucose metabolism was much more efficient in people with higher testosterone levels, which was associated with a higher percentage of muscle tissue. In our study, the 2D:4D digit ratio proved to be significant as an indicator which explained the variance of the girls' muscle mass almost in 3%. It seems to be a good indicator of muscle tissue at the age 6–13 years among girls, because the final proportion between the second and the fourth finger is established in girls at the age of about 2 years, differently from boys, in whose case the final proportion is established later [41]. This may be an explanation of the lack of significance of the 2D:4D digit ratio in boys in our study. Noteworthy, Trivers et al. [42] but also Richards et al. [3] claimed that 2D:4D ratio is age dependent and increased slightly throughout the childhood and decrease during adulthood.

However, the results of studies performed on adult men are inconclusive. Similarly, studies of men living in the Philippines showed no significant impact of the 2D:4D digit ratio on the content of muscle tissue [43]. Moreover, there are studies indicating a significant relationship between the 2D:4D digit ratio and the amount of muscle tissue and strength in groups of adult women and men [19]. It is possible that prenatal hormonal regulation affects an altered muscle composition in another postnatal ontogenetic phase depending on sex. Similar research is missing for children at the prepubertal and early pubertal age – the age at which eating habits as well as habits of physical activity are shaped. Moreover early pubertal age is a stage in the human ontogenesis when sex hormone concentrations change quite drastically. For this reason, our study is all the more important.

Apart from the 2D:4D digit ratio considered in this paper, the influence of the other independent variables was also controlled, which also proved to be significant in explaining the variability of body composition.

An important result is also the fact that the maternal education and weight gain during pregnancy modified the muscle mass, the fat mass as well as the BMI of the examined girls. Maternal higher education is associated with more muscle mass, a lower fat mass and a lower BMI of girls, thus it seems to be an independent modifier that "protects" against excess body weight and its composition, leading to early obesity. Better educated mothers are more aware of how to prepare wholesome meals to develop muscle mass [44,45]. Those mothers also more often encourage children to take up physical activity, which also involves additional expenses, and better educated mothers are often people with a better financial status. Daughters of mothers whose body mass was characterised by a higher growth during pregnancy, showed higher values of adipose tissue, which is associated with genetic predispositions, but also with epigenetic regulation caused by too much food supplied during the prenatal period [46].

Interestingly, the BMI of the girls in the age group 6–13 is not related to the WHR. There is a positive relationship of the WHR with the BMI in boys. This may be explained by different body proportions among boys and girls. Despite a larger BMI, girls may not show such differences in the WHR, because adipose tissue tends to gather in them mainly around the hips, so the WHR can remain at a low level. The WHR in girls is dependent on the amount of adipose tissue, while in boys, it is positively correlated with a higher BMI.

We have also proved that maternal weight gain during pregnancy may be linked with acceleration of fat tissue distribution among girls at the age 6–13 years. Similar results were obtained by other scientific teams [47,48]. Moreover, some authors concluded that maternal weight gain during pregnancy affected not only a higher BMI of the offspring, but also a metabolic syndrome or an increase of blood pressure [49,50].

In conclusion, it should be noted that the concentration of sex hormones in the prenatal period, reflected by the 2D:4D digit ratio, may significantly influences the body composition of girls at the age 6–13 years, regulating their muscle mass. We have found no such correlations in the group of boys. In this group, the composition of the body seems to be more correlated with simultaneously formed phenotypic traits: BMI, WHR and fat mass. Apart from the importance of the 2D:4D digit ratio, maternal factors (maternal education and maternal weight gain during pregnancy) play an important role in explaining the variability of body composition and body proportions.

Due to the growing problem of childhood obesity [34], also among Polish children [51] and the fact that 80% of obese children become obese adults in the future [28], it is very important from an epidemiological point of view to indicate simple determinants of phenotypic disorders of body composition.

4.1. Limitations

The investigation did not tackle the problem of age differences in order to standardising all dependent variables on sex to enlarge the investigated group.

We did not include in the investigation information about the time of the menarche among girls thus some of the included girls may be during the early pubertal period, when proportion of the sex hormones is changing.

5. Conclusions

The 2D:4D digit ratio (in the right hand) in the analysed group of children at the age 6–13 years is significantly differentiated depending on sex; boys are characterised by lower values of the indicator.

The muscle mass variability of girls is also dependent on the proportion of sex hormones in prenatal life, which is visible as the value of the 2D:4D digit ratio.

We have found no relationship between the 2D:4D digit ratio values and the relative body weight and the WHR in both sexes what is inconsistent result with some previous studies.

Maternal traits, such as maternal education and maternal weight gain during pregnancy, significantly modify the body composition in the group of girls aged 6–13 years.

Contributors

PP-P, analysed the data, prepared the draft and final version of the manuscript and collected the material. EŻ designed the study, prepared the manuscript and provided critical comments on the manuscript. AS, IR designed the study, collected the material and provided critical comments on the final manuscript. DS designed the study and collected the material. MS, MS-K, collected the material.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.earlhumdev.2018.08.001.

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Załącznik 4 - "The association between socioeconomic status, duration of breastfeeding, parental age and birth parameters with BMI, body fat and muscle mass among prepubertal children in Poland"

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The association between socioeconomic status, duration of breastfeeding, parental age and birth parameters with BMI, body fat and muscle mass among prepubertal children in Poland

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With 4 figures and 5 tables

Abstract: *Objectives:* We aimed to indicate simple determinants of abnormal body composition in children, such as socio-economic status (SES), duration of breastfeeding, parental age and birth parameters. *Methods:* The final data set consisted of 469 healthy prepubertal individuals (247 girls and 222 boys). We studied body mass, body height, and parameters of body composition such as muscle mass and fat mass. The birth parameters and gestational age were obtained from the children's medical record books held by the parents which were completed by medical personnel immediately following birth. Information about socio-economic status (SES), duration of breastfeeding and parental age was obtained by questionnaire. The statistical methods included forward multiple regression and generalized linear models (GLZ) or general linear model (GLM). *Results:* Higher fat mass (FM) (%) was connected with shorter duration of breastfeeding (< 2 months and lower SES ($p < 0.05$). Lower muscle mass (MM) (%) was linked with lower SES ($p < 0.05$) and lower birth weight ($p < 0.05$). Higher body mass index (BMI) was connected with higher birth weight (p < 0.05), shorter duration of breastfeeding (< 2 months) and lower SES ($p < 0.05$). Moreover interaction effects were observed in the case of the FM (%) (breastfeeding x SES; breastfeeding x parental age) and the BMI (breastfeeding x paternal age). *Conclusions:* Body composition can be linked with the duration of breastfeeding, SES, parental age, birth weight and birth length.

Keywords: children; abnormal body composition; birth parameters; socio-economic status SES; breastfeeding

Introduction

Variability of body composition among children is nowadays an important issue, especially due to the rising problem of overweight and obesity. Many factors are associated with body composition, for example: genetic factors (Maes et al. 1997), socioeconomic status (Dinsa et al. 2012; Freeston et al. 2017), prenatal sex hormones concentration (Pruszkowska-Przybylska et al. 2018) or maternal life-style during pregnancy (Althuizen et al. 2006).

It is known that longer duration of breastfeeding may be protective against overweight in further stages of ontogenesis (Toschke et al. 2002; Eny et al. 2018; Sirkka et al. 2018). Some authors claimed that despite inconsistent associations between breastfeeding and body mass, breastfeeding may moderate familial factors decreasing the risk of overweight among children (Hediger et al. 2001). Breastfed children have not only lower risk of overweight and obesity, but also lower risk of underweight (Grummer-Strawn & Mei 2004). However, there are also opinions that breastfeeding does not affect body size (Morgen et al. 2018). Probably the effect of breastfeeding on body mass and body composition is minimal in comparison to parental factors such as parent's overweight or lifestyle (Dewey 2003).

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Despite postnatal catch-up, body mass of the lightest newborns tends to be significantly lower during the next years compared with heavier newborns (Eny et al. 2018). There is a positive association between birth weight and body mass in the later stages of life (Mitchell et al. 2017). There is also evidence that longer newborns have lower BMI in the future life (Pruszkowska-Przybylska et al. 2018).

Some authors try to find out how strong is the association between SES and body composition. In the high developed countries lower obesity rate (Dinsa et al. 2012) can be linked with higher SES, higher income, frequently attending gyms and fitness clubs and higher awareness of a healthy life (Freeston et al. 2017).

There is a lack of consistent results in the case of association of parental age with body composition. Toftemo et al. (2018) suggested that lower maternal age is associated with offspring overweight in later life. Fall et al. (2015) gave evidences that offspring of younger (≤ 19 years) and older (≥ 35 years) mothers had significantly higher glucose level that may be indirectly linked with body mass. There is still no data about the relation between body composition and parental age. The aim of this study was to examine the association between the BMI, fat and muscle mass, and SES, duration of breastfeeding, parental age, birth weight and birth length of Polish children aged 6–13 years. We hypothesized that Polish children aged 6–13 years with high SES have lower fat mass, lower BMI and higher muscle mass. We also predicted that longer duration of breastfeeding is associated with higher muscle mass, lower fat mass and lower BMI. Moreover, we considered that higher birth body weight is connected with higher fat mass, higher BMI and lower muscle mass. Finally, we hypothesized that older parents may affect higher fat mass, higher BMI and lower muscle mass.

Material and methods

The collected material included information and measurements of 607 healthy individuals (317 girls, 280 boys) aged 6–13 years who were randomly recruited in years 2015–2018 in primary schools in Łódź (city in central Poland, approximately 700,000 inhabitants). Information was obtained from anthropometric measurements and questionnaires filled in by the children's parents. According to the standard anthropometric procedure of Martin (Knussmann et al. 1988) all measurements were conducted by the qualified staff of the Department of Anthropology and the Biobank Laboratory (Strapagiel et al. 2016) of the University of Łódź. The following parameters were measured: body weight (with an accuracy of 0.1 kg) using electronic scale, body height (with an accuracy of 0.001 m) using an anthropometer and fat (FM%) and muscle mass (MM%) using the bioelectrical impedance vector analysis (BIA-101 ASE, Akern, Italy). Information about socio-economic status (SES), duration of breastfeeding and parental age was obtained by using questionnaires.

The duration of breastfeeding was divided into three categories: children who were formula-fed or breastfed for a period shorter than 2 months, children who were breastfed between 2 and 6 months, and children who were breastfed for longer than 6 months.

The level of parental education was categorised as: (1) basic or vocational education (8 years at obligatory primary school plus 3 years at vocational school); (2) secondary education (4–5 years at secondary school) or bachelor degree (3 years of education after secondary school); and (3) higher education (full university degree – master of science degree).

Parental age was divided into 3 groups using quartiles value: the youngest parents (mothers < 27; fathers < 29 years), medium age parents (mothers 27–33; fathers 29–35 years), the oldest parents (mothers > 33; fathers > 35 years).

Regarding standard of life the following definitions were used in the questionnaires: (1) low standard of life (we live very poorly, insufficient resources for basic needs or we live modestly, we have to be very economical on a daily basis), (2) medium standard of life (we live on average, it is enough for us every day but we have to save on more serious purchases), (3) high standard of life (we live well enough for us without much special savings or we live very well we can afford full luxury).

The factor analysis was used to create a single variable which describes socioeconomic status based on parental education and standard of life.

The data about birth parameters, birth length (cm), birth weight (kg) and gestational age came from the children's medical records.

All the children were divided into separate groups with the same calendar age. The z-score values for the FM (%), the MM (%), the BMI, the birth weight and the birth length were calculated using own data. Z-score values for the FM (%), the MM (%) and the BMI were standardised for calendar age (years) among investigated individuals. The birth weight and the birth length were standardised for sex and gestational age (months).

The following selection criteria were used: only single term born individuals (37^{th}–42^{nd} gestational week) with normal birth weight (> 2500 g); only pre-menarcheal girls.

Due to a lack of full response in questionnaires and chosen selection criteria the final data set consisted of 469 children (247 girls and 222 boys) aged 6–13 years representing the first level of education in Poland (primary school). The percentage of missing data was 23% in total including: breastfeeding 1.65%, maternal education 0.33%, paternal education 1.81%, maternal age 1.30%, paternal age 5.28%, standard of life 1.49%, gestational age 9.70% (including individuals who were born preterm), birth weight 2.02% (including individuals with birth weight < 2500 g), birth length 2.48% and 3.78% of individuals were precluded due

Table 1. The sex differences between boys and girls in terms of analyzed features.

Variable		Total N = 469	Boys N = 222	Girls N = 247	Sex differences *p*-value *
Breastfeeding (months) n (%)	< = 2	86 (18.34)	46 (20.72)	40 (16.19)	0.440[a]
	2–6	137 (29.21)	62 (27.93)	75 (30.36)	
	> 6	246 (52.45)	114 (51.31)	132 (53.44)	
Maternal education n (%)	Basic or vocational	25 (5.33)	14 (6.31)	11 (4.45)	0.391[a]
	Secondary or post-secondary	124 (26.44)	63 (28.38)	61 (24.70)	
	Higher	320 (68.23)	145 (65.32)	175 (70.85)	
Paternal education n (%)	Basic or vocational	58 (12.37)	30 (13.51)	28 (11.34)	0.713[a]
	Secondary or post-secondary	167 (35.61)	76 (34.23)	91 (36.84)	
	Higher	244 (52.03)	116 (52.25)	128 (51.82)	
Standard of life	Low	23 (4.90)	13 (5.86)	10 (4.05)	0.664[a]
	Medium	269 (57.36)	126 (56.86)	143 (57.89)	
	Hight	177 (37.74)	83 (37.39)	94 (38.06)	
Socioeconomic status (based on parental education and standard of life)	Mean	−1.57E-15	−0.05	0.04	0.507[c]
	SD	1.00	1.04	0.96	
	Median	0.43	0.32	0.43	
	Q1	−0.39	−0.89	−0.39	
	Q3	0.54	0.54	0.54	
Maternal age	Mean	30.06	29.92	30.02	0.590[b]
	SD	4.30	4.22	4.37	
	Median	29.93	29.77	29.94	
	Q1	27.30	27.44	27.12	
	Q3	33.00	32.54	33.13	
Paternal age	Mean	32.13	32.25	32.02	0.682[c]
	SD	5.17	5.21	5.15	
	Median	31.57	31.62	31.53	
	Q1	28.77	28.79	28.59	
	Q3	35.12	35.31	34.88	
Birth body weight (g)	Mean	3326.45	3473.22	3194.53	< 0.001[c]
	SD	500.35	510.78	452.74	
	Median	3320.00	3465.00	3220.00	
	Q1	3000.00	3150.00	2960.00	
	Q3	3610.00	3830.00	3500.00	
Birth body weight z-score	Mean	0.00	−0.00	0.00	0.999[b]
	SD	1.0	1.0	1.00	
	Median	−0.01	−0.01	0.03	
	Q1	−0.63	−0.65	−0.58	
	Q3	0.66	0.74	0.63	
Birth body length (cm)	Mean	54.97	55.97	54.05	< 0.001[c]
	SD	3.14	0.03	3.15	
	Median	55.00	0.97	54.00	
	Q1	53.00	0.99	53.00	
	Q3	57.00	0.95	56.00	

Variable		Total N = 469	Boys N = 222	Girls N = 247	Sex differences *p*-value *
Birth body lenght z-score	Mean	0.00	−0.00	0.00	0.598[c]
	SD	0.99	0.98	1.00	
	Median	0.12	0.12	−0.12	
	Q1	−0.64	−0.64	−0.47	
	Q3	0.57	0.67	0.59	
MM (%)	Mean	50.38	50.67	50.11	0.515[c]
	SD	5.63	5.68	5.58	
	Median	50.30	50.40	50.30	
	Q1	46.50	46.60	46.40	
	Q3	54.40	54.80	54.00	
MM (%) z-score	Mean	−0.00	0.04	−0.04	0.217[b]
	SD	0.99	1.03	0.95	
	Median	0.01	0.03	−0.01	
	Q1	−0.69	−0.72	−0.66	
	Q3	0.71	0.77	0.63	
FM (%)	Mean	19.84	19.60	20.06	0.625[c]
	SD	7.84	7.75	7.93	
	Median	19.00	18.90	19.10	
	Q1	14.20	14.00	14.40	
	Q3	25.20	25.50	25.00	
FM (%) z-score	Mean	−0.00	−0.02	0.01	0.677[c]
	SD	0.99	1.03	0.96	
	Median	−0.08	−0.09	−0.05	
	Q1	−0.69	−0.71	−0.67	
	Q3	0.71	0.76	0.59	
BMI	Mean	17.41	17.84	17.01	0.001[c]
	SD	2.91	3.07	2.70	
	Median	16.71	16.93	16.45	
	Q1	15.31	15.80	15.00	
	Q3	18.88	19.53	18.42	
BMI z-score	Mean	−0.00	0.15	−0.14	0.001[c]
	SD	0.99	1.07	0.89	
	Median	−0.23	−0.09	−0.32	
	Q1	−0.67	−0.55	−0.72	
	Q3	0.43	0.64	0.31	

* Sex differences were assessed using the Pearson's Chi-square[a], the Student's t-test[b] or the Mann-Whitney U test[c]. Socioeconomic status (based on parental education and standard of life)

to presence of menarche. There were not statistically significant differences between excluded data and analysed samples in: the FM(%) (Z adj. = 0.24; p = 0.81), the MM(%) (t = −0.34; p = 0.97) and the BMI (Z adj. = 0.19; p = 0.85).

The study was approved by the Ethical Commission at the University of Lodz (nr 19/KBBN-UŁ/II/2016).

Statistical analysis

Distribution of all continuous variables was examined using the Shapiro-Wilk test. The variables maternal age, birth weight z-score and MM (%) z-score were normally distributed, thus in further analyses parametric tests were used. In the case of

Table 2. A forward stepwise multiple regression model containing all the most important independent variables explaining FM (%) z-score, MM (%) z-score, BMI z-score, (standardised for calendar age) sex adjusted variation among investigated individuals.

Variable	Independent variables	Beta	SE Beta	b	SE b	t	p
MM (%) z-score	Intercept			−0.0050	0.0648	−0.0774	0.938
	SES	0.1682	0.0459	0.1670	0.0456	3.6650	0.000
	birth body weight z-score	−0.1049	0.0455	−0.1044	0.0453	−2.3066	0.022
	breastfeeding < 2 (months) [a]	−0.0887	0.0462	−0.2273	0.1184	−1.9202	0.056
	sex (boys) [b]	0.0497	0.0453	0.0986	0.0900	1.0960	0.274
FM (%) z-score	Intercept			−0.0603	0.0495	−1.2186	0.2236
	SES	−0.1798	0.0456	−0.1784	0.0452	−3.9434	0.0001
	breastfeeding < 2 (months) [a]	0.1283	0.0458	0.3288	0.1175	2.7995	0.0053
	birth body weight z-score	0.0834	0.0452	0.0830	0.0450	1.8453	0.0656
BMI z-score	Intercept			−0.1860	0.0626	−2.9703	0.0031
	SES	−0.2280	0.0446	−0.2263	0.0443	−5.1102	0.0000
	sex (boys) [b]	0.1295	0.0438	0.2572	0.0869	2.9584	0.0033
	breastfeeding < 2 (months) [a]	0.1368	0.0448	0.3505	0.1148	3.0525	0.0024
	birth body weight z-score	0.1851	0.0590	0.1843	0.0588	3.1369	0.0018
	birth body length z-score	−0.1045	0.0594	−0.1041	0.0592	−1.7588	0.0793

Reference values: [a] > 6 (months); [b] girls
FM (%) z-score: R = 0.2475; R^2 = 0.0613; Adjusted R^2 = 0.0552; F(3.465) = 10.114; p < 0.001; Std.Error of estimate: 0.9647, MM (%) z-score: R = 0.2268; R^2 = 0.0514; Adjusted R^2 = 0.0433; F(4.464) = 6.290; p < 0.001; Std.Error of estimate: 0.9708, BMI z-score: R = 0.3417; R^2 = 0.1168; Adjusted R^2 = 0.1072; F(5.463) = 12.242; p < 0.001; Std.Error of estimate: 0.9378
Categories for: breastfeeding (months): < 2; 2–6; > 6

the other variables which were not normally distributed non-parametric tests were applied. To check the sex differences of all included variables the Chi² test (categorical variable), the Student t-test and the U Mann-Whitney test were used. To verify the association between all dependent variables (the MM (%) z-score, the FM (%) z-score and the BMI z-score) and the duration of breastfeeding the ANOVA and the Kruskal-Wallis tests were used. To find the most important variables which are associated with the MM (%) z-score, the FM (%) z-score and the BMI z-score the forward stepwise multiple regression were applied. In order to detect interactions between the analysed variables, the GLM and the GLZ analyses were done in the case of all dependent variables (the MM (%) z-score, the FM (%) z-score, the BMI z-score).

The Statistica ver. 13.0 software was used to perform all calculations.

Results

There were no differences in all investigated variables between boys and girls (p > 0.05) (Table 1). The duration of breastfeeding was not statistically associated with muscle mass (MM %) z-score (F = 2.686, p = 0.069, ANOVA test). Children who were breastfed shorter than 2 months or not breastfed had significantly larger fat mass and higher BMI than children who were breastfed longer (respectively H = 9.047 p = 0.011; H = 11.953 p = 0.003, Kruskal-Wallis test).

The forward multiple regression models were prepared for each dependent variable (the MM (%) z-score, the FM (%) z-score and the BMI z-score) including the following independent variables: sex, duration of breastfeeding, SES, maternal and paternal age, birth weight z-score and birth length z-score.

The variables which remained in the model explaining 6.13% of the MM (%) z-score variability were SES, birth weight z-score, duration of breastfeeding and sex. SES was positively associated with MM (%) z-score (Beta = 0.168; p < 0.001) and negatively with birth weight z-score (Beta = −0.105; p = 0.022). 5.14% of variance of the FM (%) z-score was explained by SES, duration of breastfeeding and birth weight z-score. SES was negatively (Beta = −0.180; p < 0.001) connected with FM (%) z-score, whereas duration of breastfeeding < 2 months was positively (Beta = 0.128; p = 0.005) linked with FM (%) z-score. The forward regression model for BMI z-score included SES, sex, duration of breastfeeding, birth weight z-score and birth length z-score which explained 10.72% of the BMI z-score variance. The BMI z-score was negatively associated with SES (Beta = −0.228; p < 0.001), and birth length z-score (Beta = −0.105; p = 0.0793) as well as positively with: sex (boys) (Beta = 0.130; p = 0.003), duration of breastfeeding < 2 months

(Beta = 0.137; $p < 0.002$) and birth weight z-score (Beta = 0.190; $p < 0.002$) (Table 2).

Subsequently we assessed the interactions between the duration of breastfeeding and SES and parental age for the dependent variables. Important interaction effects were observed for FM (%) z-score (breastfeeding × SES, breastfeeding × paternal age, breastfeeding × maternal age) and BMI z-score (breastfeeding × paternal age) (Table 3). Those children who were breastfed longer than 6 months and belonged to the group with high SES had lower FM (%) z-scores than low SES children who were breastfed shorter than 2 months (Fig. 1; Tables 4–5). Independently of paternal age, fathers' offspring breastfed shorter than 2 months had higher FM (%) z-scores than children who were breastfed longer than 6 months (Fig. 2; Tables 4–5). The interaction between duration of breastfeeding and maternal age indicated that children who had the youngest (< 27 years) and the oldest mothers (> 33 years) and the shortest (< 2 months) and the longest duration of breastfeeding (> 6 months), FM (%) z-scores were higher (Fig. 3; Tables 4–5). The children who were breastfed shorter than 2 months compared with children who were breastfed longer than 6 months had higher BMI z-scores in respect to their fathers' age (Fig. 4; Table 4–5).

Table 3. The log-normal models (GLM or GLZ) for important interaction effects independent variables adjusted on the main effects. The dependent variables: MM (%) z-score, FM (%) z-score, BMI z-score.

Dependent variables	Independent variables	*p*-value 1993–1997
MM (%) z-score*	breastfeeding[a] × SES[b]	0.8356
	breastfeeding[a] × paternal age[c]	0.6992
	breastfeeding[a] × maternal age[d]	0.9895
FM (%) z-score**	breastfeeding[a] × SES[b]	< 0.001
	breastfeeding[a] × paternal age[c]	< 0.001
	breastfeeding[a] × maternal age[d]	< 0.001
BMI z-score**	breastfeeding [a] × SES[b]	–
	breastfeeding[a] × parental age[c]	< 0.001
	breastfeeding[a] × maternal age[d]	0.7402

[a] < 2; 2–6; > 6; [b] low; medium; high; [c] < 27 years; 27–33 years; > 33 years, [d] < 29 years; 29–35 years; > 35 years, Tests of all effects, **p*-value for F statistics.***p*-value for Wald statistics.
*GLM
**GLZ

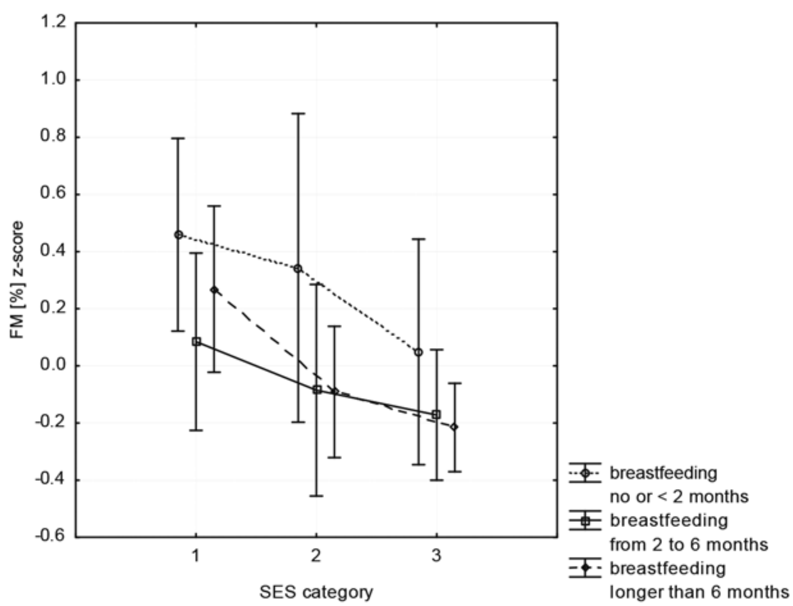

Fig. 1. Interaction between duration of breastfeeding and SES categories among children aged 6–13 years. Statistically important effect: < 2 vs > 6 months × low (1) vs high (3) (Beta = −38.3; $p < 0.001$). SES categories: 1 – low; 2 – medium; 3 – high.

Table 4. Evaluations of parameters in the of the independent variables statistically important for weight and BMI.

Dependent variables	Independent variables	Level of effects	β [a]	Std.Err.	−95% CI	+95% CI	*p*-value [a]
FM (%) z-score	breastfeeding × SES	< 2 vs > 6 months × low vs high	−38.283	2.0184	−42.239	−34.327	< 0.001
	breastfeeding × paternal age	< 2 vs > 6 months × < 29 vs > 35 years	−6.400	0.7677	−7.905	−4.896	< 0.001
		< 2 vs > 6 months × 29–35 vs > 35 years	4.996	0.8237	3.381	6.610	< 0.001
	breastfeeding × maternal age	< 2 vs > 6 months × < 27 vs > 33 years	−27.954	1.1068	−30.123	−25.785	< 0.001
		2–6 vs > 6 months × < 27 vs > 33 years	−5.505	1.4269	−8.301	−2.708	< 0.001
BMI z-score	breastfeeding × paternal age	< 2 vs > 6 months × < 29 vs > 35 years	−133.264	0.2597	−133.773	−132.755	< 0.001
		< 2 vs > 6 months × 29−35 vs > 35 years	−0.887	0.2566	−1.391	−0.384	< 0.001

[a] beta coefficient from the generalized linear model for the variable indicated, which is the log of the mean differences in the FM and BMI *p*-value for beta.

Table 5. Descriptive statistics for statistically important dependent variables.

Dependent variables	Independent variables	Level of Factor		N	Mean	SD	Std.Err.	−95% CI	+95% CI
		Duration of breastfeeding	SES/paternal age/maternal age						
FM (%) z-score	breastfeeding × SES	< 2 months	low	39	0.4593	1.0402	0.1666	0.1221	0.7965
		< 2 months	high	24	0.0492	0.9347	0.1908	−0.3455	0.4439
		> 6 months	low	57	0.2685	1.0968	0.1453	−0.0225	0.5595
		> 6 months	high	122	−0.2159	0.8632	0.0782	−0.3706	−0.0611
	breastfeeding × paternal age	< 2 months	< 29 years	20	0.5924	0.8440	0.1887	0.1974	0.9874
		< 2 months	29–35 years	24	0.2964	1.1084	0.2262	−0.1717	0.7644
		< 2 months	> 35 years	42	0.1909	1.1486	0.1772	−0.1670	0.5488
		> 6 months	< 29 years	55	−0.0416	0.9620	0.1297	−0.3017	0.2184
		> 6 months	29–35 years	62	−0.0502	0.9544	0.1212	−0.2926	0.1922
		> 6 months	> 35 years	129	−0.0910	0.9671	0.0851	−0.2595	0.0774
	breastfeeding × maternal age	< 2 months	< 27 years	22	0.5215	1.0350	0.2207	0.0627	0.9804
		< 2 months	> 33 years	43	0.2495	1.1097	0.1692	−0.0920	0.5910
		from 2 since 6 months	< 27 years	40	−0.0416	0.9083	0.1436	−0.3321	0.2489
		from 2 since 6 months	> 33 years	59	−0.1249	1.0544	0.1373	−0.3996	0.1499
		> 6 months	< 27 years	56	0.0079	1.0224	0.1366	−0.2659	0.2818
		> 6 months	> 33 years	132	−0.1224	0.9555	0.0832	−0.2869	0.0422
BMI z–score	breastfeeding × paternal age	< 2 months	< 29 years	20	0.5561	1.0740	0.2402	0.0534	1.0587
		< 2 months	29–35 years	24	0.2950	1.3012	0.2656	−0.2545	0.8444
		< 2 months	> 35 years	42	0.3234	1.0258	0.1583	0.0038	0.6431
		> 6 months	< 29 years	55	−0.0907	0.9279	0.1251	−0.3416	0.1601
		> 6 months	29–35 years	62	−0.1064	0.8943	0.1136	−0.3336	0.1207
		> 6 months	> 35 years	129	−0.0599	1.0830	0.0954	−0.2486	0.1287

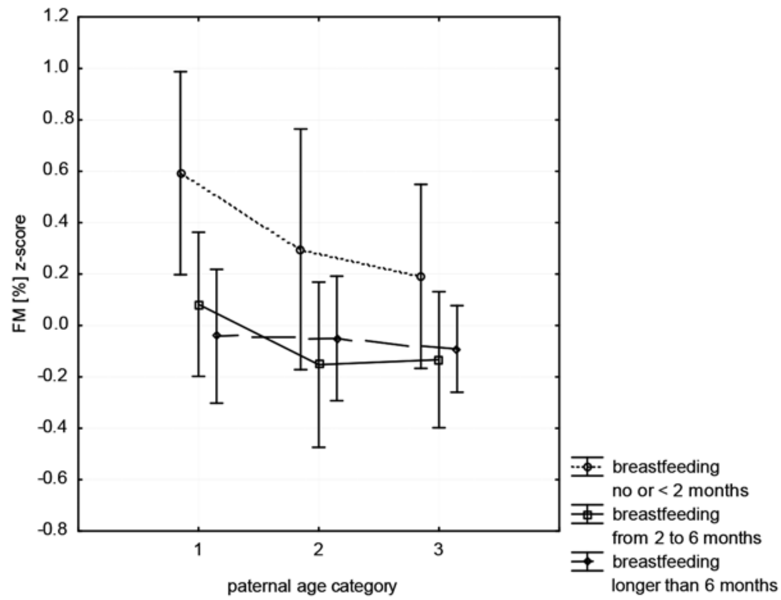

Fig. 2. Interaction between duration of breastfeeding and paternal age categories among children aged 6–13 years for FM (%) z-score. Statistically important effects: < 2 vs > 6 months × < 29 vs > 35 years (Beta = −6.4; $p < 0.001$); < 2 vs > 6 months × 29–35 vs > 35 years (Beta = 5.0; $p < 0.001$); paternal age categories: 1 – < 29; 2 – 29–35; 3 – > 35.

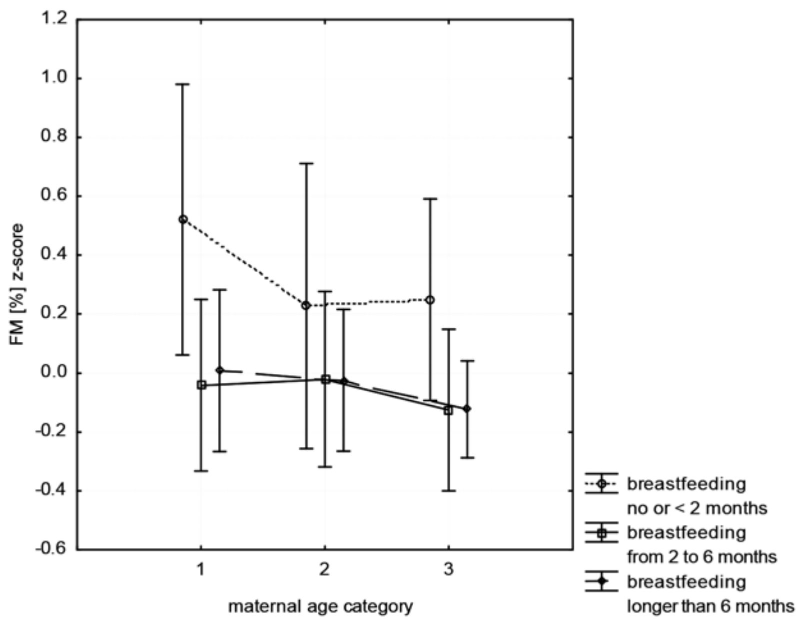

Fig. 3. Interaction between duration of breastfeeding and paternal age categories among children aged 6–13 years for FM (%) z-score. Statistically important effects < 2 vs > 6 months × < 27 vs > 33 years (Beta = −27.95; $p < 0.001$); 2–6 vs > 6 months × < 27 vs > 33 years (Beta = −5.5; $p < 0.001$); maternal age categories: 1 – < 27; 2 – 27–33; 3 – > 33.

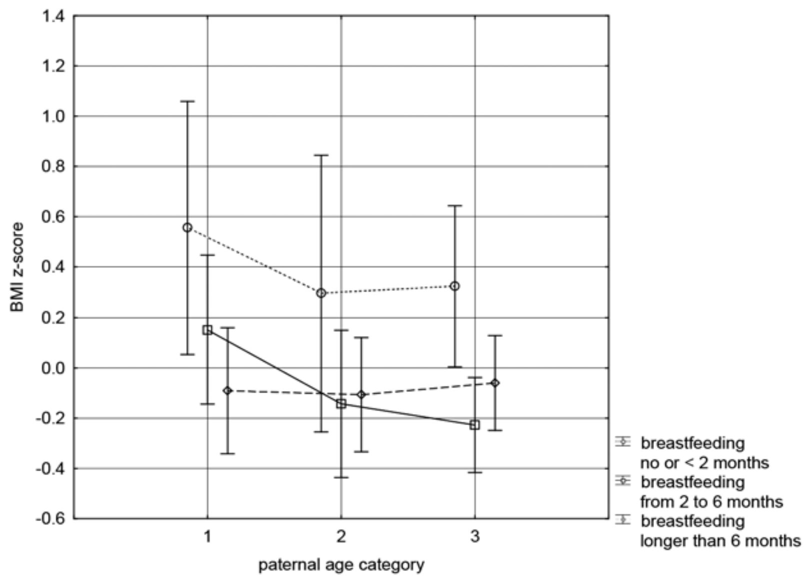

Fig. 4. Interaction between duration of breastfeeding and paternal age categories among children aged 6–13 years for BMI z-score. Statistically important effects: < 2 vs > 6 months × < 29 vs > 35 years (Beta = −133.3; p < 0.001); < 2 vs > 6 months × 29–35 vs > 35 years (Beta = −0.9; p < 0.001); paternal age categories: 1 – < 29; 2 – 29–35; 3 – > 35.

Discussion

In line with observations from other highly developed countries (Dinsa et al. 2012; Drewnowski et al. 2014) we hypothesized that Polish children aged 6–13 years from high SES, have lower fat mass and lower BMI. We also hypothesized that high SES Polish children have more muscle mass, as already observed by Sandercock et al. (2017) in Colombian children. The reason for this phenomena may be a link between SES and awareness of the quality of life and sufficient income to afford various types of physical activities, healthy food etc. (Fitzpatrick et al. 2015; Freeston et al. 2017). Moreover according to Lee et al. (2012) the percent of body fat is connected with the type of maternal work. When mothers work temporary their daughters have lower percent of body fat in contrast to the girls whose mothers have full time jobs. Furthermore, linking this investigation with our results, which showed statistically significant interaction between the duration of breastfeeding and the SES, we may presume that probably women who are in a better material position more frequently choose temporary work. Therefore, they can breastfeed their children longer affecting their lower fat mass in comparison to women characterised by lower SES who have to work longer and their duration of breastfeeding may be shortened, causing higher fat mass of their offspring.

The most appropriate age of mothers and fathers for proper body composition of their offspring is not known. Our results suggest that those young (< 27 years) and the old mothers (> 33 years) who breastfed the shortest (< 2 months) and the longest time (> 6 months) had offspring with higher value of the FM (%) z-score (Fig. 3; Tables 4–5). Probably too young parents are not sufficiently prepared to take care of their children (Moffitt et al. 2002). In the case of the oldest parents the problems with adequate body composition among their offspring can stem from ageing process (Sharma et al. 2015; Girchenko et al. 2017). The interaction between the lowest and the highest parental age and the duration of breastfeeding may indicate that children of older and younger parents are more sensitive to the duration of breastfeeding in respect to later acquisition of fat mass. There are no other studies known to the authors that discuss the duration of breastfeeding and parental age simultaneously.

In our studies we assumed that longer duration of breastfeeding can be beneficial for fat mass reduction among children. Similar results were obtained in the other studies (Modrek et al. 2017; Pruszkowska-Przybylska et al. 2018). The present data do not allow to define an optimal duration of breastfeeding in respect to body mass and composition of children.

The birth parameters are commonly associated with later body composition. We found that high birth weight was associated with high BMI at later age, confirming data published by Mitchell et al. (2017). High birth weight also appears to be associated with lower muscle mass. Similar observations were published by Sachdev et al. (2005) – they claimed that birth weight was positively associated with adiposity in women.

Conclusions

Short duration of breastfeeding (< 2 months) was connected with high FM% and high BMI in children aged 6–13 years. High birth weight was connected with low MM% and high BMI. High socioeconomic status was connected with high MM% , low FM% and low BMI.

We observed interactions between longer duration of breastfeeding with decreases in FM% in children from high and low SES. Long duration (< 2 vs. 2–6 months) of breastfeeding may decrease FM% also in children with very old, or very young mothers. However, long breastfeeding of more than 6 months may increase the FM%. High BMI was observed in children who were breastfed shorter than 2 months compared with children who were breastfed longer than 6 months independent of paternal age.

Limitations

We did not include the information about birth order which could be a valuable variable for explaining the fat and muscle mass and BMI.

Some of the investigated boys (n = 34, aged 12–13 years) may be on early pubertal stage of development in which proportion of sex hormones is changing that affects their body composition.

We did not add to the investigation information about number of mothers' children which may be connected with explaining variability of dependent variables.

In the questionnaire we included question about duration of breastfeeding, but we did not include questions about time of introducing the other types of nutrition (infant formulas, animal milks or fruit and vegetable juices) which may be also fundamental to properly infer about influence of breastfeeding on body composition.

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Association of saliva 25(OH)D concentration with body composition and proportion among pre-pubertal and pubertal Polish children

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Abstract

Objectives: Due to increasing problems with obesity and vitamin D deficiency among children, studies that tackle both problems together are needed.

Methods: Data were collected from 182 randomly selected children aged 6-13 years in primary schools in central Poland. Measures included anthropometric dimensions, body composition, questionnaires completed by participants' parents, and saliva samples. The level of 25(OH)D was assessed from the saliva samples using an enzyme-linked immunosorbent assay kit. The children were divided into two groups: pre-pubertal (girls below 10 years and boys below 11 years) and pubertal individuals (girls above 10 years and boys above 11 years).

Results: The 25(OH)D concentrations were higher in late spring (June) among pre-pubertal children than in the autumn (November-December) among pubertal children. The level of 25(OH)D was positively correlated with body cell mass (BCM,%) among all children (pubertal: $R = 0.20$, $P = .044$; pre-pubertal: $R = 0.23$, $P = .041$) and inversely associated with waist-to-hip ratio (WHR) among pubertal children of both sexes ($R = -0.25$; $P = .031$). The stepwise regression analysis revealed that investigation in spring (June) and breastfeeding was associated with increased muscle mass (MM, %) (beta = 0.253, $P = .003$ and beta = 0.225, $P = .005$, respectively) and total body water (TBW, %) (beta = 0.276, $P = .004$ and beta = 0.246, $P = .011$, respectively) and was associated with decreased body mass index (BMI; beta = −0.222, $P = .024$ and beta = −0.269, $P = .009$, respectively) and fat mass (%) (beta = −0.288, $P = .003$ and beta = −0.266, $P = .005$, respectively).

Conclusions: Season of salivary sampling and breastfeeding status were more strongly associated with body components, BMI and WHR, than 25(OH)D concentrations.

1 | INTRODUCTION

Vitamin D is commonly associated with bone health by enhanced intestine calcium absorption (Reid, 2003). Recently, numerous studies point to a pandemic of vitamin D deficiency both in children and adults (Holick & Chen, 2008). The diversity of scientific works connected with vitamin D status is noticed. There are also scientific papers which connected season of birth with current vitamin D status (Żądzińska et al., 2013; Pruszkowska-Przybylska, Nieczuja-Dwojacka, & Żądzińska, 2018).Vitamin D deficiency understood as a value below 10 ng/mL is mainly associated with abnormal bone metabolism and may result in rickets in children and osteoporosis among adults (UK Scientific Advisory Committee on Nutrition, 2016). Additionally, there are studies assessing the relationship of vitamin D and its various forms not only with the incidence of rickets, but also tumors of the colon, prostate, breast, and so on (Apperly, 1941; Garland et al., 1989; Giovannucci et al., 2006; Gorham et al., 2005; Grant, 2002; Grant & Garland, 2006; Hanchette & Schwartz, 1992). A connection with vitamin D deficiency and development of autoimmune diseases such as type 1 diabetes (Zipitis & Akobeng, 2008) and Crohn's disease (Koutkia, Lu, Chen, & Holick, 2001) has also been shown. Moreover, the development of some mental illnesses such as depression (Parker, Brotchie, & Graham, 2017) and schizophrenia (McGrath, Selten, & Chant, 2002) is associated with deficiency of vitamin D. However, more and more scientific research indicates a significant effect of vitamin D level on body composition, especially fat tissue in children as well as in adults.

Even though the first work linking vitamin D concentration with levels of adipose tissue dates from 40 years ago (Walicka et al., 2008), the mechanism is still not completely explained. Current studies have confirmed this relationship, for example, Parikh et al. (2004) showed a negative correlation between the concentration of 25(OH)D and body mass index (BMI) and adiposity. Additionally, Jackson et al. (2016) and Holmlund-Suila et al. (2016) observed significantly lower levels of 25(OH)D in individuals with larger waist circumferences and higher BMIs.

The most alarming data are those among children. The literature indicates simultaneously increasing rates of obesity and vitamin D deficiency. Some of the studies indicate a negative correlation of 25(OH)D concentration with parameters such as BMI, percentage body fatness, and the waist-to-hip ratio (WHR) index among children (Lee et al., 2013). Lourenço, Qi, Willett, and Cardoso (2014) showed that low levels of 25(OH)D influence the expression in the FTO (fat mass and obesity-associated) gene, which has been linked to obesity. In contrast, Foo et al. (2009) did not find any association between 25(OH)D concentration and fat tissue. Vimaleswaran et al. (2013) found that obesity was associated with vitamin D deficiency, but the proposed mechanism was unclear. Others, such as Vanlint (2013), showed that vitamin D deficiency and obesity may not be causally related to each other—they may both, rather, be caused by lack of exercise and poor diet.

There is no clear evidence for direct influence of 25(OH)D concentration on body composition among children. Thus, the aim of this study was to evaluate the association between 25(OH)D status and body composition and proportion among pre-pubertal and pubertal children in Poland.

2 | MATERIALS AND METHODS

The research material was collected between December 2016 and December 2017 in two seasons: spring (June 13, 2017) and autumn (from November 14, 2016 to December 9, 2016 and December 11, 2017) from randomly selected children in primary schools in Lodz (central Poland 700 000 inhabitants) that were willing to participate. The study material includes anthropometric measurements, questionnaire filled in by the participants' parents, and saliva samples from 182 children (85 boys and 97girls) aged 6 to 13 years (mean = 9.35 for girls and mean = 10.10 for boys).

All anthropometric measurements were conducted by qualified members of the staff of the Department of Anthropology according to the procedures introduced by Martin Knussmann (1988). The following measurements were recorded: body weight (with an accuracy of 0.1 kg) using electronic scale, body height (with an accuracy of 0.001 m) using an anthropometer and waist and hip circumference using an anthropometric tape (with an accuracy of 0.001 m). Body composition measurements were performed by the staff of the Biobank Laboratory of the University of Lodz including percent of fat mass (FM, %), percent of muscle mass (MM, %), percent of body cellular mass (BCM, %), and percent of total body water (TBW, %) using the bioelectrical impedance vector analysis (BIA-101 ASE, Akern, Italy). Via questionnaires, information about the type of feeding after birth and parental education was obtained.

Due to missing answers in questionnaires—2.75% in total (breastfeeding 1.09% and paternal education 1.66%) 176 individuals were included in the final analyses.

The breastfeeding status was presented as two categories: no (lack of breastfeeding, only formula feeding) and yes (at least 1 month of breastfeeding or longer).

The level of parental education was included as a widely known indicator of socioeconomic status (Currie, Elton, Todd, & Platt, 1997). Additionally, parental education seemed to be an important control factor in body composition and proportion investigations (Pruszkowska-Przybylska et al., 2018; Pruszkowska-Przybylska et al., 2019), and it was therefore applied in this investigation to authenticate the relation between body composition and proportion and vitamin D concentration. The level of parental education was categorized as: (a) basic or vocational education (8 years in obligatory primary school through 3 years in vocational school); (b) secondary education (4-5 years in secondary school through bachelor degree, 3 years of education after secondary school); and (c) higher education (full university degree—master of science degree through doctorate).

2.1 | Saliva sample collection and preparation

The participants were not allowed to eat, drink, chew gums, or brush teeth for 30 minutes before sampling. Saliva samples were collected using special saliva sampling sterile Falcon tubes (Nest Biotechnology). Immediately after collection, samples were stored at temperature 2°C- 8°C, frozen at −20°C at the same day and stored no longer than 1.5 years before testing. Before testing, the samples were thawed and centrifuged 2000 rpm at least once in order to separate the mucins.

The level of 25(OH)D was assessed in the saliva samples using an enzyme-linked immunosorbent assay (ELISA) kit (Human Vitamin D[VD]ELISA Kit, SunRed Biotechnology Company), which uses a double-antibody sandwich. Bahramian et al. (2018) confirmed that saliva 25(OH)D concentration correlates with serum 25(OH)D concentration. Nonetheless, measurements performed on saliva and serum do not give identical results. Thus, the range for vitamin D deficiency level in blood cannot be used in the case of saliva. For this reasons, we treated vitamin D as a continuous variable in our calculations due to the existence of correlation between serum and saliva vitamin D concentration but lack of specified range of vitamin D deficiency for saliva samples.

Each assay included six standard concentrations and every sample was run in duplicate. The concentration was assessed using a microplate reader (SpectraMax i3, Molecular Devices) measuring the absorbance at 450 nm. Standard curves were created using the 4PL method using the open source software (https://www.aatbio.com/tools/four-parameter-logistic-4pl-curve-regression-online-calculator/) to estimate the 25(OH)D concentration in ng/ml.

All samples were within assay range in accordance with the information supplied by kit producer. Inter-assay CVs of less than 15% are acceptable. Intra-assay CVs were less than 10%.

2.2 | Statistical methods

The *z*-score values for the BMI, WHR and the following body composition components: FM (%), MM (%), BCM (%) and TBW (%) were calculated standardizing them for calendar age and sex. The calculations were done using the following equation: z-score = $(x-\mu)/\sigma$, where: x is the individual's value for the analyzed parameter; μ is the mean of the analyzed group; and σ is the SD of the designed group (the same sex and age).

The children were divided into two age groups: prepubertal individuals (girls below 10 years [mean = 7.90 years] and boys below 11 years [mean = 8.48 years]) and pubertal individuals (girls above or equal 10 years [mean = 11.53 years] and boys above or equal 11 years [mean = 11.94]).

The student *t*-tests were used to investigate the relationships between 25(OH)D concentration and sex, season of investigation, and level of maturity. Spearman tests were applied to measure the correlations between 25(OH)D concentration and: BMI, WHR, BCM (%), FM (%), MM (%), and TBW (%).

ANOVAs with the post-hoc Tukey RIR-tests were applied to assess the influence of season of investigation, age group, and sex on 25(OH)D concentrations.

The forward stepwise multiple regression model was applied to estimate the importance of the 25(OH)D concentration on body composition and body proportion with presence of other variables.

The Statistica ver. 12.0 software was used to perform all calculations.

3 | RESULTS

3.1 | Vitamin D concentration variability

There was no statistically significant difference in 25(OH)D concentration between boys and girls (t = 0.07; P = .941). However, there was statistically significant difference in 25(OH)D concentration between children tested in the spring (t = −3.76; P < .001) (mean = 34.78) and in the autumn (mean = 25.09). The concentration of 25(OH)D was statistically significantly lower in children's saliva collected in the autumn than in the spring. Additionally, a statistically significant difference in 25(OH)D concentration ($t = -3.58$; $P < .001$) was observed between pubertal (mean = 23.82) and pre-pubertal (mean = 33.03) children (see Table 1).

3.2 | The analysis of variance of the 25(OH)D concentration

The analysis of variance of the 25(OH)D concentration including age, season of investigation, and sex (Table 2; Figure 1) showed statistically significant interactions ($F = 5.365$; $P < .001$). The pre-pubertal girls investigated in the spring had a significantly higher concentration of 25(OH)D (mean = 42.958) than those investigated in the autumn (mean = 25.435; $P = .001$). The higher concentration of 25(OH)D was observed among pre-pubertal girls investigated in the spring (mean = 42.958) in comparison to: pubertal girls investigated in the autumn (mean = 19.530; $P < .001$), pubertal boys investigated in the autumn (mean = 22.580; $P < .001$), and pubertal girls investigated in the spring (mean = 24.130; $P < .001$).

3.3 | Correlations between 25(OH)D concentration and body components, BMI, and WHR

Table 3 shows the correlations between 25(OH)D concentration and body components as well as BMI and WHR among pre-pubertal and pubertal children. There were statistically significant correlations between 25(OH)D concentration and BCM (%) among both pre-pubertal and pubertal children ($R = 0.20$; $P = .044$ and $R = 0.23$; $P = .041$, respectively). There was statistically significant correlation between 25(OH)D concentration and WHR among pubertal children ($R = -0.25$; $P = .031$) (Table 3).

TABLE 1 Vitamin D concentration stratified according to sex, pubertal stage, and season of investigation

Variables	25(OH)D concentration (ng/ml)								
		n	Mean	Median	SD	Q_1	Q_3	*t*	*P*
Sex	Boys	81	29.16	29.37	16.89	14.33	41.96	0.07	.941
	Girls	95	28.96	27.34	18.00	13.25	43.11		
Age	Pre-pubertal children	100	33.03	33.60	18.36	17.96	47.13	−3.58	<.001
	Pubertal children	76	23.82	19.53	14.72	12.53	35.28		
Season of investigation	Autumn	104	25.09	18.73	17.36	11.56	39.33	−3.76	<.001
	Spring	72	34.78	34.70	16.03	21.21	45.69		
Total		176	29.05	27.53	17.45	13.47	42.26		

Abbreviations: n, sample size; SD, standard deviation; Q_1, lower quartile; Q_3, upper quartile; t, student t test.

TABLE 2 Variability of vitamin D concentration depending on season of investigation, age, and sex

Season of investigation 0, autumn; 1, late spring	Maturity 0, prepuberty; 1, puberty	Sex 0, girls; 1, boys	*N*	Mean	SD	Q_1	Median	Q_3
0	0	0	29	25.435*	17.091	12.142	21.006	39.616
0	0	1	28	31.444	19.554	16.878	30.947	42.340
0	1	0	23	19.530*	15.866	11.127	13.547	22.447
0	1	1	24	22.580*	14.739	10.605	15.214	37.958
1	0	0	28	42.958*	14.581	33.137	39.735	57.549
1	0	1	15	32.143	18.157	18.438	29.455	48.999
1	1	0	15	24.130*	14.045	12.793	19.892	32.992
1	1	1	14	32.668	10.349	25.685	33.923	40.259
Total			176	29.053	17.449	13.472	27.527	42.264

Note: Statistically significant interactions (post-hoc Tukey RIR-test): autumn&prepuberty&girls vs late spring&prepuberty&girls ($P = .001$); late spring&prepuberty&girls vs autumn&puberty&girls ($P < .001$); late spring&prepuberty&girls vs autumn&puberty&boys ($P < .001$); late spring&prepuberty&girls vs late spring&puberty&girls ($P < .001$).

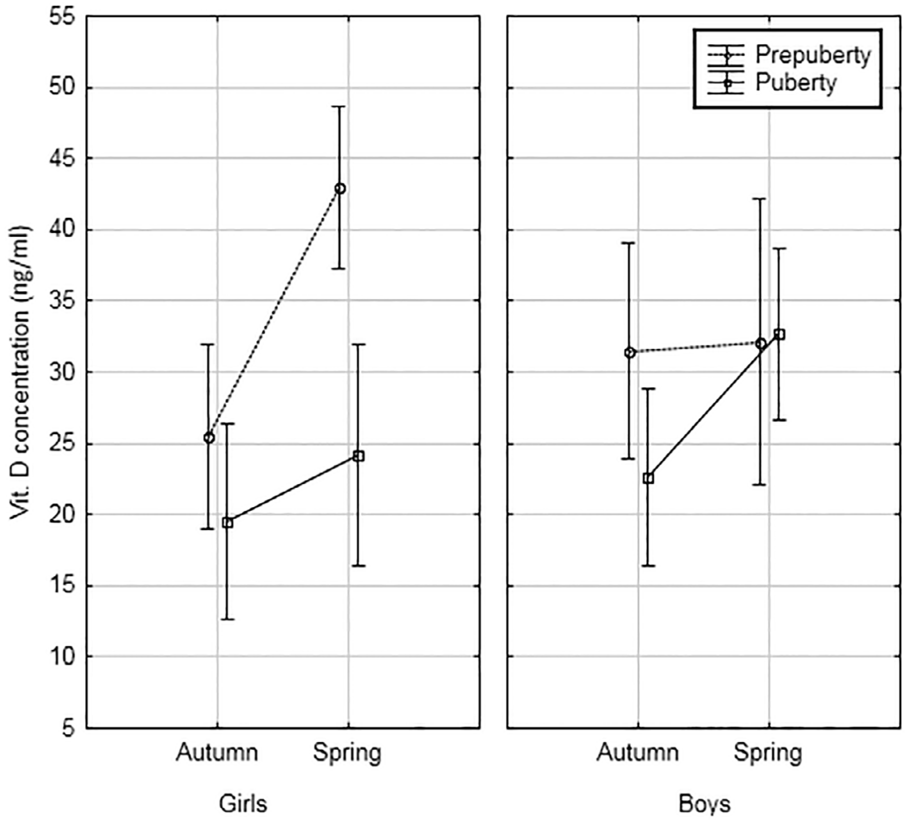

FIGURE 1 Variability of vitamin D concentration depending on season of investigation, age and sex

TABLE 3 Correlations between vitamin D concentration and body composition and proportion among pre-pubertal and pubertal children

Variables		Total (n = 176)			Pre-pubertal children (n = 100)			Pubertal children (n = 76)		
		R	t	P value	R	t	P value	R	t	P value
Vitamin D concentration	& FM%	−0.009	−0.12	.9044	−0.029	−0.29	.7711	0.039	0.33	.7390
	& BCM%	0.212	2.86	.0048	0.202	2.04	.0437	0.235	2.08	.0412
	& MM%	0.117	1.55	.1234	0.129	1.28	.2025	0.089	0.77	.4420
	& TBW%	0.004	0.05	.9601	0.047	0.46	.6435	−0.060	−0.52	.6059
	& BMI z-score	0.065	0.86	.3914	0.057	0.56	.5755	0.118	1.03	.3080
	& WHR z-score	−0.163	−2.18	.0309	−0.126	−1.26	.2101	−0.248	−2.20	.0310

Abbreviations: n, sample size; FM%, percent of fat mass; BCM%, percent of body cellular mass; MM%, percent of muscle mass; TBW%, percent of total body water; BMI, body mass index; WHR, waist-to-hip ratio; R, t, and P value, Spearman rank correlation coefficient.

3.4 | The forward stepwise multiple regression analysis

The forward stepwise multiple regression analysis was applied to investigate whether 25(OH)D concentration affects body composition and body proportion including other independent variables (type of feeding during the first year of life, level of parental education, and season of the investigation), which explains the variance of the following dependant variables: BMI, WHR, FM (%), MM (%), BCM (%), and TBW (%). Each regression model, which included the subsequent independent variables: 25(OH)D concentration, breastfeeding, parental education, and season of the investigation, was designed separately for pre-pubertal and pubertal children.

The first stepwise forward multiple regression model was constructed to explain the variability of the BMI (Table 4). In the group of pre-pubertal children, the parameters negatively associated with the BMI were breastfeeding (vs lack of breastfeeding) (beta = −0.269; P = .009) and investigation in spring (vs autumn) (beta = −0.222; P = .024) with the control of paternal

TABLE 4 A forward stepwise multiple regression model including all the most important independent variables explaining BMI, WHR, FM (%), MM (%), FMM (%), BCM (%), and TBW (%) (standardized for calendar age and sex) among pre-pubertal children

Variables	Independent variables	Beta	SE beta	*b*	SE b	*t*	*P*
BMI *z*-score	Intercept			1.043	0.339	3.075	.003
	Breastfeeding—yes[a]	−0.269	0.100	−0.853	0.319	−2.675	.009
	Season of the investigation—spring[b]	−0.222	0.097	−0.427	0.187	−2.287	.024
	Paternal education (secondary)[c]	−0.102	0.100	−0.194	0.192	−1.011	.314
WHR *z*-score	Intercept			0.948	0.338	2.807	.006
	Breastfeeding—yes[a]	−0.224	0.098	−0.702	0.307	−2.290	.024
	Vit. D concentration	−0.157	0.098	−0.008	0.005	−1.605	.112
FM (%) *z*-score	Intercept			0.997	0.278	3.586	.001
	Season of the investigation—spring[b]	−0.288	0.093	−0.550	0.178	−3.089	.003
	Breastfeeding—yes[a]	−0.266	0.093	−0.840	0.294	−2.857	.005
MM (%) *z*-score	Intercept			−1.052	0.312	−3.369	.001
	Season of the investigation—spring[b]	0.253	0.095	0.491	0.185	2.654	.009
	Breastfeeding—yes[a]	0.225	0.095	0.722	0.306	2.362	.020
	Maternal education (higher)[c]	0.116	0.095	0.254	0.208	1.224	.224
BCM (%) *z*-score	Intercept			1.665	0.931	1.788	.077
	Vit. D concentration	0.179	0.099	0.009	0.005	1.809	.074
	Maternal education (secondary)[c]	−0.995	0.433	−2.187	0.951	−2.299	.024
	Maternal education (higher)[c]	−0.892	0.434	−1.937	0.942	−2.056	.043
TBW (%) *z*-score	Intercept			−0.934	0.282	−3.316	.001
	Season of the investigation—spring[b]	0.276	0.094	0.529	0.181	2.930	.004
	Breastfeeding—yes[a]	0.246	0.094	0.778	0.298	2.609	.011

Note: BMI z-score: $R = 0.3569$; $R^2 = 0.1274$; adjusted $R^2 = 0.1001$; $F(3.96) = 4.671$; $P = .004$; SE of estimate: 0.908, WHR *z*-score: $R = 0.2710$; $R^2 = 0.0734$; adjusted $R^2 = 0.0543$; $F(2.97) = 3.845$; $P = .025$; SE of estimate: 0.920, FM *z*-score: $R = 0.4214$; $R^2 = 0.1775$; adjusted $R^2 = 0.1606$; $F(2.97) = 10.470$; $P < .001$; SE of estimate: 0.872, MM *z*-score: $R = 0.3961$; $R^2 = 0.1569$; adjusted $R^2 = 0.1305$; $F(3.96) = 5.964$; $P < .001$; SE of estimate: 0.902, BCM *z*-score: $R = 0.2932$; $R^2 = 0.0860$; adjusted $R^2 = 0.0574$; $F(3.96) = 3.010$; $P = .034$; SE of estimate: 0.929, TBW *z*-score: $R = 0.3974$; $R^2 = 0.1579$; adjusted $R^2 = 0.1406$; $F(2.97) = 9.096$; $P < .001$; SE of estimate: 0.884.
Reference values:
[a]No;
[b]Autumn;
[c]Basic or vocational.

secondary education (vs basic) explaining 10.00% of the BMI variability. The next model for the WHR among pre-pubertal children explained 5.43% of the variability of the WHR with negative association of breastfeeding (vs lack of breastfeeding) (beta = −0.224; *P* = .024) with the control of 25(OH)D concentration. The stepwise forward multiple regression models for body components among pre-pubertal children were the following: in the case of FM (%), the parameters negatively associated with FM (%) were investigation in spring (vs autumn) (beta = −0.288; *P* = .003) and breastfeeding (vs lack of breastfeeding) (beta = −0.266; *P* = .005) explaining 16.06% of the variability of FM (%); considering the MM (%), the positively linked variables were investigation in spring (vs autumn) (beta = −0.253; *P* = .009), breastfeeding (vs lack of breastfeeding) (beta = 0.224; *P* = .020), with the control of maternal education (higher vs basic or vocational) explaining 13.05% of the MM (%) variation. In the case of BCM (%), negative association was observed for maternal education (higher vs basic or vocational) (beta = −0.892; *P* = .043) and maternal education (secondary or bachelor degree vs basic or vocational) (beta = −0.995; *P* = .021) with the control of 25(OH)D concentration explaining 5.74% of the variance of BCM%. The last model for TBW (%) included breastfeeding (vs lack of breastfeeding) (beta = 0.246; *P* = .011) and investigation in spring (vs autumn) (beta = −0.276; *P* = .004) as variables positively associated with TBW (%) explaining 14.07% of the variability of TBW.

The stepwise forward regression models for pubertal children were designed accordingly (Table 5). Only in the case of BMI, the model was statistically significant.

TABLE 5 A forward stepwise multiple regression model including all the most important independent variables explaining BMI, WHR, FM (%), MM (%), FMM (%), BCM (%), and TBW (%) (standardized for calendar age and sex) among pubertal children

Variables	Independent variables	Beta	SE beta	*b*	SE *b*	*t*	*P*
BMI *z*-score	Intercept			−0.322	0.447	−0.721	.473
	Maternal education (higher)[c]	−0.268	0.112	−0.590	0.246	−2.398	.019
	Vit. D concentration	0.169	0.111	0.011	0.007	1.521	.133
	Breastfeeding—yes[a]	0.146	0.112	0.540	0.414	1.305	.196
WHR *z*-score	Intercept			0.605	0.240	2.521	.014
	Vit. D concentration	−0.201	0.115	−0.013	0.007	−1.744	.085
	Paternal education (higher)[c]	−0.198	0.112	−0.375	0.212	−1.767	.082
	Season of the investigation—spring[b]	−0.125	0.115	−0.241	0.223	−1.081	.284
FM (%) *z*-score	Intercept			−0.357	0.449	−0.796	.429
	Breastfeeding—yes[b]	0.155	0.116	0.588	0.438	1.342	.184
	Maternal education (higher)[c]	−0.117	0.116	−0.263	0.261	−1.009	.316
MM (%) *z*-score	Intercept			0.321	0.449	0.716	.476
	Breastfeeding—yes[b]	−0.166	0.115	−0.633	0.438	−1.446	.152
	Maternal education (higher)[c]	0.156	0.115	0.354	0.261	1.360	.178
BCM (%) *z*-score	Intercept			−0.341	0.207	−1.648	.104
	Vit. D concentration	0.208	0.114	0.014	0.007	1.828	.072
TBW (%) *z*-score	Intercept			−0.077	0.180	−0.426	.672
	Maternal education (secondary)[c]	−0.148	0.118	−0.397	0.315	−1.258	.212
	Paternal education (higher)[c]	0.131	0.118	0.249	0.224	1.112	.270

Note: BMI *z*-score: $R = 0.3352$; $R^2 = 0.1124$; adjusted $R^2 = 0.0754$; $F(3.72) = 3.038$; $P = .035$; SE of estimate: 0.888, WHR *z*-score: $R = 0.3200$; $R^2 = 0.1024$; adjusted $R^2 = 0.0650$; $F(3.72) = 2.738$; $P = .0496$; SE of estimate: 0.915. FM *z*-score: $R = 0.1836$; $R^2 = 0.0337$; adjusted $R^2 = 0.0072$; $F(2.73) = 1.273$; $P = .286$; SE of estimate: 0.937. MM *z*-score: $R = 0.2151$; $R^2 = 0.0463$; adjusted $R^2 = 0.0202$; $F(2.73) = 1.772$; $P = .117$; SE of estimate: 0.941, BCM *z*-score: $R = 0.2078$; $R^2 = 0.0432$; adjusted $R^2 = 0.0303$; $F(1.74) = 3.340$; $P = .072$; SE of estimate: 0.942, TBW *z*-score: $R = 0.2202$; $R^2 = 0.0485$; adjusted $R^2 = 0.0224$; $F(2.73) = 1.861$; $P = .163$; SE of estimate: 0.938.
Reference values:
[a]No;
[b]Autumn;
[c]Basic or vocational.

The model for BMI revealed that maternal education (higher vs basic or vocational) (beta = −0.268; $P = .019$) was positively associated with BMI with the control of 25(OH)D concentration and breastfeeding explaining 7.54% of the variance of the BMI.

4 | DISCUSSION

In the current sample of Polish children, we confirmed the presence of seasonal fluctuations in 25(OH)D levels as had been previously observed in other European countries (Beyitler et al., 2018; Mortensen, Mølgaard, Hauger, Kristensen, & Damsgaard, 2018). In the current sample, 25(OH)D concentrations were lower in the autumn than in the spring. In Poland, exposure to sunlight is the lowest from November to February and the highest insolation is from May to August (Woś, 1999).These findings suggest that yearly average concentrations of 25(OH)D are needed to effectively assess vitamin D status in European populations.

The level of 25(OH)D was dependent on the age of the children. Pubertal children had lower 25(OH)D concentrations than the pre-pubertal children. This may stem from changes in lifestyle and activity patterns (eg, more school duties), which may lead to less time spent outdoors. The vitamin D level may also regulate the sexual maturation process. However, there is a lack of consistent results, which may explain this association. Villamor, Marin, Mora-Plazas, and Baylin (2011) concluded that vitamin D deficiency may accelerate the time of menarche among Colombian girls, while de Bénazé, Brauner, and Souberbielle (2017) did not find an association among French girls. Moreover, Jung et al. (2018) showed that the level of vitamin D and the time of entry into puberty may be associated with metabolic syndrome in adulthood.

Our analysis suggests that both season and stage of maturity influence 25(OH)D concentrations. Levels of 25(OH)D were higher among pre-pubertal girls in the late spring, in contrast to pubertal girls investigated in the autumn and pubertal boys examined in the autumn. Kliś et al. (2016) noticed that the season of birth, which indirectly affected vitamin D levels, was associated with sexual maturation among girls, with those born in the summer having the earliest menarche. We did not find seasonal effects on the 25(OH)D concentration among pre-pubertal boys in contrast to girls and pubertal boys. Seasonal effects on the 25(OH)D concentration are the result of differences in sunshine exposure between spring and autumn. Higher exposure in spring leads to higher 25(OH)D concentration while lower exposure in autumn results in lower 25(OH)D concentration. We suspect that the reason of the lack of seasonal effects among pre-pubertal boys may be their lower outdoor physical activity than girls during spring (Beighle, Alderman, Morgan, & Masurier, 2008; Schwarzfischer et al., 2019). The pubertal boys in this study may have also spent more time outdoor than pre-pubertal boys that results in their seasonal variation of the 25(OH)D concentration.

We hypothesized that 25(OH)D concentrations would be positively correlated with BCM (%) among both pubertal and pre-pubertal children. However, after controlling for various confounding factors, our regression analyses showed no significant association. Among pre-pubertal children, maternal education seems important for explaining BCM (%) variability. The BCM is a part of the metabolically active fat free mass, which is involved in oxygen consumption and carbon dioxide production. Thus, it is a valuable measure of an individual's physical nutritional status (Roza & Shizgal, 1984; Savalle et al., 2012). Based on the present results, we believe that 25(OH)D may be important in promoting adequate nutritional status. However, more studies are needed to verify the relation between the 25(OH)D concentration and the BCM (%).

Since vitamin D is a fat-soluble molecule, the bioavailability of the vitamin is shaped by levels of body fatness and fat metabolism. With the increasing number of fat cells, vitamin D is increasingly sequestered in adipose tissue, thus reducing its bioavailability in the bloodstream (de Brito Galvao, Nagode, Schenck, & Chew, 2013). In this study, the WHR, a measure of central fat deposition, was negatively correlated with 25(OH)D concentrations among pubertal children Jackson et al. (2016) confirmed similar associations between WHR and 25(OH)D concentrations among adults, while Lee et al. (2013) found such associations among children. However, Rahmani, Eftekhari, Fallahzadeh, Fararouei, and Massoumi (2018) did not find any statistically significant association with WHR among children.

BMI, FM (%), and MM (%) were not associated with 25(OH)D concentrations among pre-pubertal children. However, we presumed that investigation in spring (June) and breastfeeding were associated with decreased BMI and FM (%) and increased MM (%).

In other studies, 25(OH)D deficiency was found to be associated with increased BMIs among Malaysian (Khor et al., 2011) and Korean (Lee et al., 2013) children. Similar results were obtained among adults by Jackson et al. (2016).

Hassan-Smith et al. (2017) and Beaudart et al. (2014) showed that greater 25(OH)D concentrations were associated with greater muscle strength among adults but not with greater muscle mass. However, no similar findings have been presented for children.

The increased MM (%) and decreased BMI and FM (%) in spring compared to autumn may be due to more intensive physical activity in spring. Beighle et al. (2008) also found that physical activity was more frequent with increased sunlight/day length during spring in a sample of children from the southern United States.

Breastfed children had higher muscle mass and lower fat mass and BMI than those who were not breastfed. The importance of breastfeeding was also observed in our previous studies (Pruszkowska-Przybylska et al., 2019; Pruszkowska-Przybylska, Sitek, et al., 2018).

TBW (%) among pre-pubertal children was not statistically significantly associated with the 25(OH)D status. However, children that were breastfed and also those measured during spring had increased water status. The TBW is crucial for an appropriate metabolic rate (Chumlea, Guo, Zeller, Reo, & Siervogel, 1999). Data about the relation between TBW (%) and 25(OH) D status are lacking. Hence, more detailed investigations are needed.

One of the limitations of this study was that the vitamin D concentrations were measured with saliva samples. While they provide an accurate index of vitamin D status, the measures are not directly comparable to those derived from plasma samples.

Overall, more studies are needed to resolve the association between vitamin D and body composition. If the hypothesized causal relationship between vitamin D and obesity exists, it has important implications for clinical medicine.

5 | CONCLUSIONS

There were statistically significant seasonal differences in the 25(OH)D concentration, with higher concentrations occurring in late spring (June) compared to autumn (November-December).

The pre-pubertal children had higher 25(OH)D concentrations than pubertal children.

The level of 25(OH)D was positively associated with BCM (%) among all children and inversely associated with WHR among pubertal children, but only when other factors were not included in the analyses.

Investigation in spring (June) and breastfeeding were associated with increased MM (%) and TBW (%) and were associated with decreased BMI and FM (%).

The effect of breastfeeding on body composition and proportion was greater among pre-pubertal than among pubertal children. The effect of breastfeeding may only be important to some stages of development.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

AUTHOR CONTRIBUTIONS

P.P.P. designed the study, performed laboratory work, analyzed the data, prepared the draft and final version of the manuscript, and collected the material. E.Ż. and N.M. designed the study, prepared the manuscript, and provided critical comments on the manuscript. A.S. and I.R. designed the study and collected the material. D.S. designed the study and collected the material. M.S. and M.S.K. collected the material.

ETHICS STATEMENT

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving research study participants were approved by the Ethical Commission at the University of Lodz (nr 19/KBBN-UŁ/II/2016). Written informed consent was obtained from all subjects' parents.

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