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1 Included papers

Paper I (Appendix 1)

Lewis JI, Mbabazi J, Friis H, Mupere E, Wells JC, Grenov B. Calibration of bioelectrical impedance analysis against deuterium dilution for body composition assessment in stunted Ugandan children. *J Nutr 2023. doi: 10.1016/j.tjnut.2022.12.028*

Paper II (Appendix 2)

Lewis JI, Mbabazi J, Mutumba R, Ritz C, Filteau S, Briend A, Michaelsen KF, Mølgaard C, Wells JC, Mupere E, Friis H, Grenov B. Correlates of body composition in children with stunting: A cross-sectional study in Uganda. (*Submitted to Journal of Nutrition)*

Paper III (Appendix 3)

Lewis JI, Christensen SH, Peerson JM, Islam M, Keya FK, Kac G, Mucci DB, Moore SE, Doel A, Njie F, Shahab-Ferdows S, Hampel D, Mølgaard C, Michaelsen KF, Allen L. Global references for human milk macronutrient concentrations: Results from the Mothers, Infants and Lactation Quality Study. (*Manuscript prepared for submission)*

1.1 Other scientific contributions

Lewis JI, Lind MV, Møller G, Hansen T, Pedersen H, Christensen MMB, Laursen JC, Nielsen S, Hansen CB, Larsen CVL, Bjerregard P, Jørgensen ME, Lauritzen L. The effect of traditional diet on glucose homeostasis depends on a common *TBC1D4* variant that affects skeletal GLUT4 translocation in Greenlandic Inuit: A randomized crossover study. Br J Nutr 2023. doi: 10.1017/S000711452300106X

Lewis JI, Christensen SH, Larnkjær A, Mølgaard C, Michaelsen KF. Early Nutrition and Its Effect on Growth, Body Composition, and Later Obesity. World Rev Nutr Diet 2022;125:138-155. doi: 10.1159/000521798.

Mutumba R, Mbabazi J, Pesu H, **Lewis JI**, Mølgaard C, Ritz C, Olsen MF, Briend A, Nabukeera-Barungi N, Wells JC, Friis H, Grenov B, Mupere E. Effect of lipid-based nutrient supplements on symptoms, inflammation, and bioelectrical impedance phase angle among stunted children: secondary analysis of a randomized trial in Uganda. Nutrients 2024 [*under review*]

Larnkjær A, Larsson MW, Wells JC, Christensen SH, **Lewis JI**, Mølgaard C, Michaelsen KF. Cohort with excessive weight gain during exclusive breastfeeding- follow-up at mid childhood of the SKOT-III cohort. J Hum Lact 2024. [*under review*]

Christensen SH, Rom AL, Greve T, **Lewis JI**, Frøkiær H, Allen LH, Mølgaard H, Renault KM and Michaelsen KF. Maternal inflammatory, lipid and metabolic markers and associations with birth and breastfeeding outcomes. Front Nutr. 2023. doi: 10.3389/fnut.2023.1223753

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Larnkjær A, **Lewis JI,** Christensen SH, Mølgaard C, Michaelsen KF. Early Nutrition and Its Effect on Growth, Body Composition, and Later Obesity. World Rev Nutr Diet 2023. doi: 10.1159/000527948

Christensen SH, **Lewis JI**, Larnkjær A, Frøkiær H, Allen LH, Mølgaard C, Michaelsen KF. Associations between maternal adiposity and appetite-regulating hormones in human milk are mediated through maternal circulating concentrations and might affect infant outcomes. Front Nutr 2022;9:1025439. doi: 10.3389/fnut.2022.1025439

Berglund NR, **Lewis JI**, Michaelsen KF, Mølgaard C, Renault KM, Carlsen EM. Birthweight z-score and fat-free mass at birth predict body composition at 3 years in Danish children born from obese mothers. Acta Paediatr 2022 Jul;111(7):1427-1434. doi: 10.1111/apa.16346

Olsen MF, Kjøller-Svarre MS, Møller G, Katzenstein TL, Nielsen BU, Pressler T, **Lewis JI,** Mathiesen IH, Mølgaard C, Faurholt-Jepsen D. Correlates of Pancreatic Enzyme Replacement Therapy Intake in Adults with Cystic Fibrosis: Results of a Cross-Sectional Study. Nutrients 2022 Mar 22;14(7):1330. doi: 10.3390/nu14071330

Pedersen H, Beaulieu K, Finlayson G, Færch K, Jørgensen ME, **Lewis JI**, Lind MV, Lauritzen L, Quist JS. Food Reward after a Traditional Inuit or a Westernised Diet in an Inuit Population in Greenland. Nutrients 2022 Jan 27;14(3):561. doi: 10.3390/nu14030561

2 Abstract

Background:

Obesity and chronic disease risk has long been a public health focus in high-income settings. This is increasingly true for low-income settings experiencing nutritional transition and an ensuing double burden of malnutrition. Nutritional exposures in early life can have lasting effects on metabolic health and chronic disease risk and alterations in growth and body composition (BC) during critical windows of development may explain some of these associations. As the earliest nutritional exposure for many infants, human milk (HM) may contribute to BC development and thus chronic disease risk. However, the study of both paediatric BC and HM are complicated by methodological difficulties, creating a challenging research environment. Bioelectrical impedance analysis (BIA) represents a feasible tool for BC assessment in low-income settings but requires population-specific calibration. HM composition is influenced by sampling and analytical protocols, and a lack of standardized tools has hindered cross-study comparisons.

Objectives:

The overall objective of this thesis was to develop improved tools for the study of HM and BC. In *paper I*, the objective was to calibrate BIA for use in Ugadan children with stunted linear growth using isotope dilution as a reference method. In *paper II*, we aimed to apply this calibrated BIA in a large cohort of Ugandan children with stunting who were enrolled to a nutritional intervention study and investigate correlates of BC at baseline. In *paper III*, the objective was to produce globally representative HM macronutrient reference values (RV) for normal mothers whose infants were growing without constraint.

Methods:

This thesis is a culmination of two separate studies. The first is a nutrition intervention trial based in Eastern Uganda, to which 750 children aged 12-59 months with stunting were enrolled. We selected a purposive subsample of 50 stunted children to represent the full range of age, sex, and nutritional statuses of the main study population and developed an equation to estimate fat-free mass (FFM) from whole-body impedance. Bioimpedance was measured using a single-frequency (50 kHz) device, and deuterium dilution analysed by isotope-ratio mass spectrometry as the reference technique (*paper I*). In *paper II* the calibration equation was applied to

bioimpedance measurements from the main study and multiple linear regression was used cross-sectionally to compare to UK BC references and investigate baseline correlates of FFM, fat mass (FM), FFM index (FFMI), FM index (FMI), and height. Correlates were chosen based on biological plausibility and included serum inflammation markers, breastfeeding practices by recall, nutritional status, and infection markers. The second study was a multi-centre cohort including mother-infant dyads from Bangladesh, Brazil, Denmark, and The Gambia (*n = 250 per site*). Dyads remained in the study if they conformed to a range of health, dietary, and growth criteria so the cohort represented a normative sample. Mothers provided full-breast HM samples at three timepoints between 1 and 8.5 months, and macronutrient concentrations analysed using infrared spectroscopy. In paper III, we used generalized additive models for location, scale, and shape (GAMLSS) to produce RVs for protein, carbohydrate, fat, and energy.

Results:

In *paper I* we developed a BIA equation that performed with a prediction error of 4.5%. for FFM. Including HAZ in the equation explained a further 1% of variation compared to the next best model which included impedance index, sex and age. In *paper II,* 750 stunted children (45% female) we found deficits in absolute FM, FFM, FMI, but not FFMI, compared to UK references. Elevated α1-acid glycoprotein (AGP) and c-reactive protein were associated with β [95% CI] 0.34 [0.26, 0.41] kg and 0.11 [0.03, 0.19] kg lower FFM, respectively, but neither were associated with FM. AGP was further associated with 1.14 [0.76, 1.52] cm lower height and 0.15 [0.07, 0.23] kg/m^2 lower FFMI. HAZ was associated inversely with FFMI, but positively with FMI. Each additional month of breastfeeding was associated with 0.02 [0.01, 0.03] kg greater FFM and 0.01 [0.00, 0.02] kg greater FM, in proportion with 0.10 [0.06, 0.13] cm greater height. A positive rapid malaria test was associated with 0.27 [0.09, 0.44] kg/m^2 greater FMI, but not with FFMI (0.01 [-0.07, 0.10]). In *paper III* we present HM macronutrient RVs produced using 2194 samples valid for inclusion from 903 mothers at 1-8.5 months postpartum. HM protein RV median reduced from 1.15 g/100 mL at 1 month, to 0.8 g/100 mL at 4 months postpartum from where it remained stable. The RV median for HM carbohydrate, fat, and energy were also relatively stable across lactation at around 6.8 g/100 mL and 3.2 g/100 mL, and 600 kcal/L, respectively. The GAMLSS framework also accounted for changing variance, skew, and kurtosis over time.

Discussion and Conclusion:

The BIA equation produced in *paper I* suggests that degree of stunting changes the relationship between whole-body impedance and FFM. We propose that characteristic alterations of limb proportions in stunting could be the mediating factor, and that although the additional variance explained by HAZ was modest, it may continue along the non-stunted range of HAZ. In the main sample, we found that systemic inflammation was associated with reduced FFM and further FFMI and height for AGP. Inflammation is known to disrupt hormonal drivers of paediatric growth and may be an ideal target for interventions seeking to improve metabolic capacity associated with lean mass, while reducing metabolic load. Longer continued breastfeeding seemed protective of growth even in this wholly stunted sample of children, reinforcing the World Health Organisation recommendations to support continued breastfeeding to two years. We also believe that there is suggestive evidence of FFM protection at the expense of linear growth and FM storage in the face of stunting, indicated by a comparable FFMI compared to UK BC, and the opposing associations of FFMI and FMI with HAZ. Brain-sparring is a well-known mechanism which may extend to other lean components of BC. Our HM macronutrient RVs were produced using HM samples collected using standardised protocols from geographically and genetically diverse populations, making them globally representative and robust. We encourage their use in future research and practice as normative values, but researchers must consider their own sampling and analytical techniques used to derive HM composition estimates.

3 Resumé (Abstract in Danish)

Baggrund:

Fedme og risiko for kronisk sygdom har længe været et folkesundhedsfokus i højindkomstmiljøer. Dette gælder i stigende grad for lavindkomstmiljøer, der oplever ernæringsmæssig overgang og en deraf følgende dobbelt byrde af underernæring. Ernæringseksponeringer i det tidlige liv kan have varige virkninger på metabolisk sundhed og risiko for kronisk sygdom, og ændringer i vækst og kropssammensætning (BC) under kritiske udviklingsvinduer kan forklare nogle af disse sammenhænge. Som den tidligste ernæringsmæssige eksponering for mange spædbørn, kan modermælk (HM) bidrage til udvikling af BC og dermed risiko for kronisk sygdom. Studiet af både pædiatrisk BC og HM er imidlertid kompliceret af metodiske vanskeligheder, hvilket skaber et udfordrende forskningsmiljø. Bioelektrisk impedansanalyse (BIA) repræsenterer et gennemførligt værktøj til BC-vurdering i lavindkomstmiljøer, men kræver populationsspecifik kalibrering. HM-sammensætning er påvirket af prøveudtagning og analytiske protokoller, og mangel på standardiserede værktøjer har hindret sammenligninger på tværs af undersøgelser.

Formål:

Det overordnede formål med denne afhandling var at udvikle forbedrede værktøjer til studiet af HM og BC. I papir I var formålet at kalibrere BIA til brug hos børn i Ugadan med hæmmet lineær vækst ved at bruge isotopfortynding som referencemetode. I papir II sigtede vi på at anvende denne kalibrerede BIA i en stor kohorte af ugandiske børn med stunting, som blev tilmeldt en ernæringsinterventionsundersøgelse og undersøge korrelater af BC ved baseline. I papir III var målet at producere globalt repræsentative HM makronæringsstof referenceværdier (RV) for normale mødre, hvis spædbørn voksede uden begrænsninger.

Metoder:

Denne afhandling er en kulmination på to separate undersøgelser. Den første er et ernæringsinterventionsforsøg baseret i det østlige Uganda, hvor 750 børn i alderen 12-59 måneder med stunting blev tilmeldt. Vi udvalgte en formålsbestemt delprøve på 50 børn med forkrøplethed til at repræsentere hele spektret af alder, køn og ernæringsstatus for hovedundersøgelsespopulationen og udviklede en ligning til at estimere fedtfri masse (FFM) fra helkropsimpedans. Bioimpedans blev målt ved hjælp af en enkeltfrekvens (50 kHz) enhed, og deuteriumfortynding blev analyseret ved isotopforhold massespektrometri som referenceteknik

(papir I). I papir II blev kalibreringsligningen anvendt på bioimpedansmålinger fra hovedundersøgelsen, og multipel lineær regression blev brugt på tværs til at sammenligne med UK BC-referencer og undersøge basislinjekorrelater af FFM, fedtmasse (FM), FFM-indeks (FFMI), FM indeks (FMI) og højde. Korrelater blev valgt baseret på biologisk plausibilitet og inkluderede seruminflammationsmarkører, ammepraksis ved tilbagekaldelse, ernæringsstatus og infektionsmarkører. Den anden undersøgelse var en multicenter-kohorte, der inkluderede mor-spædbarn-dyader fra Bangladesh, Brasilien, Danmark og Gambia (n = 250 pr. sted). Dyader forblev i undersøgelsen, hvis de var i overensstemmelse med en række sundheds-, kost- og vækstkriterier, så kohorten repræsenterede en normativ prøve. Mødre gav fuldbryst HM-prøver på tre tidspunkter mellem 1 og 8,5 måneder, og makronæringsstofkoncentrationer blev analyseret ved hjælp af infrarød spektroskopi. I papir III brugte vi generaliserede additivmodeller for placering, skala og form (GAMLSS) til at producere RV'er til protein, kulhydrat, fedt og energi.

Resultater:

I papir I udviklede vi en BIA-ligning, der fungerede med en forudsigelsesfejl på 4,5%. for FFM. Inkludering af HAZ i ligningen forklarede yderligere 1 % variation sammenlignet med den næstbedste model, som inkluderede impedansindeks, køn og alder. I papir II fandt vi underskud i absolut FM, FFM, FMI, men ikke FFMI, sammenlignet med UK-referencer. Forhøjet α1-syreglycoprotein (AGP) og c-reaktivt protein var associeret med henholdsvis β [95 % CI] 0,34 [0,26, 0,41] kg og 0,11 [0,03, 0,19] kg lavere FFM, men ingen af dem var forbundet med FM. AGP var yderligere forbundet med 1,14 [0,76, 1,52] cm lavere højde og 0,15 [0,07, 0,23] kg/m2 lavere FFMI. HAZ var omvendt forbundet med FFMI, men positivt med FMI. Hver ekstra måneds amning var forbundet med 0,02 [0,01, 0,03] kg større FFM og 0,01 [0,00, 0,02] kg større FM, i forhold til 0,10 [0,06, 0,13] cm større højde. En positiv hurtig malariatest var forbundet med 0,27 [0,09, 0,44] kg/m2 større FMI, men ikke med FFMI (0,01 [-0,07, 0,10]). I papir III præsenterer vi HM makronæringsstof RV'er produceret ved hjælp af 2194 prøver, der er gyldige til inklusion fra 903 mødre 1-8,5 måneder efter fødslen. HM protein RV median reduceret fra 1,15 g/100 ml efter 1 måned til 0,8 g/100 ml ved 4 måneder efter fødslen, hvorfra det forblev stabilt. RV-medianen for HM-kulhydrat, fedt og energi var også relativt stabil over laktation ved henholdsvis omkring 6,8 g/100 ml og 3,2 g/100 ml og 600 kcal/L. GAMLSS-rammen tegnede sig også for ændring af varians, skævhed og kurtosis over tid.

Diskussion og Konklusion:

BIA-ligningen fremstillet i papir I antyder, at graden af stunting ændrer forholdet mellem helkropsimpedans og FFM. Vi foreslår, at karakteristiske ændringer af lemmerproportioner i forkrøpning kan være den medierende faktor, og at selvom den yderligere varians, der forklares af HAZ, var beskeden, kan den fortsætte langs det ikke-forkrøplede område af HAZ. I hovedprøven fandt vi, at systemisk inflammation var forbundet med reduceret FFM og yderligere FFMI og højde for AGP. Inflammation er kendt for at forstyrre hormonelle drivkræfter for pædiatrisk vækst og kan være et ideelt mål for interventioner, der søger at forbedre metabolisk kapacitet forbundet med mager masse, samtidig med at den metaboliske belastning reduceres. Længere fortsat amning virkede beskyttende for væksten selv i denne fuldstændig forkrøblede prøve af børn, hvilket forstærkede Verdenssundhedsorganisationens anbefalinger til at støtte fortsat amning til to år. Vi mener også, at der er tegn på FFM-beskyttelse på bekostning af lineær vækst og FM-lagring i lyset af stunting, angivet ved en sammenlignelig FFMI sammenlignet med UK BC, og de modsatte foreninger af FFMI og FMI med HAZ. Hjernesparring er en velkendt mekanisme, som kan strække sig til andre magre komponenter af BC. Vores HM makronæringsstof RV'er blev produceret ved hjælp af HM prøver indsamlet ved hjælp af standardiserede protokoller fra geografisk og genetisk forskellige populationer, hvilket gør dem globalt repræsentative og robuste. Vi opfordrer til deres brug i fremtidig forskning og praksis som normative værdier, men forskere skal overveje deres egne prøveudtagnings- og analyseteknikker, der bruges til at udlede HM-sammensætningsestimater.