

Aus dem Institut für Humanernährung und Lebensmittelkunde
der Christian-Albrechts-Universität zu Kiel

**Associations between anthropometric traits, detailed body
composition, fat fraction per adipose tissue and cardiometabolic
risks: differences between children, adolescents and adults**

Dissertation
zur Erlangung des Doktorgrades
der Agrar- und Ernährungswissenschaftlichen Fakultät
der Christian-Albrechts-Universität zu Kiel

vorgelegt von
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Kiel, im Dezember 2017

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Tag der mündlichen Prüfung:	24.01.2018

Gedruckt mit Genehmigung der Agrar- und Ernährungswissenschaftlichen Fakultät der
Christian-Albrechts-Universität zu Kiel

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ABBREVIATIONS

ADP	air displacement plethysmography
AT	adipose tissue
BIA	bioelectrical impedance analysis
BMBF	Federal Ministry of Education and Research
BMC	bone mineral content
BMI	body mass index
CRP	C-reactive protein
CT	computed tomography
DBP	diastolic blood pressure
DFG	Deutsche Forschungsgemeinschaft
DXA	dual X-ray absorptiometry
D ₂ O	deuterium oxide
ECW	extracellular water
FFM	fat-free mass
FFMI	fat-free mass index
FG	Fettgewebe
FM	fat mass
FMI	fat mass index
HD	hydrodensitometry
HDL	high-density lipoprotein cholesterol
HOMA-IR	homeostasis model assessment of insulin resistance
ICW	intracellular water
IQR	interquartile range
KOPS	Kiel Obesity Prevention Study
MM	muscle mass
MRI	magnetic resonance imaging
NaBr	sodium bromide
OB	obese
OM	organ mass
OW	overweight

QMR	quantitative magnetic resonance
REE	resting energy expenditure
ROC	receiver operating characteristics
RQ	respiratory quotient
SAT	subcutaneous adipose tissue
SBP	systolic blood pressure
SDS	standard deviation score
TAT	total adipose tissue
TATI	total adipose tissue index
TBW	total body water
TG	triglycerides
TU	Taillenumfang
UWW	underwater weighing
VAT	visceral adipose tissue
WC	waist circumference
WHO	World Health Organization
4C	four-compartment

CHAPTER I

General introduction and objectives

Global epidemic of overweight and obesity

Since the end of the 1940s, obesity and its association with health and disease have become of growing research interest [1,2]. It was assumed that overweight and obesity will increasingly be present in medical practice, as people were getting older. At the population level, fat mass (FM) increased by facilitated access to high caloric food and increasing inactivity [3].

In the last four decades the prevalence of overweight and obesity have almost tripled [4,5]. In the year 2016 the worldwide prevalence of overweight and obesity were 39 % and 13 % respectively [5]. In Germany, the prevalence of overweight (men: 67.1 %, women: 53.0 %) and obesity (men: 23.3 %, women: 23.9 %) are considerably higher [6]. In children and adolescents, the increase in obesity from 1975 to 2016 has been dramatically greater than in adulthood [7].

Overweight and obesity are based on body mass index (BMI). Its use is recommended by the World Health Organization (WHO) for the definition of nutritional status in children, adolescents and adults [5].

Nutritional status defined by body mass index and waist circumference

The ratio of weight (kg) divided by height (m) squared was invented by Adolphe Quetelet in the year 1832. Termed as the BMI, Ancel Keys demonstrated that BMI was a good indirect measure to assess body fatness [8]. Thus, up today, BMI is used to assess nutritional status in children, adolescents and adults [9]. Although or even because of the definition of overweight and obesity is based on BMI only, differences become evident in children, adolescents and adults. In adults, overweight and obesity are defined as BMI higher or equal 25 kg/m² and 30 kg/m², respectively [9]. These BMI *cut-offs* are associated with increased morbidity and mortality [10–13]. By contrast, statistically calculated age- and sex specific BMI percentiles are used in children and adolescents [14]. Accordingly, overweight and obesity are defined as BMI > 90th and 97th percentile, respectively. These BMI percentiles had only been statistically extrapolated to BMI *cut-offs* for mortality risk in adults [15,16]. Besides, waist circumference (WC) is used to assess abdominal obesity [9,17,18].

Cut-offs of BMI and WC are commonly accepted and applied in clinical and research practice. However, in a forty year follow-up study, Twig et al. (2016) demonstrated that, unlike BMI of 18.3 kg/m², a BMI within the normal range in adolescence was significantly associated with elevated cardiovascular mortality in adulthood [19]. Furthermore, lower mortality rate was observed in overweight compared with normal weight adults [20]. Taking this into account, the definition of overweight and obesity in children, adolescents and adults has substantial

shortcomings. Since, the definition is BMI-based only and BMI *cut-offs* were not comparably associated with health outcomes in all age groups, commonly accepted definition of overweight and obesity and its *cut-offs* have to be re-considered and should be related to metabolic disorders in children, adolescents and adults.

Limitations of body mass index and waist circumference

In epidemiological studies, overweight and obesity increase cardiometabolic risks such as elevated blood pressure, dyslipidemia, insulin resistance and diabetes mellitus type 2 [21–24]. These results are based on BMI and WC. However, using BMI and WC to evaluate overweight and obesity have additional limitations. On the one hand, BMI cannot differentiate between the two chemical body determined compartments (FM and fat-free mass, FFM). Both compartments increase with body weight. On the other hand, the association between BMI and FM differs between normal- and overweight subjects showing strongest associations in obese group [25]. To go beyond BMI, data of a cross-sectional study on 304 adults of the German Reference Centre for Body Composition in Kiel revealed, that FM in itself did not improve BMI in disease risk predicting [26]. Nevertheless, BMI does not give any information about body fat distribution, which is characterized by WC [25,27,28]. However, WC does not allow to differentiate between abdominal subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) [29].

Beyond anthropometric traits

To go beyond BMI, WC and FM, detailed body composition and appropriate modeling take into account body function and metabolic risks [25]. Computed tomography (CT) and magnetic resonance imaging (MRI) are the gold-standard methods to determine total adipose tissue (TAT), SAT_{arms}, SAT_{trunk}, SAT_{legs} and VAT. Unlike CT, MRI is the most suitable measurement in children and adolescents because it does not expose them to radiation.

Cardiometabolic risks have been mainly related to VAT and liver fat in obese adolescents and adults [30–34]. In a cross-sectional as well as longitudinal analysis SAT_{trunk} and VAT were positively associated with cardiometabolic risks in women, whereas SAT_{legs} was inversely related to insulin resistance and triglycerides [35]. Other studies in children, adolescents and adults have found positive associations between insulin resistance, inflammation and either VAT or liver fat [36–38]. However, the associations between regional fat depots and cardiometabolic risks have only been investigated in selected age groups. Comparing different

age groups, it is imperative to analyze these associations systematically by using identical methods and outcomes in normal- and overweight children, adolescents and adults.

Methods to determine body composition

In clinical and research practice, different methods are used to assess nutritional status and body composition. Table II gives an overview about established measurement techniques. Nutritional status and central adiposity can be indirectly assessed by anthropometric parameters (e.g. BMI and WC). Measuring BMI and WC are inexpensive and fast to perform in all age groups. However, as mentioned above, no differentiation between either FM and FFM or SAT and VAT can be undertaken. Further, based on a two-compartment model, which divides the body into fat and fat-free compartments [39], FM and FFM can be indirectly determined by bioelectrical impedance analysis (BIA) and air displacement plethysmography (ADP). Although these methods are fast and applicable in children, adolescents and adults, determination of FM and FFM relies on algorithms and have a reduced accuracy in lean and obese subjects as well as under disease conditions. On the molecular level, dilution techniques (D_2O and NaBr) are used to assess total and extracellular water, respectively. Besides, bone mineral content can be measured by dual X-ray absorptiometry (DXA). Dilution techniques and DXA require an expensive equipment. Further, in Germany, DXA application is ethically not allowed in children and adolescents due to radiation exposure. Nevertheless, these methods have a high outcome precision. Combining ADP, dilution techniques and DXA results in a four-compartment (4C) model, with which FM and FFM can be calculated by minimizing shortcomings of individual methods [39]. On a tissue level, TAT, regional SAT and VAT can be directly measured by CT or MRI. These are gold-standard methods in determining adipose tissues. However, an expensive equipment is required, which is often in high demand in clinical setting. Although anthropometric traits cannot differentiate between FM and FFM and, additionally, other methods of body composition, having a higher precision of FM or TAT are available, BMI is still recommended for the definition of overweight and obesity [5].

Analyzing the associations between either anthropometric traits or body composition and cardiometabolic risks in children, adolescents and adults, we used different body composition methods to investigate their value systematically. Taking this into account, FM determined by 4C model and TAT as assessed by MRI are interchangeably used for metabolic risk assessment. However, on the molecular level, FM is one chemical body component. By contrast, on the tissue level, TAT consists of different chemical body components [40]. Therefore, the interchangeable use of FM and TAT should be considered critically.

Table II. Methods used in clinical and research practice to assess nutritional status and body composition¹

Method	Outcomes	Advantages	Shortcomings
BMI	nutritional status	quick; inexpensive equipment; convenient for all age groups; minimal training required	no differentiation between FM and FFM; affected by ethnicity; less sensitive in thin children
WC	central adiposity	quick; inexpensive equipment; minimal training required	no differentiation between SAT and VAT
Skinfolds	SAT	quick; inexpensive equipment	references based on norms during 1970s; considerable expertise and training necessary; does not describe lean body mass
BIA	resistance, reactance, phase angle	quick; inexpensive equipment; portable; applicable in children and adolescents	population specific; poor accuracy in individuals and groups; relies on prediction equations; inaccuracy in leaner and obese children
HD	body volume and density	gold standard in density measurement	expensive equipment; difficult to perform in children; estimation of respiratory volume results in largest variability
ADP	body volume and density	quick; relatively high accuracy; minimal training required; applicable in children and adolescents	expensive equipment; relies on algorithms; reduced accuracy in disease states
DXA	lean body mass, FM, bone mass and bone mineral density	minimal training required; differentiation of bone and soft tissue; high precision; low radiation exposure	expensive equipment; relies on algorithms; bias due to body size, sex, fatness; in Germany exclusion of children due to ethical reason
D ₂ O, NaBr	TBW, ECW and ICW	easy to administer isotopes	expensive equipment; needs labor for analyses
MRI	AT, SAT, VAT, MM, OM, ectopic fat in liver, skeletal muscle, pancreas	high accuracy and reproducibility for whole-body and regional adipose tissue and skeletal muscle; no radiation	expensive equipment; high level of training and expertise needed; equipment often in high demand in clinical settings; requires children to remain still for extended periods
CT	AT, SAT, VAT, MM, OM, ectopic fat in liver, skeletal muscle, pancreas	high accuracy and reproducibility for whole-body and regional adipose tissue and skeletal muscle	expensive equipment; radiation exposure; radiation safety training required; equipment often in high demand in clinical settings
QMR	FM, lean tissue, free and total water	quick; minimal training required	expensive equipment; few systems available worldwide
Ultrasound	SAT, MM thickness, OM, liver fat	moderately expensive equipment; less time consuming than MRI and CT	preperitoneal fat used as an approximation of VAT; high level of training and expertise needed; no universal guidelines for measurement of adipose tissue

Abbreviations: BMI, body mass index; FM, fat mass; FFM, fat-free mass; WC, waist circumference; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; BIA, bioelectrical impedance analysis; HD, hydrodensitometry; ADP, air displacement plethysmography; DXA, dual X-ray absorptiometry; TBW, total body water; ECW, extracellular water; ICW, intracellular water; MRI, magnetic resonance imaging; AT, adipose tissue; MM, muscle mass; OM, organ mass; CT, computed tomography; QMR, quantitative magnetic resonance.

¹Referring to the tables from Müller et al. 2016, Horan et al. 2015 and Lee and Gallagher 2008 [39,41,42].

Methods colored in green were used within this doctoral thesis.

Differentiation between fat mass and adipose tissue

FM consists of fat content in adipose tissue, organs, skeletal muscle, hands, feet and head (Figure I1). The overall density for total FM is assumed to be 0.90 kg/l [43]. Instead, fat in adipose tissue, water content, protein content and mineral content are included in TAT [43–45]. Regarding this, TAT contents have different densities. Thus, overall density for TAT (0.92 kg/l) is different from FM [46].

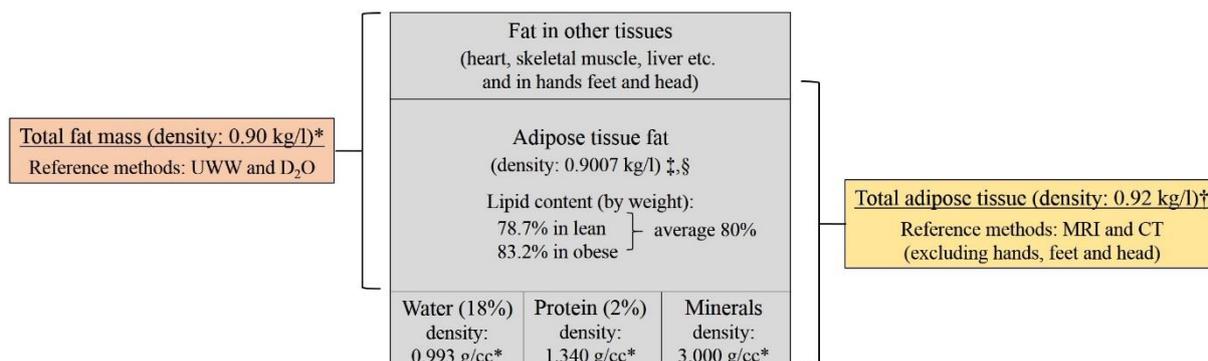


Figure I1. Relationship between total adipose tissue and total fat mass.¹

¹Referring to the figure from Al-Gindan et al. [40].

*Siri [43], ‡Fidanza et al. [44], §Brozek et al. [45], †Garrow [46]

Abbreviations: UWW, underwater weighing; D₂O, deuterium oxide; MRI, magnetic resonance imaging; CT, computed tomography.

Based on data obtained by adipose tissue biopsy, fat in adipose tissue is lower in lean subjects than in obese subjects (Figure I1) [40]. Overall, fat content in adipose tissue is assumed to be 80 %. However, as mentioned in the *Report of the task group on reference man fat fraction per adipose tissue* shows a wide variation between 62 % and 91 % [47–50]. Therefore, we questioned the idea to interchangeably use FM and TAT in relation to metabolic risk assessment.

So far, fat fraction per adipose tissue was determined by adipose tissue biopsy, which was undertaken during bariatric surgery. Nevertheless, implementing adipose tissue biopsy in healthy subjects is not allowed for ethical reason. Instead, we used a new approach based on *in vivo* body composition data on FM and TAT to determine fat fraction per adipose tissue.

Up today, the associations between either anthropometric traits or detailed body composition on cardiometabolic risks have not been systematically investigated and compared in different age groups. Moreover, it is questionable to define overweight and obesity based on BMI only. Established *cut-offs* are not comparably associated with metabolic disorders in children,

adolescents and adults. It is further doubtful to interchangeably use FM and TAT for metabolic risk assessment.

Objectives

Based on this background, overweight and obesity have an impact on public health, clinical medicine and research. Therefore, the present thesis analyzed

- (i) the associations between regional fat depots and cardiometabolic risks in normal- and overweight children, adolescents and adults,
- (ii) new *cut-offs* of BMI and WC based on body composition and insulin resistance,
- (iii) fat fraction per adipose tissue and their association with metabolic risks.

References

1. Smigell, V.K. Obesity; an enemy to health and comfort. *Journal. National Malaria Society (U.S.)* **1946**, 2, 161–163.
2. Marks, H.H. Influence of obesity on morbidity and mortality. *Bulletin of the New York Academy of Medicine* **1960**, 36, 296–312.
3. Armstrong, D.B. OBESITY AND ITS RELATION TO HEALTH AND DISEASE. *JAMA* **1951**, 147, 1007.
4. Afshin, A.; Forouzanfar, M.H.; Reitsma, M.B.; Sur, P.; Estep, K.; Lee, A.; Marczak, L.; Mokdad, A.H.; Moradi-Lakeh, M.; Naghavi, M.; *et al.* Health Effects of Overweight and Obesity in 195 Countries over 25 Years. *The New England journal of medicine* **2017**, 377, 13–27.
5. WHO. *Obesity and overweight - Fact sheet, Updated October 2017*; 2017. Available online: <http://www.who.int/mediacentre/factsheets/fs311/en/>.
6. Mensink, G.B.M.; Schienkiewitz, A.; Haftenberger, M.; Lampert, T.; Ziese, T.; Scheidt-Nave, C. Übergewicht und Adipositas in Deutschland: Ergebnisse der Studie zur Gesundheit Erwachsener in Deutschland (DEGS1). *Bundesgesundheitsblatt, Gesundheitsforschung, Gesundheitsschutz* **2013**, 56, 786–794.

7. Abarca-Gómez, L.; Abdeen, Z.A.; Hamid, Z.A.; Abu-Rmeileh, N.M.; Acosta-Cazares, B.; Acuin, C.; Adams, R.J.; Aekplakorn, W.; Afsana, K.; Aguilar-Salinas, C.A.; *et al.* Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: A pooled analysis of 2416 population-based measurement studies in 128·9 million children, adolescents, and adults. *Lancet (London, England)*.
8. Keys, A.; Fidanza, F.; Karvonen, M.J.; Kimura, N.; Taylor, H.L. Indices of relative weight and obesity. *Journal of chronic diseases* **1972**, *25*, 329–343.
9. WHO. *Obesity: Preventing and managing the global epidemic : report of a WHO consultation*; World Health Organization: Geneva, 2000.
10. Blackburn, G.L.; Dwyer, J.; Flanders, W.D.; Hill, J.O.; Kuller, L.H.; Pi-Sunyer, F.X.; St Jeor, S.T.; Willett, W.C. Report of the American Institute of Nutrition (AIN) Steering Committee on Healthy Weight. *The Journal of nutrition* **1994**, *124*, 2240–2243.
11. Manson, J.E.; Willett, W.C.; Stampfer, M.J.; Colditz, G.A.; Hunter, D.J.; Hankinson, S.E.; Hennekens, C.H.; Speizer, F.E. Body weight and mortality among women. *The New England journal of medicine* **1995**, *333*, 677–685.
12. Lindsted, K.; Tonstad, S.; Kuzma, J.W. Body mass index and patterns of mortality among Seventh-day Adventist men. *International journal of obesity* **1991**, *15*, 397–406.
13. Seidell, J.C.; Verschuren, W.M.; van Leer, E.M.; Kromhout, D. Overweight, underweight, and mortality. A prospective study of 48,287 men and women. *Archives of internal medicine* **1996**, *156*, 958–963.
14. Kromeyer-Hauschild, K.; Wabitsch, M.; Kunze, D.; Geller, F.; Geiß, H.C.; Hesse, V.; Hippel, A. von; Jaeger, U.; Johnsen, D.; Korte, W.; *et al.* Perzentile für den Body-mass-Index für das Kindes- und Jugendalter unter Heranziehung verschiedener deutscher Stichproben. *Monatsschrift Kinderheilkunde* **2001**, *149*, 807–818.
15. Cole, T.J.; Flegal, K.M.; Nicholls, D.; Jackson, A.A. Body mass index cut offs to define thinness in children and adolescents: international survey. *BMJ (Clinical research ed.)* **2007**, *335*, 194.
16. Cole, T.J.; Bellizzi, M.C.; Flegal, K.M.; Dietz, W.H. Establishing a standard definition for child overweight and obesity worldwide: international survey. *BMJ (Clinical research ed.)* **2000**, *320*, 1240–1243.

17. Wabitsch, M., Kunze, D. (responsible for AGA). *Konsensbasierte (S2) Leitlinie zur Diagnostik, Therapie und Prävention von Übergewicht und Adipositas im Kindes- und Jugendalter (Consensus-based (S2) guideline for the diagnosis, therapy and prevention of overweight and obesity in childhood and adolescence)*; 2015. Available online: http://www.adipositas-gesellschaft.de/fileadmin/PDF/Leitlinien/AGA_S2_Leitlinie.pdf.
18. Kromeyer-Hauschild, K.; Gläßer, N.; Zellner, K. Perzentile für den Taillenumfang von Jenaer Kindern im Alter von 6 bis 18 Jahren. *Akt Ernähr Med* **2008**, *33*, 116–122.
19. Twig, G.; Yaniv, G.; Levine, H.; Leiba, A.; Goldberger, N.; Derazne, E.; Ben-Ami Shor, D.; Tzur, D.; Afek, A.; Shamiss, A.; *et al.* Body-Mass Index in 2.3 Million Adolescents and Cardiovascular Death in Adulthood. *The New England journal of medicine* **2016**, *374*, 2430–2440.
20. Flegal, K.M.; Kit, B.K.; Orpana, H.; Graubard, B.I. Association of all-cause mortality with overweight and obesity using standard body mass index categories: a systematic review and meta-analysis. *JAMA* **2013**, *309*, 71–82.
21. Freedman, D.S.; Mei, Z.; Srinivasan, S.R.; Berenson, G.S.; Dietz, W.H. Cardiovascular risk factors and excess adiposity among overweight children and adolescents: the Bogalusa Heart Study. *The Journal of pediatrics* **2007**, *150*, 12-17.e2.
22. Janssen, I.; Katzmarzyk, P.T.; Srinivasan, S.R.; Chen, W.; Malina, R.M.; Bouchard, C.; Berenson, G.S. Utility of childhood BMI in the prediction of adulthood disease: comparison of national and international references. *Obesity research* **2005**, *13*, 1106–1115.
23. Collaboration, T.E.R.F. Separate and combined associations of body-mass index and abdominal adiposity with cardiovascular disease: Collaborative analysis of 58 prospective studies. *The Lancet* **2011**, *377*, 1085–1095.
24. Marcus, M.D.; Foster, G.D.; El Ghormli, L.; Baranowski, T.; Goldberg, L.; Jago, R.; Linder, B.; Steckler, A.; Treviño, R. Shifts in BMI category and associated cardiometabolic risk: prospective results from HEALTHY study. *Pediatrics* **2012**, *129*, e983-91.
25. Müller, M.J.; Braun, W.; Enderle, J.; Bosy-Westphal, A. Beyond BMI: Conceptual Issues Related to Overweight and Obese Patients. *Obesity facts* **2016**, *9*, 193–205.

26. Müller, M.J.; Lagerpusch, M.; Enderle, J.; Schautz, B.; Heller, M.; Bosy-Westphal, A. Beyond the body mass index: tracking body composition in the pathogenesis of obesity and the metabolic syndrome. *Obesity reviews : an official journal of the International Association for the Study of Obesity* **2012**, *13 Suppl 2*, 6–13.
27. Wells, J.C.K. *The evolutionary biology of human body fatness: Thrift and control*, 1st ed; Cambridge University Press: Cambridge, 2010.
28. Prentice, A.M.; Jebb, S.A. Beyond body mass index. *Obesity reviews : an official journal of the International Association for the Study of Obesity* **2001**, *2*, 141–147.
29. Eloi, J.C.; Epifanio, M.; Gonçalves, M.M. de; Pelliccioli, A.; Vieira, P.F.G.; Dias, H.B.; Bruscato, N.; Soder, R.B.; Santana, J.C.B.; Mouzaki, M.; *et al.* Quantification of Abdominal Fat in Obese and Healthy Adolescents Using 3 Tesla Magnetic Resonance Imaging and Free Software for Image Analysis. *PloS one* **2017**, *12*, e0167625.
30. Yatsuya, H.; Nihashi, T.; Li, Y.; Hotta, Y.; Matsushita, K.; Muramatsu, T.; Otsuka, R.; Matsunaga, M.; Yamashita, K.; Wang, C.; *et al.* Independent association of liver fat accumulation with insulin resistance. *Obesity research & clinical practice* **2014**, *8*, e350-5.
31. King, R.J.; Ajjan, R.A. Vascular risk in obesity: Facts, misconceptions and the unknown. *Diabetes & vascular disease research* **2017**, *14*, 2–13.
32. Kim, S.K.; Kim, H.J.; Hur, K.Y.; Choi, S.H.; Ahn, C.W.; Lim, S.K.; Kim, K.R.; Lee, H.C.; Huh, K.B.; Cha, B.S. Visceral fat thickness measured by ultrasonography can estimate not only visceral obesity but also risks of cardiovascular and metabolic diseases. *The American journal of clinical nutrition* **2004**, *79*, 593–599.
33. Kelly, A.S.; Dengel, D.R.; Hodges, J.; Zhang, L.; Moran, A.; Chow, L.; Sinaiko, A.R.; Steinberger, J. The relative contributions of the abdominal visceral and subcutaneous fat depots to cardiometabolic risk in youth. *Clinical obesity* **2014**, *4*, 101–107.
34. Gyllenhammer, L.E.; Alderete, T.L.; Toledo-Corral, C.M.; Weigensberg, M.; Goran, M.I. Saturation of subcutaneous adipose tissue expansion and accumulation of ectopic fat associated with metabolic dysfunction during late and post-pubertal growth. *International journal of obesity (2005)* **2016**, *40*, 601–606.

35. Schautz, B.; Later, W.; Heller, M.; Müller, M.J.; Bosy-Westphal, A. Associations between breast adipose tissue, body fat distribution and cardiometabolic risk in women: cross-sectional data and weight-loss intervention. *European journal of clinical nutrition* **2011**, *65*, 784–790.
36. Schwimmer, J.B.; Pardee, P.E.; Lavine, J.E.; Blumkin, A.K.; Cook, S. Cardiovascular risk factors and the metabolic syndrome in pediatric nonalcoholic fatty liver disease. *Circulation* **2008**, *118*, 277–283.
37. Shoelson, S.E.; Herrero, L.; Naaz, A. Obesity, inflammation, and insulin resistance. *Gastroenterology* **2007**, *132*, 2169–2180.
38. Tilg, H.; Moschen, A.R. Insulin resistance, inflammation, and non-alcoholic fatty liver disease. *Trends in endocrinology and metabolism: TEM* **2008**, *19*, 371–379.
39. Müller, M.J.; Braun, W.; Pourhassan, M.; Geisler, C.; Bosy-Westphal, A. Application of standards and models in body composition analysis. *The Proceedings of the Nutrition Society* **2016**, *75*, 181–187.
40. Al-Gindan, Y.Y.; Hankey, C.R.; Govan, L.; Gallagher, D.; Heymsfield, S.B.; Lean, M.E.J. Derivation and validation of simple anthropometric equations to predict adipose tissue mass and total fat mass with MRI as the reference method. *The British journal of nutrition* **2015**, *114*, 1852–1867.
41. Horan, M.; Gibney, E.; Molloy, E.; McAuliffe, F. Methodologies to assess paediatric adiposity. *Irish journal of medical science* **2015**, *184*, 53–68.
42. Lee, S.Y.; Gallagher, D. Assessment methods in human body composition. *Current opinion in clinical nutrition and metabolic care* **2008**, *11*, 566–572.
43. Siri, W.E. Body composition from fluid spaces and density: analysis of methods. 1961. *Nutrition (Burbank, Los Angeles County, Calif.)* **1993**, *9*, 480-91; discussion 480, 492.
44. Fidanza, F.; Keys, A.; ANDERSON, J.T. Density of body fat in man and other mammals. *Journal of applied physiology* **1953**, *6*, 252–256.
45. BROZEK, J.; GRANDE, F.; ANDERSON, J.T.; Keys, A. DENSITOMETRIC ANALYSIS OF BODY COMPOSITION: REVISION OF SOME QUANTITATIVE ASSUMPTIONS. *Annals of the New York Academy of Sciences* **1963**, *110*, 113–140.
46. Garrow, J.S. *Energy balance and obesity in man*; Elsevier/North-Holland Biomedical Press: Amsterdam, New York, op. 1974.

47. Report of the task group on reference man ICRP Publication 23 (1975). *Ann ICRP* **1980**, *4*, III-III.
48. Sohlström, A.; Wahlund, L.O.; Forsum, E. Adipose tissue distribution as assessed by magnetic resonance imaging and total body fat by magnetic resonance imaging, underwater weighing, and body-water dilution in healthy women. *The American journal of clinical nutrition* **1993**, *58*, 830–838.
49. Wang, Z.; Zhu, S.; Wang, J.; Pierson, R.N.; Heymsfield, S.B. Whole-body skeletal muscle mass: development and validation of total-body potassium prediction models. *The American journal of clinical nutrition* **2003**, *77*, 76–82.
50. Woodard, H.Q.; White, D.R. The composition of body tissues. *The British journal of radiology* **1986**, *59*, 1209–1218.

CHAPTER II

Association between individual fat depots and cardio-metabolic traits in normal- and overweight children, adolescents and adults

published in Nutrition & Diabetes

OPEN

Citation: *Nutrition & Diabetes* (2017) 7, e267; doi:10.1038/nutd.2017.20www.nature.com/nutd

ORIGINAL ARTICLE

Association between individual fat depots and cardio-metabolic traits in normal- and overweight children, adolescents and adults

M Hübers¹, C Geisler¹, S Plachta-Danielzik^{1,2} and MJ Müller¹**OBJECTIVES:** To determine age-related associations between fat mass (FM), regional fat depots and cardiometabolic traits in normal- and overweight children, adolescents and adults.**METHODS:** Detailed body composition (regional subcutaneous and visceral adipose tissue; SAT, VAT) by whole-body magnetic resonance imaging (MRI), FM and fat-free mass by air-displacement plethysmography, systolic and diastolic blood pressure (SBP, DBP), triglycerides (TG), high-density lipoprotein cholesterol (HDL), plasma glucose and plasma insulin were measured in 433 subjects (BMI: 23.6 (21.0–27.7)); 151 children and adolescents, aged 6–18 years, 150 young adults, aged 18–30 years and 132 adults, aged 30–60 years). Data were derived from pooled data of the 'Reference Center for Body Composition' in Kiel, Germany. Insulin resistance was determined by the homeostatic model assessment of insulin resistance (HOMA-IR). Partial correlations and multivariate linear regression analyses were used to evaluate the associations between body composition and cardiometabolic traits. A descriptive approach was used to demonstrate age-dependent differences in associations between body fat depots and insulin resistance, independent of BMI.**RESULTS:** FM, SAT, and VAT increased from childhood to adulthood with low VAT in children and adolescents. When compared to children, TG was higher in adults. HDL and DBP did not differ between age groups. Insulin resistance was highest in male adolescents and female young adults. Associations between body fat depots and cardiometabolic traits were seen after puberty with no associations in pre- and inpubertal children. When compared to FM, SAT and VAT had the strongest association with insulin resistance in adults. This association was independent of BMI.**CONCLUSIONS:** Associations between individual body fat depots and most cardiometabolic traits became evident after puberty only. The strongest associations were observed between insulin resistance and abdominal fat in adults. The impact of VAT was independent of BMI.*Nutrition & Diabetes* (2017) 7, e267; doi:10.1038/nutd.2017.20; published online 8 May 2017

INTRODUCTION

To address the impact of overweight on cardiometabolic risk most studies are based on body mass index (BMI).^{1–3} However, BMI can neither differentiate between fat mass (FM) and fat-free mass (FFM) nor give detailed information about body fat distribution.^{4,5} To go beyond BMI, detailed body composition and appropriate modeling taking into account body function have to be considered.⁶ Magnetic resonance imaging (MRI) and computed tomography are the gold-standard methods to determine trunk and peripheral subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT). In children and adolescents MRI is the most suitable measurement because it does not expose them to radiation.

Our present knowledge about the association between individual body fat depots and cardio-metabolic risk factors is mainly based on studies on adults. In addition, there is only limited knowledge about regional fat depots in children and adolescents. In a cross-sectional study SAT and VAT were lower in children compared to adults.⁷ In a longitudinal study, SAT increased during adolescence, whereas VAT remained low.⁸ By contrast, both, SAT and VAT, increased between age of 11 and 13 years.⁹ With regard to gender differences, girls compared with

boys had greater masses of VAT and SAT.¹⁰ This was contrary to other authors who observed that VAT was greater in boys than in girls.¹¹

In obese adolescents and adults cardiometabolic traits and insulin resistance have been mainly related to VAT and liver fat.^{12–16} In children and adolescents the degree of insulin resistance rises from pre- to inpuberty and decreases after puberty.^{17,18} However, the association between cardiometabolic traits and insulin resistance with detailed body composition has not been well characterized in children and adolescents. As in adults, SAT and VAT were differently associated with cardiometabolic traits in children and adolescents.^{19–22} Both, SAT and VAT were independently correlated with blood pressure,¹⁹ whereas VAT was positively associated with plasma triglycerides (TG) and negatively associated with high-density lipoprotein cholesterol (HDL).²⁰ In addition, there was a positive relationship between either VAT or SAT and insulin resistance.^{20–23}

Until now, there is no systematic study of the associations between individual body fat depots and cardiometabolic risk in different age groups. The aim of this study was to determine the associations between MRI-derived individual fat depots and cardiometabolic traits in children and adolescents compared to

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Received 31 January 2017; revised 17 March 2017; accepted 20 March 2017

young and older adults. Furthermore, we investigated the associations between insulin resistance, relative to BMI, and regional fat depots.

METHODS

All studies had been approved by the Ethical Committee of Christian-Albrechts-University of Kiel, Germany, and conducted according to the guidelines laid down in the 'Declaration of Helsinki'. Written informed consent was obtained from each subject and, in state of minority, from its legal guardian before participation.

Subjects

This investigation was a secondary analysis of data collected at the 'Reference Center for Body Composition' (Institute of Human Nutrition and Food Science at the University of Kiel, Germany) between 2005 and 2016. In this cross sectional study, data of 433 healthy Caucasian subjects (151 children and adolescents, aged 6–18 years, 150 young adults, aged 18–30 years and 132 adults, aged 30–60 years) were analyzed. Exclusion criteria were metallic implants, smoking, pregnancy, chronic or acute diseases, and medication intake that influences body composition. Subjects were recruited by notice boards postings and advertisements in the local newspaper. Study measurements of anthropometry, body composition, detailed body composition by whole body MRI and cardiometabolic variables were performed in children, adolescents and adults. Self-assessment of pubertal stage was determined according to the definition of Marshall and Tanner.²⁴ On the basis of breast and genital stages pubertal status was categorized into three groups (prepubertal: Tanner I; intrapubertal: Tanner II–III, postpubertal: Tanner ≥ IV).¹⁸ In this analysis prepubertal and intrapubertal subjects were grouped together. This was done because they did not differ in associations between body composition and cardiometabolic traits (data not shown), and because of a low number of prepubertal children ($n = 26$).

Anthropometric measures

Body weight was measured to the nearest 0.01 kg using an electronic Tanita scale coupled to the BOD POD Body Composition Tracking System (Life Measurement Instruments, Concord, CA, USA). Body height was assessed to the nearest 0.5 cm by using a stadiometer (SECA, Modell 220, Hamburg, Germany). BMI was calculated as body weight (kg)/body height (m)². To define overweight and obesity for children and adolescents (>90th percentile; >97th percentile) German age- and sex-specific references were used.²⁵ Waist circumference (WC) was measured midway between the lowest rib and the top of the iliac crest with the subjects standing in upright position. The measurement was made with a nonelastic plastic tape placed parallel to the floor at the end of a normal expiration.

Densitometry

Air-displacement plethysmography was performed by BOD POD device to assess body composition. Child-specific equations were used to determine FM, as in detail described elsewhere.²⁶ In adults, FM was calculated using the equation by Siri *et al.*²⁷ FFM was then calculated from the difference between body weight and FM. Because of reported difficulties in BOD POD calibration in one of the children studies cross-validated gender specific equations of FM were developed for children and adolescents, as procedure is described elsewhere.²⁸ Around 222 children and adolescents were randomly split into two parts; 155 (91 female, 64 male) were assigned to the derivation group, with 67 (31 female, 36 male) subjects in the cross-validation group. When compared to derivation group, the cross-validation group did not differ in age, gender, pubertal status, height, weight, BMI, WC, and FM measured by bio-impedance analysis. The correlation coefficients between FM measured by bio-impedance analysis and predicted FM based on BOD POD algorithm did not differ between the two groups. Following equations based on stepwise multiple regression

analysis were developed for children and adolescents:

$$\begin{aligned} \text{FM}_{\text{female}}(\%) &= 1.808 \times \text{BMI} - 7.7 \times 10^5 \times \text{age}^4 \\ &\quad - 0.531 \times \text{body weight (kg)} + 53.672 \times \text{height (m)} \\ &\quad + 0.416 \times \text{FM}_{\text{ADP}}(\%) + 0.139 \times \text{WC (cm)} - 86.307. \end{aligned}$$

$$\begin{aligned} \text{FM}_{\text{male}}(\%) &= 0.961 \times \text{BMI} + 7.0 \times 10^5 \times \text{age}^4 + 0.336 \times \text{FM}_{\text{ADP}}(\%) \\ &\quad - 2.853. \end{aligned}$$

On the basis of calculated FM and FFM, FM-Index (FMI) and FFM-Index (FFMI) were calculated for children and adolescents as FM (kg)/height (m)² and FFM (kg)/height (m)². Height-independent FM is confounded by age in adolescents, whereas age was not included as a significant independent variable in children. By contrast, height-independent FFM is confounded by age in children but not in adolescents. Age-adjusted power of height (n) was determined using log-log regression analysis²⁹ (FMI: $n = 6.1, 2.3$ and FFMI: $n = 2.3, 2.9$, respectively for children and adolescents). In adults FMI and FFMI were calculated, as FM (kg)/height (m)² and FFM (kg)/height (m)².

Magnetic resonance imaging (MRI)

As described previously, detailed body composition of all subjects was performed by using whole-body MRI with a 1.5T scanner (Magnetom Vision or Magnetom Avanto, Siemens Medical Systems, Erlangen, Germany).^{30,31} Briefly, participants were examined in a supine position with their arms extended above their heads and they were asked to hold their breath during measurement of abdominal and thoracic regions for image acquisition. In the present analysis volumes of SAT and VAT were manually segmented by using segmentation software (SliceOmatic 4.3 and 5.0, Tomovision, Montreal, Canada). SAT was analyzed from ankle to wrist. In this case SAT_{legs} were defined from ankle to femoral heads and SAT_{arms} were defined from humerus heads to wrist. Between femoral and humerus heads volume of SAT_{trunk} was assessed. VAT was defined as intra-abdominal fat between femoral heads and the top of the liver. Volumes of SAT and VAT were determined by the sum of all adipose tissue areas (cm²) multiplied by slice thickness. Volume data were then transformed into tissue masses by using the following density: 0.923 g cm⁻³ for SAT and VAT.

Cardiometabolic risk factors

Systolic and diastolic blood pressure were measured by manual sphygmomanometer in a seated position. After an 8-h overnight fast blood samples were taken and analyzed by standard procedures. HDL was assessed enzymatically by Konelab-20i-Analyzer (Konelab, Espoo, Finland). Triglyceride and plasma glucose were analyzed enzymatically using a Konelab-Test-Kit (Thermo Clinical Labsystems, Frankfurt, Germany). Plasma insulin was measured by radioimmunoassay (Adaltis, Freiburg, Germany). Insulin resistance was then calculated by homeostasis model assessment of insulin resistance (HOMA-IR):

$$\text{HOMA-IR} = \text{plasma glucose (mmol/L)} \times \text{plasma insulin (}\mu\text{U/ml)} / 22.5.^{32}$$

Statistical analysis

Statistical analyses were performed with SPSS statistical software (SPSS 24.0, Inc., Chicago, Illinois, USA). All data are given as median and interquartile range because of not normally distributed data. Kruskal-wallis test with bonferroni correction was performed for more than two groups (pre- and intrapubertal subjects, postpubertal subjects, young adults and adults). To examine the association of individual body components on cardiometabolic traits, age- and gender-adjusted partial correlations were performed. Differences in correlation coefficients between age groups were tested by using the method of Eid, Gollwitzer, Schmitt.³³ Further, stepwise multivariate linear regression analyses were done. Four models were conducted. Model 1 included FMI, Model 2 SAT_{trunk}, Model 3 VAT and Model 4 SAT_{trunk} and VAT as independent variables. All models were adjusted for age and gender. In addition, a descriptive approach was used to demonstrate age-dependent differences in individual body components between three different HOMA-IR per BMI groups (low, normal and high). These three groups were stratified according to their age dependent deviation from the regression line. Cut-offs were chosen from tertiles between HOMA-IR and BMI (low: residuals < -0.48, < -0.90, < -0.92, < -0.69; normal: residuals ≤ 0.16 and ≥ -0.48, ≤ 0.25 and ≥ -0.90, ≤ 0.00 and ≥ -0.92, ≤ 0.29 and ≥ -0.69; high: residuals > 0.16, > 0.25, > 0.00, > 0.29, respectively for children, adolescents, young adults, and adults). All tests were two-sided and level of significance was set at $P < 0.05$.

RESULTS**Detailed body composition: differences between age groups**

The characteristics of the study population are shown in Table 1. When compared with children, height, weight, FM, and FFM were significantly higher in adolescents and with no significant differences to adults. When compared with young adults, VAT was lower in children and adolescents. In males, VAT was significantly higher in older compared with young adults. In females, SAT_{total}, SAT_{arms}, and SAT_{trunk} were higher in young adults compared with children and adolescents. By contrast, SAT_{trunk} was the only subcutaneous fat depot in males which was significantly higher in young adults compared to children and adolescents.

Cardiometabolic risks: Differences between age groups

In females, plasma insulin and HOMA-IR were highest in young adults when compared to other age groups (Table 1). In males, plasma insulin and HOMA-IR were highest in adolescents. In both genders, HOMA-IR did not differ between adolescents and adults. There were no differences in SBP, DBP and HDL between the different age groups, except for SBP in male adolescents, and high TG level in female young adults and male adults.

Associations between body composition and cardiometabolic traits: Differences between age groups

Age- and gender-adjusted partial correlation coefficients between individual body fat depots and cardiometabolic traits are shown in Table 2. In general, correlation coefficients were low to moderate. As for SBP, DBP, TG and plasma glucose, significant associations were seen after puberty only. The associations between SAT_{trunk} and either SBP or DBP or TG did not differ in adolescents, young adults and adults. Strongest associations were observed between plasma insulin levels or HOMA-IR and truncal fat depots (SAT_{trunk}, VAT). When compared with adults, the associations between individual body fat depots and HDL were higher in children and adolescents.

Using stepwise multivariate linear regression analysis, the explained variance and respective beta coefficients of cardiometabolic traits as dependent variables and FMI, SAT_{trunk}, VAT and the combination of SAT_{trunk} and VAT, as independent variables are shown in Table 3. The explained variance of cardiometabolic traits was moderate, maximally, 35.2% of the variance of HOMA-IR was explained by SAT_{trunk} and VAT in adults.

When compared with adolescents and adults, the explained variances of SBP, DBP, TG and plasma glucose by body fat depots were low ($\leq 5.3\%$) in children. However, the explained variances of HOMA-IR and plasma insulin by parameters of body composition differed only slightly between children, adolescents and adults. The explained variance of HDL by body fat depots decreased from children to adults.

When compared to FMI, the explained variance of plasma insulin and HOMA-IR by SAT_{trunk} and VAT were moderately increased in young and older adults. When compared to FMI, SAT_{trunk} and VAT did not exceed the explained variance of HDL, whereas abdominal fat depots added to the variance of TG in adolescents.

Association between detailed body composition and insulin resistance

Investigating subjects with low HOMA-IR (insulin sensitive), normal HOMA-IR or high HOMA-IR (insulin resistance) relative to their BMI, age-dependent differences in individual body components between these groups became evident (Table 4). In children and adolescents, no differences were found in body fat depots between groups differing in insulin resistance. By contrast, in young adults, adipose tissue, SAT_{total}, SAT_{trunk} and SAT_{legs} were

significantly higher in insulin resistant subjects. In older adults, VAT was highest in insulin resistant subjects.

DISCUSSION

Up to now, there are limited data on the association between regional fat depots and metabolic disturbance in normal- and overweight children and adolescents compared with adults.^{34,35} To our knowledge, the present study is first to systematically investigate detailed body composition by age group and gender as well as its associations to cardiometabolic traits in 6–60 year old subjects.

Detailed body Composition in different age groups

In both genders, VAT was low in children and adolescents compared with adults (Table 1). This is in line with previous data.^{7,8} However, other authors reported that during childhood, growth of VAT decelerated with increasing age.³⁴ In our study sexual dimorphism was apparent after puberty only with males having more VAT than females. By contrast, other authors observed higher masses of VAT in girls compared to boys.¹⁰

Cardiometabolic risks in different age groups

As shown in Table 1, in males, insulin resistance increased from pre- and intrapuberty to postpuberty, with changes to young adulthood in females only. These findings support previous data.^{36,37} By contrast, other authors reported that the increase in insulin resistance from childhood to adulthood was independent of gender.³⁸ Studies on blood pressure showed that SBP and DBP increased with age.^{38,39} These results are contrary to our findings.

Associations between body composition and cardiometabolic traits in different age groups

Low and moderate associations were observed between cardiometabolic traits and body fat depots in normal- and overweight children, adolescents and adults (Table 2). This is in line with previous data.³⁸ However, moderate associations were seen after puberty only. These findings indicate that in pre- and intrapubertal children individual body components are not related to cardiometabolic risk, as it is in older subjects. Accordingly, a study on pre- and intrapubertal children found no associations between abdominal fat depots vs blood pressure and blood lipids in European Americans.⁴⁰ Positive associations became evident between SAT_{trunk}, VAT, and cardiometabolic traits in adolescents.¹⁹

Overall, there was a limited explained variance of cardiometabolic traits by FMI and different regional fat depots (Table 3). When compared to FMI, abdominal fat depots, especially VAT, were stronger associated to insulin resistance in adults than in children and adolescents. The associations between HDL and body fat depots decreased with age. As HDL is positively associated to physical activity in children, adolescents and adults,^{41,42} a possible explanation may be increasing sedentary behavior with age which is due to school entry and entering the workplace.^{43,44} This is in line with the finding that children improved HDL by increased physical activity which was independent of weight change.⁴⁵

Associations between detailed body composition and insulin resistance

To address the role of individual body fat depots on insulin resistance, independent of BMI, differences in age groups became evident (Table 4). In children and adolescents, there were no differences in body fat depots between groups differing in insulin resistance. By contrast, regional SAT depots were significantly different in young adults, with VAT as associated to insulin resistance in older adults only. Our findings suggest that VAT

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Table 1. Characteristics of the study population stratified by age group and gender

	(1) Children		(2) Adolescents		(3) Young Adults		(4) Adults	
	Females	Males	Females	Males	Females	Males	Females	Males
<i>n</i>	37	43	41	30	69	81	69	63
Age (y)	10.9 (9.4–13.4)	12.3 (9.4–14.1)	15.8 (15.3–16.3)	16.3 (15.6–17.3)	26.0 (24.0–26.0) ^{b,c}	25.0 (23.0–27.0) ^{b,c}	44.0 (36.0–50.0) ^{b,c,d}	41.0 (35.0–50.0) ^{b,c,d}
Height (m)	1.49 (1.34–1.61)	1.58 (1.42–1.70)	1.68 (1.65–1.72) ^b	1.78 (1.73–1.83) ^b	1.70 (1.65–1.75) ^b	1.81 (1.76–1.87) ^b	1.67 (1.63–1.72) ^b	1.79 (1.76–1.84) ^b
Weight (kg)	43.4 (29.4–56.9)	53.1 (32.3–72.5)	65.6 (55.0–80.7) ^b	73.0 (64.0–101.6) ^b	69.5 (61.0–87.2) ^b	78.6 (71.1–85.1) ^b	68.0 (61.4–79.6) ^b	81.2 (73.5–92.3) ^b
OW (OB) (%)	35.1 (27.0)	34.9 (16.3)	43.9 (36.6)	43.3 (36.7)	37.7 (29.0)	33.3 (8.6)	40.6 (18.8)	46.0 (17.5)
BMI (kg/m ²)	19.3 (16.5–25.5)	19.0 (15.7–24.8)	21.8 (19.8–29.6)	23.4 (20.0–31.1) ^b	23.9 (21.7–31.7) ^b	23.4 (22.1–25.7) ^b	24.3 (21.7–27.7) ^b	24.5 (23.2–28.7) ^b
FFM (kg)	9.4 (4.7–19.9)	9.9 (5.0–19.4)	16.8 (12.4–31.9) ^b	14.0 (9.3–29.6) ^b	22.1 (17.4–37.8) ^b	13.2 (10.1–18.9)	23.4 (16.7–30.5) ^b	17.2 (12.9–24.4) ^b
FM (kg)	32.5 (24.5–39.3)	40.6 (27.4–50.0)	47.6 (41.6–51.1) ^b	57.7 (54.8–68.2) ^b	47.5 (43.6–52.0) ^b	64.5 (59.0–68.7) ^b	45.0 (41.8–50.2) ^b	65.3 (59.4–69.3) ^b
FFMI (kg/m ⁶)	0.95 (0.55–1.56)	0.70 (0.48–0.98)	4.8 (3.9–10.2)	4.0 (2.6–8.2)	7.8 (6.0–14.2)	3.8 (3.0–5.8)	8.3 (6.3–10.9)	5.3 (3.9–7.3)
Adipose tissue (kg)	13.0 (12.1–14.5)	12.9 (11.8–15.1)	10.1 (9.3–11.4)	11.1 (10.5–12.0)	16.4 (15.5–17.9)	19.6 (18.5–20.5)	16.0 (15.0–17.8)	19.9 (18.7–21.5)
SAT total (kg)	9.5 (7.1–19.3)	9.0 (4.7–18.5)	15.0 (11.9–29.1)	11.6 (8.4–25.0)	21.5 (17.5–34.6) ^b	12.9 (10.8–18.6) ^b	22.6 (17.0–30.8) ^b	18.2 (14.0–24.5) ^{b,d}
SAT arms (kg)	1.2 (1.0–2.2)	1.2 (0.7–2.1)	1.7 (1.4–3.2)	1.4 (1.1–3.0)	2.2 (1.8–3.6) ^b	1.5 (1.4–2.2)	2.3 (1.7–3.0) ^b	1.9 (1.6–2.2) ^b
SAT trunk (kg)	2.9 (2.1–6.3)	2.6 (1.3–6.7)	4.7 (3.7–13.5)	3.8 (2.8–10.4)	8.6 (6.2–14.3) ^b	4.7 (3.7–8.1) ^b	9.1 (6.3–13.3) ^b	6.8 (4.9–9.0) ^b
SAT legs (kg)	5.3 (3.8–8.7)	4.6 (2.6–8.4)	8.0 (6.4–12.5) ^b	6.0 (4.4–11.0)	9.8 (8.2–13.9) ^b	5.6 (4.8–7.3)	8.9 (7.0–11.9) ^b	6.3 (5.3–7.7) ^b
VAT (kg)	0.36 (0.22–0.87)	0.29 (0.16–0.88)	0.47 (0.34–0.96)	0.64 (0.37–1.30)	1.11 (0.60–1.71) ^{b,c}	1.13 (0.70–2.15) ^b	1.36 (0.68–2.29) ^{b,c}	2.50 (1.69–5.17) ^{b,c,d}
SBP (mm Hg)	120 (108–121)	120 (110–125)	118 (110–120)	128 (120–134) ^b	115 (110–125)	120 (110–125) ^c	118 (110–125)	120 (115–130)
DBP (mm Hg)	70 (70–80)	75 (70–80)	75 (70–80)	76 (70–85)	80 (70–82)	80 (75–85)	80 (70–80)	80 (75–85)
TG (mg/dl)	75 (59–96)	67 (47–121)	85 (70–104)	86 (69–114)	102 (73–145) ^b	91 (70–127) ^b	86 (70–108)	106 (78–143) ^b
HDL (mg/dl)	54 (46–60)	53 (46–62)	52 (43–66)	51 (42–59)	55 (46–72)	51 (40–57)	65 (48–73)	46 (33–58)
Glucose (mg/dl)	89 (84–94)	90 (85–96)	87 (82–93)	91 (86–94)	88 (83–92)	86 (78–94)	94 (86–99) ^{c,d}	99 (94–105) ^{b,c,d}
Insulin (µU/ml)	8.7 (4.9–11.1)	7.7 (4.4–11.6)	12.1 (6.9–15.3)	10.7 (6.9–14.5)	12.5 (8.0–18.6) ^b	7.8 (6.3–10.1)	9.2 (6.8–12.3) ^d	7.7 (5.9–14.1)
HOMA-IR	1.92 (0.98–2.36)	1.84 (0.92–2.79)	2.49 (1.46–3.11)	2.34 (1.46–3.08)	2.68 (1.62–4.34) ^b	1.64 (1.20–2.15)	2.20 (1.53–2.84)	1.98 (1.40–3.31)

Abbreviations: DBP, diastolic blood pressure; FFM, fat-free mass; FFMi, fat-free mass index; FM, fat mass; FMI, fat mass index; HDL, high-density lipoprotein cholesterol; OB, obese; OW, overweight; SAT, subcutaneous adipose tissue; SBP, systolic blood pressure; TG, triglyceride; VAT, visceral adipose tissue. ^a(1), pre- and intrapubertal children; (2), postpubertal adolescents; (3), young adults (18–30 years old); (4), adults (30–60 years old). ^bSignificantly different from gender-dependent pre- and intrapubertal subjects. ^cSignificantly different from gender-dependent postpubertal subjects. ^dSignificantly different from gender-dependent young adults (Kruskal-wallis test with bonferroni correction, *P* < 0.05). ^ePower of height which is unequal in pre- and intrapubertal, postpubertal and adult stage (FMI: 6.1, 2.3, 2.0, 2.0; FFMi: 2.3, 2.9, 2.0, 2.0, respectively). Data are given as median and interquartile range.

Table 2. Age- and gender-adjusted partial correlations between parameters of body composition and cardiometabolic traits in children, adolescents and adults

Age groups ^a	Correlation coefficient (r)															
	FMI				SAT _{trunk}				SAT _{legs}				VAT			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
n	80	71	150	132	80	71	150	132	80	71	150	132	80	71	150	132
SBP	0.17 ^{b,c}	0.31	0.43	0.42	0.29	0.27	0.25	0.43	0.29	0.29	0.36	0.30	0.19 ^c	0.27^c	0.27^c	0.48
DBP	0.14 ^{b,c,d}	0.42	0.41	0.37	0.33	0.43	0.29	0.40	0.30	0.37	0.34	0.23	0.24^c	0.42	0.26^c	0.45
TG	0.00 ^{b,c,d}	0.37	0.35	0.24	-0.05 ^{b,d}	0.34	0.31	0.16	0.02 ^{b,d}	0.34	0.37	0.24	0.12	0.54	0.36	0.41
HDL	-0.53 ^{b,c}	-0.42	-0.27	-0.31	-0.50 ^{b,c}	-0.39	-0.26	-0.24	-0.52 ^b	-0.41	-0.28	-0.33	-0.21	-0.50 ^{b,c}	-0.29	-0.30
Glucose	-0.15 ^c	-0.23 ^{b,c}	0.07 ^c	0.30	-0.17 ^{b,c}	-0.25 ^{b,c}	0.15	0.30	-0.24 ^{b,c}	-0.27 ^{b,c}	0.18	0.30	0.05 ^c	-0.26 ^{b,c}	0.35	0.37
Insulin	0.55	0.54	0.48	0.48	0.55	0.52	0.52	0.39	0.57	0.55	0.56	0.53	0.34	0.64	0.55	0.50
HOMA-IR	0.51	0.51	0.45	0.51	0.52	0.48	0.50	0.41	0.52	0.51	0.54	0.55	0.37	0.59	0.52	0.55

Abbreviations: DBP, diastolic blood pressure; FMI, fat mass index; HDL, high-density lipoprotein cholesterol; SAT, subcutaneous adipose tissue; SBP, systolic blood pressure; TG, triglyceride; VAT, visceral adipose tissue. ^a1, pre- and intrapubertal children; 2, postpubertal adolescents; 3, young adults (18–30 years old); 4, adults (30–60 years old). ^bSignificantly different from correlation coefficient in young adults. ^cSignificantly different from correlation coefficient in adults, *P* < 0.05. ^dSignificantly different from correlation coefficient in postpubertal adolescents (method of Eid, Gollwitzer, Schmitt³³). Significant correlation coefficients in bold, *P* < 0.05.

Table 3. Multivariate linear regression on cardiometabolic traits as dependent variables

Independent variables	FMI ^a												SAT _{trunk} ^a												VAT ^a												SAT _{trunk} +VAT ^a											
	age group ^b				FMI ^a				SAT _{trunk} ^a				VAT ^a				SAT _{trunk} +VAT ^a				age group ^b				FMI ^a				SAT _{trunk} ^a				VAT ^a				SAT _{trunk} +VAT ^a											
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4								
n	80	71	150	132	80	71	150	132	80	71	150	132	80	71	150	132	80	71	150	132	80	71	150	132	80	71	150	132	80	71	150	132	80	71	150	132	80	71	150	132								
SBP	0.0	0.18	0.59	0.27	0.17	0.50	0.50	0.50	0.44	0.35	0.26	0.63	0.27	0.17	0.47	0.15	0.41	0.0	0.20	0.46	0.24	0.65	0.28	20.6	0.53	3.5	0.02	6.3	0.02	17.7	-0.06	19.4	0.52															
DBP	0.0	0.16	0.15	0.42	0.16	0.47	0.12	0.40	0.40	0.53	0.31	0.67	0.42	0.36	0.41	0.48	0.41	4.2	0.26	16.1	0.41	6.3	0.27	19.0	0.51	5.3	0.09	16.7	0.20	13.6	0.00	18.4	0.50															
TG	0.0	0.00	0.11	0.36	0.11	0.36	0.11	0.36	0.45	0.25	0.0	0.02	9.9	0.33	12.6	0.40	4.6	0.24	0.0	0.17	27.2	0.53	11.8	0.37	15.2	0.46	0.0	0.17	32.6	0.96	12.6	0.20	15.8	0.47														
HDL	25.4	-0.54	15.1	-0.41	5.6	-0.29	6.8	-0.30	23.2	-0.60	14.7	-0.40	6.3	-0.29	7.6	-0.30	21.6	-0.54	12.2	-0.37	6.6	-0.28	6.7	-0.31	23.2	-0.25	14.7	-0.09	6.6	-0.28	7.6	-0.17																
Glucose	0.0	-0.14	0.0	-0.23	0.0	0.08	7.1	0.31	3.7	-0.27	5.8	-0.27	2.7	0.20	7.1	0.29	4.8	-0.28	0.0	-0.17	11.5	0.36	11.0	0.39	4.8	-0.28	5.8	0.24	11.5	0.36	11.3	0.40																
Insulin	19.4	0.47	27.4	0.54	18.5	0.51	22.8	0.54	20.7	0.56	29.1	0.55	25.6	0.57	27.9	0.55	26.4	0.59	29.3	0.49	24.8	0.58	26.4	0.49	24.8	0.58	26.4	0.59	29.3	0.55	28.2	0.24	31.0	0.31														
HOMA-IR	16.3	0.43	24.4	0.50	16.3	0.48	25.6	0.57	16.8	0.51	24.9	0.50	24.4	0.55	21.2	0.53	30.0	0.57	21.2	0.53	26.7	0.52	23.3	0.50	29.8	0.63	21.2	0.53	27.9	0.52	27.9	0.28	35.2	0.64														

Abbreviations: DBP, diastolic blood pressure; FMI, fat mass index; HDL, high-density lipoprotein cholesterol; SAT, subcutaneous adipose tissue; SBP, systolic blood pressure; TG, triglyceride; VAT, visceral adipose tissue. ^aModel with independent variable/variables in bold adjusted for age and gender. ^bModel with independent variable/variables in bold explained variance (%) by independent variable/variables which is/are in bold compared to the base model which includes age and gender. ^c1, pre- and intrapubertal children; 2, postpubertal adolescents; 3, young adults (18–30 years old); 4, adults (30–60 years old). ^dβ-coefficient of VAT.

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Table 4. Differences in body fat depots between three groups (low HOMA-IR per BMI, normal HOMA-IR per BMI and high HOMA-IR per BMI) stratified by age group

	HOMA-IR/BMI ↓	HOMA-IR/BMI =	HOMA-IR/BMI ↑	P-value
Children				
n	26	27	26	
FM (kg)	14.8 (4.7–23.4)	7.1 (4.2–12.8)	10.7 (7.6–20.5)	0.038
FFM (kg)	36.7 (27.4–49.8)	28.4 (23.6–38.9)	42.1 (32.4–47.1)	0.011
Adipose tissue (kg)	11.9 (4.7–22.9)	8.3 (4.7–12.6)	9.8 (8.5–22.5)	0.111
SAT total (kg)	11.5 (4.5–22.1)	7.9 (4.5–12.0)	9.4 (8.3–21.9)	0.121
SAT arms (kg)	1.5 (0.7–2.5)	1.1 (0.7–1.7)	1.3 (1.0–2.3)	0.193
SAT trunk (kg)	3.9 (1.3–9.3)	2.6 (1.3–3.9)	3.1 (2.4–8.0)	0.132
SAT legs (kg)	6.1 (2.6–10.5)	4.5 (2.5–6.4)	5.1 (4.5–9.4)	0.102
VAT (kg)	0.53 (0.18–0.97)	0.29 (0.16–0.47)	0.36 (0.26–1.02)	0.171
HOMA-IR	0.84 (0.57–2.05)	1.60 (0.97–1.91)	2.74 (2.07–4.10)	< 0.001
Adolescents				
n	23	24	23	
FM (kg)	24.2 (12.5–35.5)	15.5 (9.9–29.6)	13.5 (10.8–31.9)	0.174
FFM (kg)	56.9 (49.3–64.9)	50.4 (44.4–57.3)	49.3 (43.9–52.1)	0.058
Adipose tissue (kg)	22.4 (11.9–31.3)	12.0 (9.7–24.6)	13.3 (11.1–30.2)	0.222
SAT total (kg)	21.1 (11.6–30.1)	11.6 (9.3–23.2)	12.9 (10.6–24.4)	0.222
SAT arms (kg)	2.6 (1.3–3.8)	1.4 (1.2–2.8)	1.5 (1.3–3.1)	0.338
SAT trunk (kg)	8.1 (3.6–13.5)	3.8 (3.2–9.7)	4.5 (3.1–13.9)	0.190
SAT legs (kg)	10.0 (6.2–13.7)	6.9 (4.7–11.3)	6.8 (5.8–12.5)	0.265
VAT (kg)	0.72 (0.44–1.20)	0.43 (0.31–0.81)	0.51 (0.32–1.20)	0.306
HOMA-IR	1.62 (0.97–2.49)	2.02 (1.38–2.89)	3.25 (2.72–5.49)	< 0.001
Young adults				
n	47	47	47	
FM (kg)	17.6 (12.3–37.8)	15.6 (10.3–19.7)	20.8 (14.8–26.1)	0.034
FFM (kg)	62.1 (49.4–68.7)	58.7 (50.3–65.4)	53.6 (44.3–63.1)	0.010
Adipose tissue (kg)	16.5 (11.1–34.6)	16.0 (11.0–19.6)	20.4 (14.8–26.1)	0.013
SAT total (kg)	15.2 (10.4–33.3)	14.4 (10.3–19.3)	18.9 (14.2–25.0)	0.008
SAT arms (kg)	1.9 (1.4–3.1)	1.7 (1.4–2.2)	2.2 (1.6–2.8)	0.066
SAT trunk (kg)	5.9 (3.8–14.6)	5.8 (3.8–8.1)	8.8 (6.2–12.0)	0.006
SAT legs (kg)	8.1 (5.2–13.9)	6.4 (5.0–8.9)	8.7 (6.2–10.3)	0.021
VAT (kg)	0.96 (0.53–2.29)	1.25 (0.76–1.85)	1.1 (0.89–2.60)	0.411
HOMA-IR	1.21 (0.84–1.95)	1.68 (1.49–2.04)	4.00 (2.78–5.55)	< 0.001
Adults				
n	44	44	44	
FM (kg)	20.9 (14.6–27.5)	19.5 (15.5–24.6)	22.5 (15.1–30.6)	0.519
FFM (kg)	58.2 (46.4–68.4)	51.3 (43.0–59.8)	53.1 (44.2–64.4)	0.051
Adipose tissue (kg)	18.9 (15.7–29.3)	18.3 (14.6–24.6)	21.7 (16.0–31.4)	0.277
SAT total (kg)	16.9 (13.8–24.8)	17.3 (13.7–22.1)	18.6 (14.9–27.6)	0.433
SAT arms (kg)	2.1 (1.7–2.8)	2.0 (1.6–2.4)	2.2 (1.7–2.8)	0.339
SAT trunk (kg)	7.7 (5.9–11.0)	7.1 (5.2–9.9)	8.4 (5.5–13.1)	0.380
SAT legs (kg)	6.9 (5.7–10.4)	7.3 (6.3–10.1)	8.3 (6.5–10.8)	0.643
VAT (kg)	2.07 (1.32–3.59)	1.57 (0.75–2.39)	2.39 (1.23–4.90)	0.013
HOMA-IR	1.39 (1.06–1.66)	1.84 (1.63–2.28)	3.75 (2.67–4.88)	< 0.001

Abbreviations: FM, fat mass; FFM, fat-free mass; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue. (1), pre- and intrapubertal children; (2), postpubertal adolescents; (3), young adults (18–30 years old); (4), adults (30–60 years old). Group 1: HOMA-IR/BMI ↓, residuals (1) < -0.48, (2) < -0.90, (3) < -0.92, (4) < -0.69; Group 2: HOMA-IR/BMI =, residuals (1) ≤ 0.16 and ≥ -0.48, (2) ≤ 0.25 and ≥ -0.90, (3) ≤ 0.00 and ≥ -0.92, (4) ≤ 0.29 and ≥ -0.69; Group 3: HOMA-IR/BMI ↑, residuals (1) > 0.16, (2) > 0.25, (3) > 0.00, (4) > 0.29. Significant correlation coefficients in bold (Kruskal-wallis test with bonferroni correction, $P < 0.05$)

becomes an important determinant of insulin resistance as age increased to adulthood. In our study, VAT was very low in children and adolescents and started to increase in young adults (Table 1). This is in line with other data that VAT is a primary determinant of insulin resistance in adults only.^{46–48}

Study strengths and limitations

To our knowledge, this is the first study to systematically investigate age-related associations between individual body components and cardiometabolic traits in children and adolescents compared to adults. Detailed body composition was measured by MRI. This is the gold-standard method of body composition. We have used two different approaches. First, an analytic approach was chosen to examine age-dependent associations between cardiometabolic traits and body fat depots.

Second, a descriptive approach was conducted to investigate age-dependent differences in body fat depots in groups differing in insulin resistance. A limitation of this study is that we have used cross-sectional data only. Thus, no causal relationships can be derived from our data.

CONCLUSION

Associations between regional fat depots and cardiometabolic traits were seen after puberty only. The impact of FMI on cardiometabolic traits is limited. With regard to HOMA-IR, this was improved by measuring individual fat depots in adults. The strongest associations were observed between abdominal fat depots and insulin resistance. When compared with adults, VAT was low in normal- and overweight children and adolescents. VAT

has no association with insulin resistance groups in children, adolescents and young adults. The role of VAT is apparent in adults only.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank Britta Jux and the Clinic for Diagnostic Radiology, University Medical Center Schleswig-Holstein, Kiel (Germany) for the help with MRI scanning. We acknowledge financial support by Land Schleswig-Holstein within the funding programme Open Access Publikationsfonds. This work was supported by the Deutsche Forschungsgemeinschaft (DFG), the Federal Ministry of Education and Research (BMBF) (DFG Mü 714/8-3; DFG Bo 3296/1-1; BMBF 01EA1336, 01GI1121A, 01GI1125, 01GI0821) and OMRON, Kyoto, Japan.

REFERENCES

- Janssen I, Katzmarzyk PT, Srinivasan SR, Chen W, Malina RM, Bouchard C *et al.* Utility of childhood BMI in the prediction of adulthood disease: comparison of national and international references. *Obes Res* 2005; **13**: 1106–1115.
- Goodman E, Dolan LM, Morrison JA, Daniels SR. Factor analysis of clustered cardiovascular risks in adolescence: obesity is the predominant correlate of risk among youth. *Circulation* 2005; **111**: 1970–1977.
- Blüher S, Molz E, Wiegand S, Otto K-P, Sergeev E, Tuschy S *et al.* Body mass index, waist circumference, and waist-to-height ratio as predictors of cardiometabolic risk in childhood obesity depending on pubertal development. *J Clin Endocrinol Metab* 2013; **98**: 3384–3393.
- Wells JCK. *The Evolutionary Biology of Human Body Fatness: Thrift and Control* 1st edn. Cambridge University Press: Cambridge, UK, 2010.
- Prentice AM, Jebb SA. Beyond body mass index. *Obes Rev* 2001; **2**: 141–147.
- Müller MJ, Braun W, Enderle J, Bösby-Westphal A. Beyond BMI: conceptual issues related to overweight and obese patients. *Obes Facts* 2016; **9**: 193–205.
- Shen W, Punyanitya M, Silva AM, Chen J, Gallagher D, Sardinha LB *et al.* Sexual dimorphism of adipose tissue distribution across the lifespan: a cross-sectional whole-body magnetic resonance imaging study. *Nutr Metab (Lond)* 2009; **6**: 17.
- Brambilla P, Manzoni P, Agostini G, Beccaria L, Ruotolo G, Sironi S *et al.* Persisting obesity starting before puberty is associated with stable intraabdominal fat during adolescence. *Int J Obes Relat Metab Disord* 1999; **23**: 299–303.
- Fox KR, Peters DM, Sharpe P, Bell M. Assessment of abdominal fat development in young adolescents using magnetic resonance imaging. *Int J Obes Relat Metab Disord* 2000; **24**: 1653–1659.
- Benfield LL, Fox KR, Peters DM, Blake H, Rogers I, Grant C *et al.* Magnetic resonance imaging of abdominal adiposity in a large cohort of British children. *Int J Obes (Lond)* 2008; **32**: 91–99.
- Staiano AE, Broyles ST, Gupta AK, Katzmarzyk PT. Ethnic and sex differences in visceral, subcutaneous, and total body fat in children and adolescents. *Obesity* 2013; **21**: 1251–1255.
- Gyllenhammer LE, Alderete TL, Toledo-Corral CM, Weigensberg M, Goran MI. Saturation of subcutaneous adipose tissue expansion and accumulation of ectopic fat associated with metabolic dysfunction during late and post-pubertal growth. *Int J Obes (Lond)* 2016; **40**: 601–606.
- Kelly AS, Dengel DR, Hodges J, Zhang L, Moran A, Chow L *et al.* The relative contributions of the abdominal visceral and subcutaneous fat depots to cardio-metabolic risk in youth. *Clin Obes* 2014; **4**: 101–107.
- Kim SK, Kim HJ, Hur KY, Choi SH, Ahn CW, Lim SK *et al.* Visceral fat thickness measured by ultrasonography can estimate not only visceral obesity but also risks of cardiovascular and metabolic diseases. *Am J Clin Nutr* 2004; **79**: 593–599.
- King RJ, Ajan RA. Vascular risk in obesity: facts, misconceptions and the unknown. *Diab Vasc Dis Res* 2017; **14**: 2–13.
- Yatsuya H, Nishihashi T, Li Y, Hotta Y, Matsushita K, Muramatsu T *et al.* Independent association of liver fat accumulation with insulin resistance. *Obes Res Clin Pract* 2014; **8**: e350–e355.
- Tobisch B, Blatniczky L, Barkai L. Cardiometabolic risk factors and insulin resistance in obese children and adolescents: relation to puberty. *Pediatr Obes* 2015; **10**: 37–44.
- Reinehr T, Wolters B, Knop C, Lass N, Holl RW. Strong effect of pubertal status on metabolic health in obese children: a longitudinal study. *J Clin Endocrinol Metab* 2015; **100**: 301–308.
- Kim JA, Park HS. Association of abdominal fat distribution and cardiometabolic risk factors among obese Korean adolescents. *Diabetes Metab* 2008; **34**: 126–130.
- Owens S, Gutin B, Ferguson M, Allison J, Karp W, Le NA. Visceral adipose tissue and cardiovascular risk factors in obese children. *J Pediatr* 1998; **133**: 41–45.
- Syme C, Abrahamowicz M, Leonard GT, Perron M, Pitiot A, Qiu X *et al.* Intra-abdominal adiposity and individual components of the metabolic syndrome in adolescence: sex differences and underlying mechanisms. *Arch Pediatr Adolesc Med* 2008; **162**: 453–461.
- He F, Rodriguez-Colon S, Fernandez-Mendoza J, Vgontzas AN, Bixler EO, Berg A *et al.* Abdominal obesity and metabolic syndrome burden in adolescents—Penn State Children Cohort study. *J Clin Densitom* 2015; **18**: 30–36.
- Maffei C, Manfredi R, Trombetta M, Sordelli S, Storti M, Benuzzi T *et al.* Insulin sensitivity is correlated with subcutaneous but not visceral body fat in overweight and obese prepubertal children. *J Clin Endocrinol Metab* 2008; **93**: 2122–2128.
- Duke PM, Litt IF, Gross RT. Adolescents' self-assessment of sexual maturation. *Pediatrics* 1980; **66**: 918–920.
- Kromeyer-Hauschild K, Wabitsch M, Kunze D, Geller F, Geiß HC, Hesse V *et al.* Perzentile für den Body-mass-Index für das Kindes- und Jugendalter unter Heranziehung verschiedener deutscher Stichproben. *Monatsschrift Kinderheilkunde* 2001; **149**: 807–818.
- Bösby-Westphal A, Danielzik S, Becker C, Geisler C, Onur S, Korth O *et al.* Need for optimal body composition data analysis using air-displacement plethysmography in children and adolescents. *J Nutr* 2005; **135**: 2257–2262.
- Siri WE. Body composition from fluid spaces and density: analysis of methods. 1961. *Nutrition* 1993; **9**: 480–491 discussion 480, 492.
- Huang TT-K, Watkins MP, Goran MI. Predicting total body fat from anthropometry in Latino children. *Obes Res* 2003; **11**: 1192–1199.
- Wells JCK, Cole TJ. Adjustment of fat-free mass and fat mass for height in children aged 8 y. *Int J Obes Relat Metab Disord* 2002; **26**: 947–952.
- Bösby-Westphal A, Booke C-A, Blocker T, Kossel E, Goele K, Later W *et al.* Measurement site for waist circumference affects its accuracy as an index of visceral and abdominal subcutaneous fat in a Caucasian population. *J Nutr* 2010; **140**: 954–961.
- Schautz B, Later W, Heller M, Müller MJ, Bösby-Westphal A. Total and regional relationship between lean and fat mass with increasing adiposity—impact for the diagnosis of sarcopenic obesity. *Eur J Clin Nutr* 2012; **66**: 1356–1361.
- Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; **28**: 412–419.
- Eid M, Gollwitzer M, Schmitt M. *Statistik und Forschungsmethoden: Lehrbuch; mit Online-Materialien* 2nd edn. Beltz: Weinheim, 2011.
- Huang TT, Johnson MS, Figueroa-Colon R, Dwyer JH, Goran MI. Growth of visceral fat, subcutaneous abdominal fat, and total body fat in children. *Obes Res* 2001; **9**: 283–289.
- Johnson MS, Figueroa-Colon R, Huang TT, Dwyer JH, Goran MI. Longitudinal changes in body fat in African American and Caucasian children: influence of fasting insulin and insulin sensitivity. *J Clin Endocrinol Metab* 2001; **86**: 3182–3187.
- Raab J, Haupt F, Kordonouri O, Scholz M, Wosch A, Ried C *et al.* Continuous rise of insulin resistance before and after the onset of puberty in children at increased risk for type 1 diabetes—a cross-sectional analysis. *Diabetes Metab Res Rev* 2013; **29**: 631–635.
- Ballerini MG, Bergada I, Rodriguez ME, Keselman A, Bengolea VS, Pipman V *et al.* Concentracion de insulina e indices de insulinosensibilidad en niños y adolescentes sanos. *Arch Argent Pediatr* 2016; **114**: 329–336.
- Ali O, Cerjak D, Kent JW JR, James R, Blangero J, Zhang Y. Obesity, central adiposity and cardiometabolic risk factors in children and adolescents: a family-based study. *Pediatr Obes* 2014; **9**: e58–e62.
- Wills AK, Lawlor DA, Matthews FE, Sayer AA, Bakra E, Ben-Shlomo Y *et al.* Life course trajectories of systolic blood pressure using longitudinal data from eight UK cohorts. *PLoS Med* 2011; **8**: e1000440.
- Casazza K, Dulin-Keita A, Gower BA, Fernandez JR. Intraabdominal fat is related to metabolic risk factors in Hispanic Americans, African Americans and in girls. *Acta Paediatr* 2009; **98**: 1965–1971.
- Heshmat R, Qorbani M, Shahr Babaki AE, Djalalinia S, Ataei-Jafari A, Motlagh ME *et al.* Joint Association of Screen Time and Physical Activity with Cardiometabolic Risk Factors in a National Sample of Iranian Adolescents: The CASPIANIII Study. *PLoS One* 2016; **11**: e0154502.
- Bakrania K, Edwardson CL, Bodicoat DH, Esliger DW, Gill JMR, Kazi A *et al.* Associations of mutually exclusive categories of physical activity and sedentary time with markers of cardiometabolic health in English adults: a cross-sectional analysis of the Health Survey for England. *BMC Public Health* 2016; **16**: 25.
- von Kries R, Beyerlein A, Müller MJ, Heinrich J, Landsberg B, Bolte G *et al.* Different age-specific incidence and remission rates in pre-school and primary school

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- suggest need for targeted obesity prevention in childhood. *Int J Obes (Lond)* 2012; **36**: 505–510.
- 44 Mackenzie K, Goyder E, Eves F. Acceptability and feasibility of a low-cost, theory-based and co-produced intervention to reduce workplace sitting time in desk-based university employees. *BMC Public Health* 2015; **15**: 1294.
- 45 Lee YH, Song YW, Kim HS, Lee SY, Jeong HS, Suh S-H *et al*. The effects of an exercise program on anthropometric, metabolic, and cardiovascular parameters in obese children. *Korean Circ J* 2010; **40**: 179–184.
- 46 Faria AN, Ribeiro Filho FF, Gouveia Ferreira SR, Zanella MT. Impact of visceral fat on blood pressure and insulin sensitivity in hypertensive obese women. *Obes Res* 2002; **10**: 1203–1206.
- 47 Matsuzawa Y, Shimomura I, Nakamura T, Keno Y, Kotani K, Tokunaga K. Pathophysiology and pathogenesis of visceral fat obesity. *Obes Res* 1995; **3** (Suppl 2): 187S–194S.
- 48 Phillips GB, Jing T, Heymsfield SB. Relationships in men of sex hormones, insulin, adiposity, and risk factors for myocardial infarction. *Metabolism* 2003; **52**: 784–790.



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Abstract***Objectives***

To determine age-related associations between fat mass (FM), regional fat depots and cardiometabolic traits in normal- and overweight children, adolescents and adults.

Methods

Detailed body composition (regional subcutaneous and visceral adipose tissue; SAT, VAT) by whole-body magnetic resonance imaging (MRI), FM and fat-free mass by air-displacement plethysmography, systolic and diastolic blood pressure (SBP, DBP), triglycerides (TG), high-density lipoprotein cholesterol (HDL), plasma glucose and plasma insulin were measured in 433 subjects (BMI: 23.6 (21.0–27.7)); 151 children and adolescents, aged 6–18 years, 150 young adults, aged 18–30 years and 132 adults, aged 30–60 years). Data were derived from pooled data of the ‘Reference Center for Body Composition’ in Kiel, Germany. Insulin resistance was determined by the homeostatic model assessment of insulin resistance (HOMA-IR). Partial correlations and multivariate linear regression analyses were used to evaluate the associations between body composition and cardiometabolic traits. A descriptive approach was used to demonstrate age-dependent differences in associations between body fat depots and insulin resistance, independent of BMI.

Results

FM, SAT, and VAT increased from childhood to adulthood with low VAT in children and adolescents. When compared to children, TG was higher in adults. HDL and DBP did not differ between age groups. Insulin resistance was highest in male adolescents and female young adults. Associations between body fat depots and cardiometabolic traits were seen after puberty with no associations in pre- and intrapubertal children. When compared to FM, SAT and VAT had the strongest association with insulin resistance in adults. This association was independent of BMI.

Conclusions

Associations between individual body fat depots and most cardiometabolic traits became evident after puberty only. The strongest associations were observed between insulin resistance and abdominal fat in adults. The impact of VAT was independent of BMI.

Introduction

To address the impact of overweight on cardiometabolic risk most studies are based on body mass index (BMI).¹⁻³ However, BMI can neither differentiate between fat mass (FM) and fat-free mass (FFM) nor give detailed information about body fat distribution.^{4,5} To go beyond BMI, detailed body composition and appropriate modeling taking into account body function have to be considered.⁶ Magnetic resonance imaging (MRI) and computed tomography are the gold-standard methods to determine trunk and peripheral subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT). In children and adolescents, MRI is the most suitable measurement because it does not expose them to radiation.

Our present knowledge about the association between individual body fat depots and cardio-metabolic risk factors is mainly based on studies on adults. In addition, there is only limited knowledge about regional fat depots in children and adolescents. In a cross-sectional study SAT and VAT were lower in children compared to adults.⁷ In a longitudinal study, SAT increased during adolescence, whereas VAT remained low.⁸ By contrast, both, SAT and VAT, increased between age of 11 to 13 years.⁹ With regard to gender differences, girls compared with boys had greater masses of VAT and SAT.¹⁰ This was contrary to other authors who observed that VAT was greater in boys than in girls.¹¹

In obese adolescents and adults cardiometabolic traits and insulin resistance have been mainly related to VAT and liver fat.¹²⁻¹⁶ In children and adolescents the degree of insulin resistance rises from pre- to intrapuberty and decreases after puberty.^{17,18} However, the association between cardiometabolic traits and insulin resistance with detailed body composition has not been well characterized in children and adolescents. As in adults, SAT and VAT were differently associated with cardiometabolic traits in children and adolescents.¹⁹⁻²² Both, SAT and VAT were independently correlated with blood pressure,¹⁹ whereas VAT was positively associated with plasma triglycerides (TG) and negatively associated with high-density lipoprotein cholesterol (HDL).²⁰ In addition, there was a positive relationship between either VAT or SAT and insulin resistance.²⁰⁻²³

Until now, there is no systematic study of the associations between individual body fat depots and cardiometabolic risk in different age groups. The aim of this study was to determine the associations between MRI-derived individual fat depots and cardiometabolic traits in children and adolescents compared to young and older adults. Furthermore, we investigated the associations between insulin resistance, relative to BMI, and regional fat depots.

Methods

All studies had been approved by the Ethical Committee of Christian-Albrechts-University of Kiel, Germany, and conducted according to the guidelines laid down in the “Declaration of Helsinki”. Written informed consent was obtained from each subject and, in state of minority, from its legal guardian before participation.

Subjects

This investigation was a secondary analysis of data collected at the “Reference Center for Body Composition” (Institute of Human Nutrition and Food Science at the University of Kiel, Germany) between 2005 and 2016. In this cross sectional study, data of 433 healthy Caucasian subjects (151 children and adolescents, aged 6-18 years, 150 young adults, aged 18-30 years and 132 adults, aged 30-60 years) were analyzed. Exclusion criteria were metallic implants, smoking, pregnancy, chronic or acute diseases, and medication intake that influences body composition. Subjects were recruited by notice boards postings and advertisements in the local newspaper. Study measurements of anthropometry, body composition, detailed body composition by whole body MRI and cardiometabolic variables were performed in children, adolescents and adults. Self-assessment of pubertal stage was determined according to the definition of Marshall and Tanner.²⁴ Based on breast and genital stages pubertal status was categorized into three groups (prepubertal: Tanner I; intrapubertal: Tanner II-III, postpubertal: Tanner \geq IV).¹⁸ In this analysis prepubertal and intrapubertal subjects were grouped together. This was done because they did not differ in associations between body composition and cardiometabolic traits (data not shown) and because of a low number of prepubertal children (n = 26).

Anthropometric measures

Body weight was measured to the nearest 0.01 kg using an electronic Tanita scale coupled to the BOD POD[®] Body Composition Tracking System (Life Measurement Instruments, Concord, California, USA). Body height was assessed to the nearest 0.5 cm by using a stadiometer (SECA, Modell 220, Hamburg, Germany). BMI was calculated as body weight (kg) / body height (m)². To define overweight and obesity for children and adolescents (>90th percentile; >97th percentile) German age- and sex-specific references were used.²⁵ Waist circumference (WC) was measured midway between the lowest rib and the top of the iliac crest with the subjects standing in upright position. The measurement was made with a nonelastic plastic tape placed parallel to the floor at the end of a normal expiration.

Densitometry

Air-displacement plethysmography (ADP) was performed by BOD POD[®] device to assess body composition. Child-specific equations were used to determine FM as in detail described elsewhere.²⁶ In adults, FM was calculated using the equation by Siri et al.²⁷ FFM was then calculated from the difference between body weight and FM. Because of reported difficulties in BOD POD calibration in one of the children studies cross-validated gender specific equations of FM were developed for children and adolescents as procedure is described elsewhere.²⁸ 222 children and adolescents were randomly split into two parts. 155 (91 female, 64 male) were assigned to the generation group, with 67 (31 female, 36 male) subjects in the cross-validation group. When compared to generation group, the cross-validation group did not differ in age, gender, pubertal status, height, weight, BMI, WC and FM measured by bio-impedance analysis. The correlation coefficients between FM measured by bio-impedance analysis and predicted FM based on BOD POD algorithm did not differ between the two groups. Following equations based on stepwise multiple regression analysis were developed for children and adolescents:

$$FM_{\text{female}} (\%) = 1.808 * BMI - 7.7 * 10^5 * \text{age}^4 - 0.531 * \text{body weight (kg)} + 53.672 * \text{height (m)} + 0.416 * FM_{\text{ADP}}(\%) + 0.139 * WC \text{ (cm)} - 86.307.$$

$$FM_{\text{male}} (\%) = 0.961 * BMI + 7.0 * 10^5 * \text{age}^4 + 0.336 * FM_{\text{ADP}} (\%) - 2.853.$$

Based on calculated FM and FFM, FM-Index (FMI) and FFM-Index (FFMI) were calculated for children and adolescents as FM (kg) / height (m)ⁿ and FFM (kg) / height (m)ⁿ. Height-independent FM is confounded by age in adolescents, whereas age was not included as a significant independent variable in children. By contrast, height-independent FFM is confounded by age in children but not in adolescents. Age-adjusted power of height (*n*) was determined using log – log regression analysis²⁹ (FMI: *n* = 6.1, 2.3 and FFMI: *n* = 2.3, 2.9, respectively for children and adolescents). In adults FMI and FFMI were calculated as FM (kg) / height (m)² and FFM (kg) / height (m)².

Magnetic resonance imaging (MRI)

As described previously, detailed body composition of all subjects was performed by using whole-body MRI with a 1.5T scanner (Magnetom Vision or Magnetom Avanto, Siemens Medical Systems, Erlangen, Germany).^{30,31} Briefly, participants were examined in a supine position with their arms extended above their heads and they were asked to hold their breath during measurement of abdominal and thoracic regions for image acquisition. In the present analysis volumes of SAT and VAT were manually segmented by using segmentation software (SliceOmatic 4.3 and 5.0, Tomovision, Montreal, Canada). SAT was analyzed from ankle to

wrist. In this case SAT_{legs} were defined from ankle to femoral heads and SAT_{arms} were defined from humerus heads to wrist. Between femoral and humerus heads volume of SAT_{trunk} was assessed. VAT was defined as intra-abdominal fat between femoral heads and the top of the liver. Volumes of SAT and VAT were determined by the sum of all adipose tissue areas (cm²) multiplied by slice thickness. Volume data were then transformed into tissue masses by using the following density: 0.923 g/cm³ for SAT and VAT.

Cardiometabolic risk factors

Systolic and diastolic blood pressure were measured by manual sphygmomanometer in a seated position. After an 8-h overnight fast blood samples were taken and analyzed by standard procedures. HDL was assessed enzymatically by Konelab-20i-Analyzer (Konelab, Espoo, Finland). Triglyceride and plasma glucose were analyzed enzymatically using a Konelab-Test-Kit (Thermo Clinical LabSystems, Frankfurt, Germany). Plasma insulin was measured by radioimmunoassay (Adaltis, Freiburg, Germany). Insulin resistance was then calculated by homeostasis model assessment of insulin resistance (HOMA-IR):

$$\text{HOMA-IR} = \text{plasma glucose (mmol/L)} \times \text{plasma insulin (\mu U/ml)} / 22.5 \text{ (ref.32)}$$

Statistical analysis

Statistical analyses were performed with SPSS statistical software (SPSS 24.0, Inc., Chicago, Illinois, USA). All data are given as median and interquartil range (IQR) because of not normally distributed data. Kruskal-wallis test with bonferroni correction was performed for more than two groups (pre- and intrapubertal subjects, postpubertal subjects, young adults and adults). To examine the association of individual body components on cardiometabolic traits, age- and gender-adjusted partial correlations were performed. Differences in correlation coefficients between age groups were tested by using the method of Eid, Gollwitzer, Schmitt³³. Further, stepwise multivariate linear regression analyses were done. Four models were conducted. Model 1 included FMI, Model 2 SAT_{trunk}, Model 3 VAT and Model 4 SAT_{trunk} and VAT as independent variables. All models were adjusted for age and gender. In addition, a descriptive approach was used to demonstrate age-dependent differences in individual body components between three different HOMA-IR per BMI groups (low, normal and high). These three groups were stratified according to their age dependent deviation from the regression line. Cut-offs were chosen from tertiles between HOMA-IR and BMI (low: residuals < -0.48, < -0.90, < -0.92, < -0.69; normal: residuals ≤ 0.16 and ≥ -0.48, ≤ 0.25 and ≥ -0.90, ≤ 0.00 and ≥ -0.92, ≤ 0.29 and ≥ -0.69; high: residuals > 0.16, > 0.25, > 0.00, > 0.29, respectively for children,

adolescents, young adults and adults). All tests were two-sided and level of significance was set at $P < 0.05$.

Results

Detailed body composition: differences between age groups

The characteristics of the study population are shown in **Table 1**. When compared with children, height, weight, FM and FFM were significantly higher in adolescents and with no significant differences to adults. When compared with young adults, VAT was lower in children and adolescents. In males, VAT was significantly higher in older compared with young adults. In females, SAT_{total} , SAT_{arms} and SAT_{trunk} were higher in young adults compared with children and adolescents. By contrast, SAT_{trunk} was the only subcutaneous fat depot in males which was significantly higher in young adults compared to children and adolescents.

Cardiometabolic risks: differences between age groups

In females, plasma insulin and HOMA-IR were highest in young adults when compared to other age groups (**Table 1**). In males, plasma insulin and HOMA-IR were highest in adolescents. In both genders, HOMA-IR did not differ between adolescents and adults. There were no differences in SBP, DBP and HDL between the different age groups, except for SBP in male adolescents, and high TG level in female young adults and male adults.

Associations between body composition and cardiometabolic traits: differences between age groups

Age- and gender-adjusted partial correlation coefficients between individual body fat depots and cardiometabolic traits are shown in **Table 2**. In general, correlation coefficients were low to moderate. As for SBP, DBP, TG and plasma glucose, significant associations were seen after puberty only. The associations between SAT_{trunk} and either SBP or DBP or TG did not differ in adolescents, young adults and adults. Strongest associations were observed between plasma insulin levels or HOMA-IR and truncal fat depots (SAT_{trunk} , VAT). When compared with adults, the associations between individual body fat depots and HDL were higher in children and adolescents.

Table 1. Characteristics of the study population stratified by age group and gender

age groups [#]	(1) Children		(2) Adolescents		(3) Young Adults		(4) Adults	
	Females	Males	Females	Males	Females	Males	Females	Males
n	37	43	41	30	69	81	69	63
Age (y)	10.9 (9.4 – 13.4)	12.3 (9.4 – 14.1)	15.8 (15.3 – 16.3)	16.3 (15.6 – 17.3)	26.0 (24.0 – 26.0) ^{1,2}	25.0 (23.0 – 27.0) ^{1,2}	44.0 (36.0 – 50.0) ^{1,2,3}	41.0 (35.0 – 50.0) ^{1,2,3}
Height (m)	1.49 (1.34 – 1.61)	1.58 (1.42 – 1.70)	1.68 (1.65 – 1.72) ¹	1.78 (1.73 – 1.83) ¹	1.70 (1.65 – 1.75) ¹	1.81 (1.76 – 1.87) ¹	1.67 (1.63 – 1.72) ¹	1.79 (1.76 – 1.84) ¹
Weight (kg)	43.4 (29.4 – 56.9)	53.1 (32.3 – 72.5)	65.6 (55.0 – 80.7) ¹	73.0 (64.0 – 101.6) ¹	69.5 (61.0 – 87.2) ¹	78.6 (71.1 – 85.1) ¹	68.0 (61.4 – 79.6) ¹	81.2 (73.5 – 92.3) ¹
OW (OB) (%)	35.1 (27.0)	34.9 (16.3)	43.9 (36.6)	43.3 (36.7)	37.7 (29.0)	33.3 (8.6)	40.6 (18.8)	46.0 (17.5)
BMI (kg/m ²)	19.3 (16.5 – 25.5)	19.0 (15.7 – 24.8)	21.8 (19.8 – 29.6)	23.4 (20.0 – 31.1) ¹	23.9 (21.7 – 31.7) ¹	23.4 (22.1 – 25.7) ¹	24.3 (21.7 – 27.7) ¹	24.5 (23.2 – 28.7) ¹
FM (kg)	9.4 (4.7 – 19.9)	9.9 (5.0 – 19.4)	16.8 (12.4 – 31.9) ¹	14.0 (9.3 – 29.6) ¹	22.1 (17.4 – 37.8) ¹	13.2 (10.1 – 18.9)	23.4 (16.7 – 30.5) ¹	17.2 (12.9 – 24.4) ¹
FFM (kg)	32.5 (24.5 – 39.3)	40.6 (27.4 – 50.0)	47.6 (41.6 – 51.1) ¹	57.7 (54.8 – 68.2) ¹	47.5 (43.6 – 52.0) ¹	64.5 (59.0 – 68.7) ¹	45.0 (41.8 – 50.2) ¹	65.3 (59.4 – 69.3) ¹
FMI (kg/m ³)	0.95 (0.55 – 1.56)	0.70 (0.48 – 0.98)	4.8 (3.9 – 10.2)	4.0 (2.6 – 8.2)	7.8 (6.0 – 14.2)	3.8 (3.0 – 5.8)	8.3 (6.3 – 10.9)	5.3 (3.9 – 7.3)
FFMI (kg/m ³)	13.0 (12.1 – 14.5)	12.9 (11.8 – 15.1)	10.1 (9.3 – 11.4)	11.1 (10.5 – 12.0)	16.4 (15.5 – 17.9)	19.6 (18.5 – 20.5)	16.0 (15.0 – 17.8)	19.9 (18.7 – 21.5)
Adipose tissue (kg)	9.5 (7.1 – 19.3)	9.0 (4.7 – 18.5)	15.0 (11.9 – 29.1)	11.6 (8.4 – 25.0)	21.5 (17.5 – 34.6) ¹	12.9 (10.8 – 18.6) ¹	22.6 (17.0 – 30.8) ¹	18.2 (14.0 – 24.5) ^{1,3}
SAT total (kg)	9.1 (6.9 – 18.8)	8.7 (4.6 – 17.9)	14.5 (11.5 – 27.9)	11.3 (8.1 – 23.2)	20.0 (17.0 – 33.3) ¹	11.8 (10.2 – 17.2)	21.4 (16.1 – 28.3) ¹	14.8 (11.7 – 18.6) ¹
SAT arms (kg)	1.2 (1.0 – 2.2)	1.2 (0.7 – 2.1)	1.7 (1.4 – 3.2)	1.4 (1.1 – 3.0)	2.2 (1.8 – 3.6) ¹	1.5 (1.4 – 2.2)	2.3 (1.7 – 3.0) ¹	1.9 (1.6 – 2.2) ¹
SAT trunk (kg)	2.9 (2.1 – 6.3)	2.6 (1.3 – 6.7)	4.7 (3.7 – 13.5)	3.8 (2.8 – 10.4)	8.6 (6.2 – 14.3) ¹	4.7 (3.7 – 8.1) ¹	9.1 (6.3 – 13.3) ¹	6.8 (4.9 – 9.0) ¹
SAT legs (kg)	5.3 (3.8 – 8.7)	4.6 (2.6 – 8.4)	8.0 (6.4 – 12.5) ¹	6.0 (4.4 – 11.0)	9.8 (8.2 – 13.9) ¹	5.6 (4.8 – 7.3)	8.9 (7.0 – 11.9) ¹	6.3 (5.3 – 7.7) ¹
VAT (kg)	0.36 (0.22 – 0.87)	0.29 (0.16 – 0.88)	0.47 (0.34 – 0.96)	0.64 (0.37 – 1.30)	1.11 (0.60 – 1.71) ^{1,2}	1.13 (0.70 – 2.15) ¹	1.36 (0.68 – 2.29) ^{1,2}	2.50 (1.69 – 5.17) ^{1,2,3}
SBP (mmHg)	120 (108 – 121)	120 (110 – 125)	118 (110 – 120)	128 (120 – 134) ¹	115 (110 – 125)	120 (110 – 125) ²	118 (110 – 125)	120 (115 – 130)
DBP (mmHg)	70 (70 – 80)	75 (70 – 80)	75 (70 – 80)	76 (70 – 85)	80 (70 – 82)	80 (75 – 85)	80 (70 – 80)	80 (75 – 85)
TG (mg/dl)	75 (59 – 96)	67 (47 – 121)	85 (70 – 104)	86 (69 – 114)	102 (73 – 145) ¹	91 (70 – 127) ¹	86 (70 – 108)	106 (78 – 143) ¹
HDL (mg/dl)	54 (46 – 60)	53 (46 – 62)	52 (43 – 66)	51 (42 – 59)	55 (46 – 72)	51 (40 – 57)	65 (48 – 73)	46 (33 – 58)
Glucose (mg/dl)	89 (84 – 94)	90 (85 – 96)	87 (82 – 93)	91 (86 – 94)	88 (83 – 92)	86 (78 – 94)	94 (86 – 99) ^{2,3}	99 (94 – 105) ^{1,2,3}
Insulin (μU/ml)	8.7 (4.9 – 11.1)	7.7 (4.4 – 11.6)	12.1 (6.9 – 15.3)	10.7 (6.9 – 14.5)	12.5 (8.0 – 18.6) ¹	7.8 (6.3 – 10.1)	9.2 (6.8 – 12.3) ³	7.7 (5.9 – 14.1)
HOMA-IR	1.92 (0.98 – 2.36)	1.84 (0.92 – 2.79)	2.49 (1.46 – 3.11)	2.34 (1.46 – 3.08)	2.68 (1.62 – 4.34) ¹	1.64 (1.20 – 2.15)	2.20 (1.53 – 2.84)	1.98 (1.40 – 3.31)

Abbreviations: OW, overweight; OB, obese; FM, fat mass; FFM, fat-free mass; FMI, fat mass index; FFMI, fat-free mass index; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; HDL, high-density lipoprotein cholesterol.
Data are given as median and IQR.
[#](1), pre- and intrapubertal children; (2), postpubertal adolescents; (3), young adults (18-30 years old); (4), adults (30-60 years old).
¹power of height which is unequal in pre- and intrapubertal, postpubertal and adult stage (FMI: 6.1, 2.3, 2.0, 2.0; FFMI: 2.3, 2.9, 2.0, 2.0, respectively).
¹significantly different from gender-dependent pre- and intrapubertal subjects; ²significantly different from gender-dependent postpubertal subjects; ³significantly different from gender-dependent young adults (Kruskal-wallis test with bonferroni correction, $P < 0.05$).

Table 2. Age- and gender-adjusted partial correlations between parameters of body composition and cardiometabolic traits in children, adolescents and adults

age groups [#]	Correlation coefficient (<i>r</i>)																			
	FMI				SAT _{arms}				SAT _{trunk}				SAT _{legs}				VAT			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
n	80	71	150	132	80	71	150	132	80	71	150	132	80	71	150	132	80	71	150	132
SBP	0.17 ^{b,c}	0.31	0.43	0.42	0.29	0.27	0.39	0.27	0.25	0.31	0.43	0.42	0.29	0.28	0.36	0.30	0.19 ^c	0.27^c	0.27^c	0.48
DBP	0.14 ^{a,b,c}	0.42	0.41	0.37	0.33	0.43	0.35	0.25	0.29	0.42	0.38	0.40	0.30	0.37	0.34	0.23	0.24^c	0.42	0.26^c	0.45
TG	0.00 ^{a,b,c}	0.37	0.35	0.24	-0.05 ^{a,b}	0.34	0.31	0.16	0.02 ^{a,b}	0.34	0.37	0.24	-0.12 ^{a,b,c}	0.29	0.26	0.12	0.16 ^{a,c}	0.54	0.36	0.41
HDL	-0.53^{b,c}	-0.42	-0.27	-0.31	-0.50^{b,c}	-0.39	-0.26	-0.24	-0.52^b	-0.41	-0.28	-0.33	-0.52^{b,c}	-0.33	-0.21	-0.22	-0.50^{b,c}	-0.37	-0.29	-0.30
Glucose	-0.15 ^c	-0.23^{b,c}	0.07 ^c	0.30	-0.17 ^{b,c}	-0.25^{b,c}	0.15	0.30	-0.24^{b,c}	-0.27^{b,c}	0.18	0.30	-0.19 ^{b,c}	-0.22 ^{b,c}	0.05 ^c	0.33	-0.26^{b,c}	-0.17 ^{b,c}	0.35	0.37
Insulin	0.55	0.54	0.48	0.48	0.55	0.52	0.52	0.39	0.57	0.55	0.56	0.53	0.53	0.55^c	0.37	0.34	0.64	0.55	0.53	0.50
HOMA-IR	0.51	0.51	0.45	0.51	0.52	0.48	0.50	0.41	0.52	0.51	0.54	0.55	0.50	0.52	0.34	0.37	0.59	0.52	0.53	0.55

Abbreviations: FMI, fat mass index; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; HDL, high-density lipoprotein cholesterol.

[#]1, pre- and intrapubertal children; 2, postpubertal adolescents; 3, young adults (18-30 years old); 4, adults (30-60 years old).

significant correlation coefficients in bold, $P < 0.05$

^asignificantly different from correlation coefficient in postpubertal adolescents, ^bsignificantly different from correlation coefficient in young adults, ^csignificantly different from correlation coefficient in adults, $P < 0.05$ [method of Eid, Gollwitzer, Schmitt³⁶]

Using stepwise multivariate linear regression analysis, the explained variance and respective beta coefficients of cardiometabolic traits as dependent variables and FMI, SAT_{trunk}, VAT and the combination of SAT_{trunk} and VAT as independent variables are shown in **Table 3**. The explained variance of cardiometabolic traits was moderate, maximally, 35.2% of the variance of HOMA-IR was explained by SAT_{trunk} and VAT in adults.

When compared with adolescents and adults, the explained variances of SBP, DBP, TG and plasma glucose by body fat depots were low ($\leq 5.3\%$) in children. However, the explained variances of HOMA-IR and plasma insulin by parameters of body composition differed only slightly between children, adolescents and adults. The explained variance of HDL by body fat depots decreased from children to adults.

When compared to FMI, the explained variance of plasma insulin and HOMA-IR by SAT_{trunk} and VAT were moderately increased in young and older adults. When compared to FMI, SAT_{trunk} and VAT did not exceed the explained variance of HDL, whereas abdominal fat depots added to the variance of TG in adolescents.

Association between detailed body composition and insulin resistance

Investigating subjects with low HOMA-IR (insulin sensitive), normal HOMA-IR or high HOMA-IR (insulin resistance) relative to their BMI, age-dependent differences in individual body components between these groups became evident (**Table 4**). In children and adolescents, no differences were found in body fat depots between groups differing in insulin resistance. By contrast, in young adults, adipose tissue, SAT_{total}, SAT_{trunk} and SAT_{legs} were significantly higher in insulin resistant subjects. In older adults, VAT was highest in insulin resistant subjects.

Table 3. Multivariate linear regression on cardiometabolic traits as dependent variables

independent variables	<i>FMI^a</i>				<i>SAT_{trunk}^a</i>				<i>VAT^a</i>				<i>SAT_{trunk} + VAT^a</i>																			
	1		2		3		4		1		2		3		4		1		2		3		4									
age groups [#]	R ²	β	R ²	β	R ²	β	R ²	β	R ²	β	R ²	β	R ²	β	R ²	β	R ²	β ^b														
n	80		71		150		132		80		71		150		132		80		71		150		132									
SBP	0.0	0.18	5.9	0.27	17.9	0.50	15.0	0.44	3.5	0.26	6.3	0.27	17.7	0.47	15.5	0.41	0.0	0.20	4.6	0.24	6.5	0.28	20.6	0.53	3.5	0.02	6.3	0.02	17.7	-0.06	19.4	0.52
DBP	0.0	0.16	15.7	0.42	16.1	0.47	12.4	0.40	5.3	0.31	16.7	0.42	13.6	0.41	14.8	0.41	4.2	0.26	16.1	0.41	6.3	0.27	19.0	0.51	5.3	0.09	16.7	0.20	13.6	0.00	18.4	0.50
TG	0.0	0.00	11.7	0.36	11.3	0.40	4.5	0.25	0.0	0.02	9.9	0.33	12.6	0.40	4.6	0.24	0.0	0.17	27.2	0.53	11.8	0.37	15.2	0.46	0.0	0.17	32.6	0.96	12.6	0.20	15.8	0.47
HDL	25.4	-0.54	15.1	-0.41	5.6	-0.29	6.8	-0.30	23.2	-0.60	14.7	-0.40	6.3	-0.29	7.6	-0.30	21.6	-0.54	12.2	-0.37	6.6	-0.28	6.7	-0.31	23.2	-0.25	14.7	-0.09	6.6	-0.28	7.6	-0.17
Glucose	0.0	-0.14	0.0	-0.23	0.0	0.08	7.1	0.31	3.7	-0.27	5.8	-0.27	2.7	0.20	7.1	0.29	4.8	-0.28	0.0	-0.17	11.5	0.36	11.0	0.39	4.8	-0.28	5.8	0.24	11.5	0.36	11.3	0.40
Insulin	19.4	0.47	27.4	0.54	18.5	0.51	22.8	0.54	20.7	0.56	29.1	0.55	25.6	0.57	27.9	0.55	26.4	0.59	29.3	0.55	22.3	0.49	24.8	0.58	26.4	0.59	29.3	0.55	28.2	0.24	31.0	0.31
HOMA-IR	16.3	0.43	24.4	0.50	16.3	0.48	25.6	0.57	16.8	0.51	24.9	0.50	24.4	0.55	30.0	0.57	21.2	0.53	26.7	0.52	23.3	0.50	29.8	0.63	21.2	0.53	26.7	0.52	27.9	0.28	35.2	0.64

Abbreviations: FMI, fat mass index; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; HDL, high-density lipoprotein cholesterol.

[#]1, pre- and intrapubertal children; 2, postpubertal adolescents; 3, young adults (18-30 years old); 4, adults (30-60 years old).

^amodel with independent variable/variables in bold adjusted for age and gender

R², additional explained variance (%) by independent variable/variables which is/are in bold compared to the base model which includes age and gender

^bβ-coefficient of VAT.

Table 4. Differences in body fat depots between three groups (low HOMA-IR per BMI, normal HOMA-IR per BMI and high HOMA-IR per BMI) stratified by age group

	HOMA-IR/BMI ↓	HOMA-IR/BMI =	HOMA-IR/BMI ↑	<i>p</i> -value
(1) Children				
n	26	27	26	
FM (kg)	14.8 (4.7 – 23.4)	7.1 (4.2 – 12.8)	10.7 (7.6 – 20.5)	0.038
FFM (kg)	36.7 (27.4 – 49.8)	28.4 (23.6 – 38.9)	42.1 (32.4 – 47.1)	0.011
Adipose tissue (kg)	11.9 (4.7 – 22.9)	8.3 (4.7 – 12.6)	9.8 (8.5 – 22.5)	0.111
SAT total (kg)	11.5 (4.5 – 22.1)	7.9 (4.5 – 12.0)	9.4 (8.3 – 21.9)	0.121
SAT arms (kg)	1.5 (0.7 – 2.5)	1.1 (0.7 – 1.7)	1.3 (1.0 – 2.3)	0.193
SAT trunk (kg)	3.9 (1.3 – 9.3)	2.6 (1.3 – 3.9)	3.1 (2.4 – 8.0)	0.132
SAT legs (kg)	6.1 (2.6 – 10.5)	4.5 (2.5 – 6.4)	5.1 (4.5 – 9.4)	0.102
VAT (kg)	0.53 (0.18 – 0.97)	0.29 (0.16 – 0.47)	0.36 (0.26 – 1.02)	0.171
HOMA-IR	0.84 (0.57 – 2.05)	1.60 (0.97 – 1.91)	2.74 (2.07 – 4.10)	< 0.001
(2) Adolescents				
n	23	24	23	
FM (kg)	24.2 (12.5 – 35.5)	15.5 (9.9 – 29.6)	13.5 (10.8 – 31.9)	0.174
FFM (kg)	56.9 (49.3 – 64.9)	50.4 (44.4 – 57.3)	49.3 (43.9 – 52.1)	0.058
Adipose tissue (kg)	22.4 (11.9 – 31.3)	12.0 (9.7 – 24.6)	13.3 (11.1 – 30.2)	0.222
SAT total (kg)	21.1 (11.6 – 30.1)	11.6 (9.3 – 23.2)	12.9 (10.6 – 24.4)	0.222
SAT arms (kg)	2.6 (1.3 – 3.8)	1.4 (1.2 – 2.8)	1.5 (1.3 – 3.1)	0.338
SAT trunk (kg)	8.1 (3.6 – 13.5)	3.8 (3.2 – 9.7)	4.5 (3.1 – 13.9)	0.190
SAT legs (kg)	10.0 (6.2 – 13.7)	6.9 (4.7 – 11.3)	6.8 (5.8 – 12.5)	0.265
VAT (kg)	0.72 (0.44 – 1.20)	0.43 (0.31 – 0.81)	0.51 (0.32 – 1.20)	0.306
HOMA-IR	1.62 (0.97 – 2.49)	2.02 (1.38 – 2.89)	3.25 (2.72 – 5.49)	< 0.001
(3) Young Adults				
n	47	47	47	
FM (kg)	17.6 (12.3 – 37.8)	15.6 (10.3 – 19.7)	20.8 (14.8 – 26.1)	0.034
FFM (kg)	62.1 (49.4 – 68.7)	58.7 (50.3 – 65.4)	53.6 (44.3 – 63.1)	0.010
Adipose tissue (kg)	16.5 (11.1 – 34.6)	16.0 (11.0 – 19.6)	20.4 (14.8 – 26.1)	0.013
SAT total (kg)	15.2 (10.4 – 33.3)	14.4 (10.3 – 19.3)	18.9 (14.2 – 25.0)	0.008
SAT arms (kg)	1.9 (1.4 – 3.1)	1.7 (1.4 – 2.2)	2.2 (1.6 – 2.8)	0.066
SAT trunk (kg)	5.9 (3.8 – 14.6)	5.8 (3.8 – 8.1)	8.8 (6.2 – 12.0)	0.006
SAT legs (kg)	8.1 (5.2 – 13.9)	6.4 (5.0 – 8.9)	8.7 (6.2 – 10.3)	0.021
VAT (kg)	0.96 (0.53 – 2.29)	1.25 (0.76 – 1.85)	1.1 (0.89 – 2.60)	0.411
HOMA-IR	1.21 (0.84 – 1.95)	1.68 (1.49 – 2.04)	4.00 (2.78 – 5.55)	< 0.001
(4) Adults				
n	44	44	44	
FM (kg)	20.9 (14.6 – 27.5)	19.5 (15.5 – 24.6)	22.5 (15.1 – 30.6)	0.519
FFM (kg)	58.2 (46.4 – 68.4)	51.3 (43.0 – 59.8)	53.1 (44.2 – 64.4)	0.051
Adipose tissue (kg)	18.9 (15.7 – 29.3)	18.3 (14.6 – 24.6)	21.7 (16.0 – 31.4)	0.277
SAT total (kg)	16.9 (13.8 – 24.8)	17.3 (13.7 – 22.1)	18.6 (14.9 – 27.6)	0.433
SAT arms (kg)	2.1 (1.7 – 2.8)	2.0 (1.6 – 2.4)	2.2 (1.7 – 2.8)	0.339
SAT trunk (kg)	7.7 (5.9 – 11.0)	7.1 (5.2 – 9.9)	8.4 (5.5 – 13.1)	0.380
SAT legs (kg)	6.9 (5.7 – 10.4)	7.3 (6.3 – 10.1)	8.3 (6.5 – 10.8)	0.643
VAT (kg)	2.07 (1.32 – 3.59)	1.57 (0.75 – 2.39)	2.39 (1.23 – 4.90)	0.013
HOMA-IR	1.39 (1.06 – 1.66)	1.84 (1.63 – 2.28)	3.75 (2.67 – 4.88)	< 0.001

Abbreviations: FM, fat mass; FFM, fat-free mass; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

(1), pre- and inpubertal children; (2), postpubertal adolescents; (3), young adults (18-30 years old); (4), adults (30-60 years old). Group 1: HOMA-IR/BMI ↓, residuals (1) < -0.48, (2) < -0.90, (3) < -0.92, (4) < -0.69; Group 2: HOMA-IR/BMI =, residuals (1) ≤ 0.16 and ≥ -0.48, (2) ≤ 0.25 and ≥ -0.90, (3) ≤ 0.00 and ≥ -0.92, (4) ≤ 0.29 and ≥ -0.69; Group 3: HOMA-IR/BMI ↑, residuals (1) > 0.16, (2) > 0.25, (3) > 0.00, (4) > 0.29.

significant correlation coefficients in bold (Kruskal-wallis test with bonferroni correction, $P < 0.05$)

Discussion

Up to now, there are limited data on the association between regional fat depots and metabolic disturbance in normal- and overweight children and adolescents compared with adults.^{34,35} To our knowledge, the present study is first to systematically investigate detailed body composition by age group and gender as well as its associations to cardiometabolic traits in 6 to 60 year old subjects.

Detailed body composition in different age groups

In both genders, VAT was low in children and adolescents compared with adults (Table 1). This is in line with previous data.^{7,8} However, other authors reported that during childhood, growth of VAT decelerated with increasing age.³⁴ In our study sexual dimorphism was apparent after puberty only with males having more VAT than females. By contrast, other authors observed higher masses of VAT in girls compared to boys.¹⁰

Cardiometabolic risks in different age groups

As shown in Table 1, in males, insulin resistance increased from pre- and inrapuberty to postpuberty, with changes to young adulthood in females only. These findings support previous data.^{36,37} By contrast, other authors reported that the increase in insulin resistance from childhood to adulthood was independent of gender.³⁸ Studies on blood pressure showed that SBP and DBP increased with age.^{38,39} These results are contrary to our findings.

Associations between body composition and cardiometabolic traits in different age groups

Low and moderate associations were observed between cardiometabolic traits and body fat depots in normal- and overweight children, adolescents and adults (Table 2). This is in line with previous data.³⁸ However, moderate associations were seen after puberty only. These findings indicate that in pre- and inrapubertal children individual body components are not related to cardiometabolic risk as it is in older subjects. Accordingly, a study on pre- and inrapubertal children found no associations between abdominal fat depots vs. blood pressure and blood lipids in European Americans.⁴⁰ Positive associations became evident between SAT_{trunk}, VAT and cardiometabolic traits in adolescents.¹⁹

Overall, there was a limited explained variance of cardiometabolic traits by FMI and different regional fat depots (Table 3). When compared to FMI, abdominal fat depots, especially VAT, were stronger associated to insulin resistance in adults than in children and adolescents. The associations between HDL and body fat depots decreased with age. As HDL is positively

associated to physical activity in children, adolescents and adults,^{41,42} a possible explanation may be increasing sedentary behavior with age which is due to school entry and entering the workplace.^{43,44} This is in line with the finding that children improved HDL by increased physical activity which was independent of weight change.⁴⁵

Associations between detailed body composition and insulin resistance

To address the role of individual body fat depots on insulin resistance, independent of BMI, differences in age groups became evident (Table 4). In children and adolescents, there were no differences in body fat depots between groups differing in insulin resistance. By contrast, regional SAT depots were significantly different in young adults, with VAT as associated to insulin resistance in older adults only. Our findings suggest that VAT becomes an important determinant of insulin resistance as age increased to adulthood. In our study, VAT was very low in children and adolescents and started to increase in young adults (Table 1). This is in line with other data that VAT is a primary determinant of insulin resistance in adults only.⁴⁶⁻⁴⁸

Study Strengths and Limitations

To our knowledge, this is the first study to systematically investigate age-related associations between individual body components and cardiometabolic traits in children and adolescents compared to adults. Detailed body composition was measured by MRI. This is the gold-standard method of body composition. We have used two different approaches. First, an analytic approach was chosen to examine age-dependent associations between cardiometabolic traits and body fat depots. Secondly, a descriptive approach was conducted to investigate age-dependent differences in body fat depots in groups differing in insulin resistance. A limitation of this study is that we have used cross-sectional data only. Thus, no causal relationships can be derived from our data.

Conclusion

Associations between regional fat depots and cardiometabolic traits were seen after puberty only. The impact of FMI on cardiometabolic traits is limited. With regard to HOMA-IR, this was improved by measuring individual fat depots in adults. The strongest associations were observed between abdominal fat depots and insulin resistance. When compared with adults, VAT was low in normal- and overweight children and adolescents. VAT has no association with insulin resistance groups in children, adolescents and young adults. The role of VAT is apparent in adults only.

Conflict of Interest: The authors declare no conflict of interest.

Acknowledgements: We thank Britta Jux and the Clinic for Diagnostic Radiology, University Medical Center Schleswig-Holstein, Kiel (Germany) for the help with MRI scanning. We acknowledge financial support by Land Schleswig-Holstein within the funding programme Open Access Publikationsfonds. This work was supported by the Deutsche Forschungsgemeinschaft (DFG), the Federal Ministry of Education and Research (BMBF) (DFG Mü 714/8-3; DFG Bo 3296/1-1; BMBF 01EA1336, 01GI1121A, 01GI1125, 01GI0821) and OMRON, Kyoto, Japan.

References

- 1 Janssen I, Katzmarzyk PT, Srinivasan SR, Chen W, Malina RM, Bouchard C et al. Utility of childhood BMI in the prediction of adulthood disease: comparison of national and international references. *Obes Res* 2005; 13(6): 1106–15.
- 2 Goodman E, Dolan LM, Morrison JA, Daniels SR. Factor analysis of clustered cardiovascular risks in adolescence: obesity is the predominant correlate of risk among youth. *Circulation* 2005; 111(15): 1970–7.
- 3 Bluher S, Molz E, Wiegand S, Otto K-P, Sergeyev E, Tuschy S et al. Body mass index, waist circumference, and waist-to-height ratio as predictors of cardiometabolic risk in childhood obesity depending on pubertal development. *J Clin Endocrinol Metab* 2013; 98(8): 3384–93.
- 4 Wells JCK. *The evolutionary biology of human body fatness: Thrift and control*, 1st edn. Cambridge University Press: Cambridge, 2010.
- 5 Prentice AM, Jebb SA. Beyond body mass index. *Obes Rev* 2001; 2(3): 141–7.
- 6 Müller MJ, Braun W, Enderle J, Bosy-Westphal A. Beyond BMI: Conceptual Issues Related to Overweight and Obese Patients. *Obes Facts* 2016; 9(3): 193–205.
- 7 Shen W, Punyanitya M, Silva AM, Chen J, Gallagher D, Sardinha LB et al. Sexual dimorphism of adipose tissue distribution across the lifespan: a cross-sectional whole-body magnetic resonance imaging study. *Nutr Metab (Lond)* 2009; 6: 17.

- 8 Brambilla P, Manzoni P, Agostini G, Beccaria L, Ruotolo G, Sironi S et al. Persisting obesity starting before puberty is associated with stable intraabdominal fat during adolescence. *Int J Obes Relat Metab Disord* 1999; 23(3): 299–303.
- 9 Fox KR, Peters DM, Sharpe P, Bell M. Assessment of abdominal fat development in young adolescents using magnetic resonance imaging. *Int J Obes Relat Metab Disord* 2000; 24(12): 1653–9.
- 10 Benfield LL, Fox KR, Peters DM, Blake H, Rogers I, Grant C et al. Magnetic resonance imaging of abdominal adiposity in a large cohort of British children. *Int J Obes (Lond)* 2008; 32(1): 91–9.
- 11 Staiano AE, Broyles ST, Gupta AK, Katzmarzyk PT. Ethnic and sex differences in visceral, subcutaneous, and total body fat in children and adolescents. *Obesity (Silver Spring)* 2013; 21(6): 1251–5.
- 12 Gyllenhammer LE, Alderete TL, Toledo-Corral CM, Weigensberg M, Goran MI. Saturation of subcutaneous adipose tissue expansion and accumulation of ectopic fat associated with metabolic dysfunction during late and post-pubertal growth. *Int J Obes (Lond)* 2016; 40(4): 601–6.
- 13 Kelly AS, Dengel DR, Hodges J, Zhang L, Moran A, Chow L et al. The relative contributions of the abdominal visceral and subcutaneous fat depots to cardiometabolic risk in youth. *Clin Obes* 2014; 4(2): 101–7.
- 14 Kim SK, Kim HJ, Hur KY, Choi SH, Ahn CW, Lim SK et al. Visceral fat thickness measured by ultrasonography can estimate not only visceral obesity but also risks of cardiovascular and metabolic diseases. *Am J Clin Nutr* 2004; 79(4): 593–9.
- 15 King RJ, Ajjan RA. Vascular risk in obesity: Facts, misconceptions and the unknown. *Diab Vasc Dis Res* 2017; 14(1): 2–13.
- 16 Yatsuya H, Nihashi T, Li Y, Hotta Y, Matsushita K, Muramatsu T et al. Independent association of liver fat accumulation with insulin resistance. *Obes Res Clin Pract* 2014; 8(4): e350-5.
- 17 Tobisch B, Blatniczky L, Barkai L. Cardiometabolic risk factors and insulin resistance in obese children and adolescents: relation to puberty. *Pediatr Obes* 2015; 10(1): 37–44.

- 18 Reinehr T, Wolters B, Knop C, Lass N, Holl RW. Strong effect of pubertal status on metabolic health in obese children: a longitudinal study. *J Clin Endocrinol Metab* 2015; 100(1): 301–8.
- 19 Kim JA, Park HS. Association of abdominal fat distribution and cardiometabolic risk factors among obese Korean adolescents. *Diabetes Metab* 2008; 34(2): 126–30.
- 20 Owens S, Gutin B, Ferguson M, Allison J, Karp W, Le NA. Visceral adipose tissue and cardiovascular risk factors in obese children. *J Pediatr* 1998; 133(1): 41–5.
- 21 Syme C, Abrahamowicz M, Leonard GT, Perron M, Pitiot A, Qiu X et al. Intra-abdominal adiposity and individual components of the metabolic syndrome in adolescence: sex differences and underlying mechanisms. *Arch Pediatr Adolesc Med* 2008; 162(5): 453–61.
- 22 He F, Rodriguez-Colon S, Fernandez-Mendoza J, Vgontzas AN, Bixler EO, Berg A et al. Abdominal obesity and metabolic syndrome burden in adolescents--Penn State Children Cohort study. *J Clin Densitom* 2015; 18(1): 30–6.
- 23 Maffei C, Manfredi R, Trombetta M, Sordelli S, Storti M, Benuzzi T et al. Insulin sensitivity is correlated with subcutaneous but not visceral body fat in overweight and obese prepubertal children. *J Clin Endocrinol Metab* 2008; 93(6): 2122–8.
- 24 Duke PM, Litt IF, Gross RT. Adolescents' self-assessment of sexual maturation. *Pediatrics* 1980; 66(6): 918–20.
- 25 Kromeyer-Hauschild K, Wabitsch M, Kunze D, Geller F, Geiß HC, Hesse V et al. Perzentile für den Body-mass-Index für das Kindes- und Jugendalter unter Heranziehung verschiedener deutscher Stichproben. *Monatsschrift Kinderheilkunde* 2001; 149(8): 807–18.
- 26 Bosy-Westphal A, Danielzik S, Becker C, Geisler C, Onur S, Korth O et al. Need for optimal body composition data analysis using air-displacement plethysmography in children and adolescents. *J Nutr* 2005; 135(9): 2257–62.
- 27 Siri WE. Body composition from fluid spaces and density: analysis of methods. 1961. *Nutrition* 1993; 9(5): 480-91; discussion 480, 492.
- 28 Huang TT-K, Watkins MP, Goran MI. Predicting total body fat from anthropometry in Latino children. *Obes Res* 2003; 11(10): 1192–9.

- 29 Wells JCK, Cole TJ. Adjustment of fat-free mass and fat mass for height in children aged 8 y. *Int J Obes Relat Metab Disord* 2002; 26(7): 947–52.
- 30 Bosy-Westphal A, Booke C-A, Blocker T, Kossel E, Goele K, Later W et al. Measurement site for waist circumference affects its accuracy as an index of visceral and abdominal subcutaneous fat in a Caucasian population. *J Nutr* 2010; 140(5): 954–61.
- 31 Schautz B, Later W, Heller M, Müller MJ, Bosy-Westphal A. Total and regional relationship between lean and fat mass with increasing adiposity--impact for the diagnosis of sarcopenic obesity. *Eur J Clin Nutr* 2012; 66(12): 1356–61.
- 32 Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; 28(7): 412–9.
- 33 Eid M, Gollwitzer M, Schmitt M. *Statistik und Forschungsmethoden: Lehrbuch ; mit Online-Materialien*, 2nd edn. Beltz: Weinheim, 2011.
- 34 Huang TT, Johnson MS, Figueroa-Colon R, Dwyer JH, Goran MI. Growth of visceral fat, subcutaneous abdominal fat, and total body fat in children. *Obes Res* 2001; 9(5): 283–9.
- 35 Johnson MS, Figueroa-Colon R, Huang TT, Dwyer JH, Goran MI. Longitudinal changes in body fat in African American and Caucasian children: influence of fasting insulin and insulin sensitivity. *J Clin Endocrinol Metab* 2001; 86(7): 3182–7.
- 36 Raab J, Haupt F, Kordonouri O, Scholz M, Wosch A, Ried C et al. Continuous rise of insulin resistance before and after the onset of puberty in children at increased risk for type 1 diabetes - a cross-sectional analysis. *Diabetes Metab Res Rev* 2013; 29(8): 631–5.
- 37 Ballerini MG, Bergada I, Rodriguez ME, Keselman A, Bengolea VS, Pipman V et al. Concentracion de insulina e indices de insulinosensibilidad en ninos y adolescentes sanos. *Arch Argent Pediatr* 2016; 114(4): 329–36.
- 38 Ali O, Cerjak D, Kent JW JR, James R, Blangero J, Zhang Y. Obesity, central adiposity and cardiometabolic risk factors in children and adolescents: a family-based study. *Pediatr Obes* 2014; 9(3): e58-62.

- 39 Wills AK, Lawlor DA, Matthews FE, Sayer AA, Bakra E, Ben-Shlomo Y et al. Life course trajectories of systolic blood pressure using longitudinal data from eight UK cohorts. *PLoS Med* 2011; 8(6): e1000440.
- 40 Casazza K, Dulin-Keita A, Gower BA, Fernandez JR. Intraabdominal fat is related to metabolic risk factors in Hispanic Americans, African Americans and in girls. *Acta Paediatr* 2009; 98(12): 1965–71.
- 41 Heshmat R, Qorbani M, Shahr Babaki AE, Djalalinia S, Ataei-Jafari A, Motlagh ME et al. Joint Association of Screen Time and Physical Activity with Cardiometabolic Risk Factors in a National Sample of Iranian Adolescents: The CASPIANIII Study. *PLoS One* 2016; 11(5): e0154502.
- 42 Bakrania K, Edwardson CL, Bodicoat DH, Esliger DW, Gill JMR, Kazi A et al. Associations of mutually exclusive categories of physical activity and sedentary time with markers of cardiometabolic health in English adults: a cross-sectional analysis of the Health Survey for England. *BMC Public Health* 2016; 16: 25.
- 43 Kries R von, Beyerlein A, Muller MJ, Heinrich J, Landsberg B, Bolte G et al. Different age-specific incidence and remission rates in pre-school and primary school suggest need for targeted obesity prevention in childhood. *Int J Obes (Lond)* 2012; 36(4): 505–10.
- 44 Mackenzie K, Goyder E, Eves F. Acceptability and feasibility of a low-cost, theory-based and co-produced intervention to reduce workplace sitting time in desk-based university employees. *BMC Public Health* 2015; 15: 1294.
- 45 Lee YH, Song YW, Kim HS, Lee SY, Jeong HS, Suh S-H et al. The effects of an exercise program on anthropometric, metabolic, and cardiovascular parameters in obese children. *Korean Circ J* 2010; 40(4): 179–84.
- 46 Faria AN, Ribeiro Filho FF, Gouveia Ferreira SR, Zanella MT. Impact of visceral fat on blood pressure and insulin sensitivity in hypertensive obese women. *Obes Res* 2002; 10(12): 1203–6.
- 47 Matsuzawa Y, Shimomura I, Nakamura T, Keno Y, Kotani K, Tokunaga K. Pathophysiology and pathogenesis of visceral fat obesity. *Obes Res* 1995; 3 Suppl 2: 187S-194S.

- 48 Phillips GB, Jing T, Heymsfield SB. Relationships in men of sex hormones, insulin, adiposity, and risk factors for myocardial infarction. *Metabolism* 2003; 52(6): 784–90.

CHAPTER III

Definition of new cut-offs of BMI and waist circumference based on body composition and insulin resistance: differences between children, adolescents and adults

published in Obesity Science & Practice

ORIGINAL ARTICLE

Definition of new cut-offs of BMI and waist circumference based on body composition and insulin resistance: differences between children, adolescents and adults

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Received 11 April 2017; revised 7 June 2017; accepted 21 June 2017

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Summary

Objective

This study aims to determine associations between anthropometric traits, regional fat depots and insulin resistance in children, adolescents and adults to define new cut-offs of body mass index (BMI) or waist circumference (WC).

Design

Cross-sectional data were assessed in 433 children, adolescents and adults (aged: 6–60 years, BMI: 23.6 [21.0–27.7] kg m⁻²). Total adipose tissue (TAT), regional subcutaneous adipose tissue (SAT_{total}, SAT_{trunk}) and visceral adipose tissue (VAT) were determined by whole-body magnetic resonance imaging, fat mass by air-displacement plethysmography. Insulin resistance was evaluated by homeostasis model assessment of insulin resistance (HOMA-IR). Bivariate as well as partial correlations and regression analyses were used. Cut-off values of BMI and WC related to regional fat depots and HOMA-IR were analysed by receiver operating characteristics curve.

Results

In adults, TAT, SAT_{total} and SAT_{trunk} increased linearly with increasing BMI and WC, whereas they followed a cubic function in children and adolescents with a steep increase at BMI and WC ≥ 1 standard deviation score and VAT at WC ≥ 2 standard deviation score. Sex differences were apparent in adults with women having higher masses of TAT and SAT and men having higher VAT. Using established BMI or WC cut-offs, correspondent masses of TAT, SAT_{total}, SAT_{trunk} and VAT increased from childhood to adulthood. In all age groups, there were positive associations between BMI, WC, SAT_{trunk}, VAT and HOMA-IR. When compared with normative cut-offs of BMI or WC, HOMA-IR-derived cut-offs of regional fat depots were lower in all age groups.

Conclusions

Associations between BMI, WC and regional fat depots varied between children, adolescents, young and older adults. When compared with BMI-derived and WC-derived values, an insulin resistance-derived cut-off corresponded to lower masses of regional fat depots. Thus, established BMI and WC cut-offs are not appropriate to assess metabolic disturbances associated with obesity; therefore, new cut-offs of BMI and WC are needed for clinical practice.

Keywords: body composition, body fat distribution, insulin resistance, obesity.

Introduction

Body mass index (BMI) and waist circumference (WC) are used to assess obesity and abdominal obesity in children, adolescents and adults (1,2). In epidemiological studies, these anthropometric traits are positively associated with cardiometabolic traits (3–6). However, anthropometric traits have considerable limitations. BMI can neither differentiate between fat mass (FM) and fat-free mass nor between regional fat depots (7–9). In addition, WC does not allow to distinguish between abdominal subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) (10). In daily practice, health-related cut-offs of BMI and WC are established in adult population (2). In children and adolescents, BMI and WC ≥ 97 age-specific and sex-specific percentiles are used as cut-offs (11,12). The cut-off data had been extrapolated to mortality risk in overweight and obese adults at BMI of 25 and 30 kg m⁻² (13,14). These references are widely used in clinical and research practice. However, the associations between anthropometric traits and regional fat depots as well as insulin resistance have not been systematically addressed and may vary between children and adolescents compared with adults. Therefore, we questioned the statistical approach based on mortality risk estimates obtained in epidemiological studies.

To go beyond simple anthropometric traits, detailed body composition as assessed by whole-body magnetic resonance imaging (MRI) or computed tomography is used (9). The aims of this study were to determine associations between simple anthropometric traits and regional fat depots in children, adolescents and adults and to compare masses of regional fat depots at either BMI or WC or insulin resistance-derived cut-offs in different age and sex groups.

Methods

Subjects

This investigation was a secondary analysis. The study sample included pooled data of 433 healthy Caucasian subjects who participated in different studies at the 'Reference Center for Body Composition' (Institute of Human Nutrition and Food Science at the University of Kiel, Germany) between 2005 and 2016 as described in detail elsewhere (15–18). Cross-sectional data of 151 children and adolescents (aged: 6–18 years; BMI: 21.0 [18.0–27.6]), 150 young adults (aged: 18–30 years; BMI: 23.5 [22.0–26.7]) and 132 adults (aged: 30–60 years; BMI: 24.4 [22.8–28.5]) were analysed. The recruitment was conducted by local advertisements and notice board

postings. Exclusion criteria were metallic implants, pregnancy, smoking, chronic or acute diseases and medication intake. Each participant underwent examinations of anthropometry and body composition at the Institute of Human Nutrition (University of Kiel, Germany). Whole-body MRI was performed at the Clinic for Diagnostic Radiology (University Medical Center Schleswig-Holstein, Kiel, Germany). Pubertal stage was self-assessed according to the definition of Marshall and Tanner (19). Breast and genital stages were used to categorize pubertal status into three groups (prepubertal: Tanner I; inrapubertal: Tanner II–III, postpubertal: Tanner \geq IV) (20). Because of a low number of prepubertal children ($n = 26$), prepubertal and inrapubertal subjects were grouped together. Before participation, written informed consent was received from each participant and, in state of minority, from its legal guardian. All studies were authorized by the ethical committee of the University Kiel and conducted according to the guidelines laid down in the 'Declaration of Helsinki'.

Anthropometric measurements

Body weight was determined to the nearest 0.01 kg using a Tanita scale coupled to the BOD POD® Body Composition Tracking System (Life Measurement Instruments, Concord, California, USA) with subjects wearing underwear. Height was assessed without shoes to the nearest 0.5 cm by using a stadiometer (SECA, Modell 220, Hamburg, Germany). BMI was calculated as body weight (kg) / body height (m)². WC was measured with the subjects standing in upright position midway between the lowest rib and the top of the iliac crest. At the end of a normal expiration, the measurement was performed with a non-elastic plastic tape positioned parallel to the floor. BMI and WC values were converted to standard deviation score (SDS) values in children and adolescents using the lambda-mu-sigma (LMS) method from Cole (21). The calculation was based on German reference data for children and adolescents (11,12). Cut-offs for the definition of obesity and abdominal obesity in children and adolescents were BMI ≥ 2 SDS and WC ≥ 2 SDS (22). In adults, obesity was defined as BMI ≥ 30 kg m⁻², whereas cut-offs for abdominal obesity were WC > 88 cm in women and WC > 102 cm in men (23).

Densitometry

Air-displacement plethysmography was performed by BOD POD® device. In children and adolescents, specific equations were used to assess FM as in detail described elsewhere (24). The equation by Siri *et al.* was used to calculate FM in adults (25).

Magnetic resonance imaging

Detailed body composition was performed by using whole-body MRI with a 1.5T scanner (Magnetom Vision or Magnetom Avanto, Siemens Medical Systems, Erlangen, Germany) as in detail described elsewhere (26,27). Briefly, participants were examined in a supine position with their arms extended above their heads. SAT_{arms} were defined from wrist to humerus heads. SAT_{legs} were defined from femoral heads to ankle. Between humerus and femoral heads, volume of SAT_{trunk} was assessed. VAT was defined from the top of the liver to femoral heads. In the present analysis, volumes of SAT and VAT were manually segmented by using segmentation software (SliceOmatic 4.3 and 5.0, Tomovision, Montreal, Canada). Tissue volumes were determined from the sum of all areas (cm²) multiplied by slice thickness. Masses of regional SAT and VAT were calculated as tissue volumes multiplied by density (0.923 g cm⁻³).

Homeostasis model assessment of insulin resistance

Blood samples were taken after an 8-h overnight fast and analysed by standard procedures. Plasma glucose was determined enzymatically using Konelab-Test-Kit (Thermo Clinical LabSystems, Frankfurt, Germany). Plasma insulin was measured by radioimmunoassay (Adaltis, Freiburg, Germany). Homeostasis model assessment of insulin resistance (HOMA-IR) was used to determine insulin resistance:

$$\text{HOMA-IR} = \frac{\text{plasma glucose (mmol L}^{-1}) \times \text{plasma insulin } (\mu\text{U ml}^{-1})}{22.5}$$
 (28). Because there is no recommended threshold for insulin resistance in children, adolescents and adults, a cut-off of 2.5 was used to separate participants into having normal or elevated HOMA-IR (29,30).

Statistical analysis

All statistical analyses were carried out with SPSS statistical software (SPSS 24.0, Inc., Chicago, Illinois, USA). Data were given as median and interquartile range because of not normally distributed data. Mann–Whitney *U* test and Kruskal–Wallis test with bonferroni correction were used to assess differences between groups. To examine the association between BMI and FM, bivariate correlations were performed. Age-adjusted and gender-adjusted partial correlations were used to determine the association between anthropometric traits, regional fat depots and insulin resistance in children, adolescents and adults. Further, regression analyses were carried

out to determine the associations between BMI, WC and regional fat depots in different age groups. Receiver operating characteristics curve analyses demonstrated the ability of regional fat depots to discriminate against elevated HOMA-IR. Further, Youden's index was used to obtain the optimal cut-off value for the detection of elevated HOMA-IR. Significance was set at $P < 0.05$ for all tests.

Results

The main characteristics of the study population are shown in Table 1. In both genders, BMI, WC, total adipose tissue (TAT), SAT_{trunk} and VAT were significantly higher in young adults when compared with children. When compared with male young adults, WC, FM, TAT and VAT were higher in male adults with no differences in women. In women, HOMA-IR was highest in young adults, whereas in men, HOMA-IR was highest in adolescents. In children, anthropometric traits and regional fat depots did not differ between men and women. By contrast, WC, FM, TAT, SAT_{total}, SAT_{trunk} and VAT differed significantly between female and male adults.

Association between body mass index and fat mass in children, adolescents and adults

Associations between BMI and FM in children, adolescents, young adults and adults are shown in Figure 1. These associations were strong in children and adolescents but moderate in young and older adults. The explained variance of FM by BMI was highest in children and decreased with increasing age. Sex differences in the BMI – FM – association became apparent in adolescents and were most obvious in adults. Sensitivity of BMI to predict FM was high in all age groups; by contrast, specificity decreased from children to adults.

Association between anthropometric traits and masses of regional fat depots

Associations between BMI, WC and regional fat depots in children, adolescents, young adults and adults are shown in Figure 2. In children and adolescents, associations between either BMI or WC and regional fat depots followed a cubic function. TAT, SAT_{total} and SAT_{trunk} were constant until about 1.0 SDS and started to increase beyond that number. VAT increased with $\text{WC} > 2$ SDS. In contrast to children and adolescents, the associations between either BMI or WC and regional fat depots were linear in young and older adults. TAT, SAT_{total}, SAT_{trunk} and VAT increased with increasing BMI or WC. Sex

Table 1 Characteristics of the study population stratified by age group and gender¹

	Children		Adolescents		Young adults		Adults	
	Female	Male	Female	Male	Female	Male	Female	Male
BMI (kg/m ²)	19.3 ² (16.5–25.5)	19.0 (15.7–24.9)	21.8 (19.8–29.6)	23.4 ³ (20.0–31.1)	23.9 ³ (21.7–31.8)	23.4 ³ (22.1–25.7)	24.3 ³ (21.7–27.7)	24.5 ³ (23.2–28.7)
WC (cm)	65.5 (57.5–83.8)	73.0 (58.0–87.5)	75.3 ³ (68.8–99.5)	78.3 (72.5–99.0)	81.0 ³ (74.0–96.8)	85.0 ³ (81.0–89.0)	83.0 ³ (77.5–94.0)	89.5 ^{3,4,5} (84.0–99.0)
FM (%)	22.6 (16.1–36.3)	20.1 (15.5–27.1)	25.9 (23.0–39.5)	20.2 (14.6–31.2)	32.0 ³ (27.9–43.4)	16.5 (13.2–22.9)	34.3 ³ (28.4–41.6)	22.1 ⁵ (16.5–27.9)
TAT (kg)	9.5 (7.1–19.3)	9.0 (4.7–18.5)	15.0 (11.9–29.1)	11.6 (8.4–25.0)	21.5 ³ (17.5–34.6)	12.9 ³ (10.8–18.6)	22.6 ³ (17.0–30.8)	18.2 ^{3,5} (14.0–24.5)
SAT _{total} (kg)	9.1 (6.9–18.8)	8.7 (4.6–17.9)	14.5 (11.5–27.9)	11.3 (8.1–23.2)	20.0 ³ (17.0–33.3)	11.8 (10.2–17.2)	21.4 ³ (16.1–28.3)	14.8 ³ (11.7–18.6)
SAT _{trunk} (kg)	2.9 (2.1–6.3)	2.6 (1.3–6.7)	4.7 (3.7–13.5)	3.8 (2.8–10.4)	8.6 ³ (6.2–14.3)	4.7 ³ (3.7–8.1)	9.1 ³ (6.3–13.3)	6.8 ³ (4.9–9.0)
VAT (kg)	0.36 (0.22–0.87)	0.29 (0.16–0.88)	0.47 (0.34–0.96)	0.64 (0.37–1.30)	1.1 ^{3,4} (0.6–1.7)	1.1 ³ (0.7–2.1)	1.4 ^{3,4} (0.7–2.3)	2.5 ^{3,4,5} (1.7–5.2)
HOMA-IR	1.9 (1.0–2.4)	1.8 (0.9–2.8)	2.5 (1.5–3.1)	2.3 (1.5–3.1)	2.7 ³ (1.6–4.3)	1.6 (1.2–2.1)	2.2 (1.5–2.8)	2.0 (1.4–3.3)

¹BMI, body mass index; FM, fat mass; HOMA-IR, homeostasis model assessment of insulin resistance; SAT, subcutaneous adipose tissue; TAT, total adipose tissue; VAT, visceral adipose tissue; WC, waist circumference.

²Median (IQR) (all such values).

³Median in bold, significantly different from women (Mann-Whitney U test, $P < 0.05$).

⁴Significantly different from children for either women or men;

⁵Significantly different from adolescents for either women or men;

⁶Significantly different from young adults for either women or men (Kruskal–Wallis test with Bonferroni correction, $P < 0.05$).

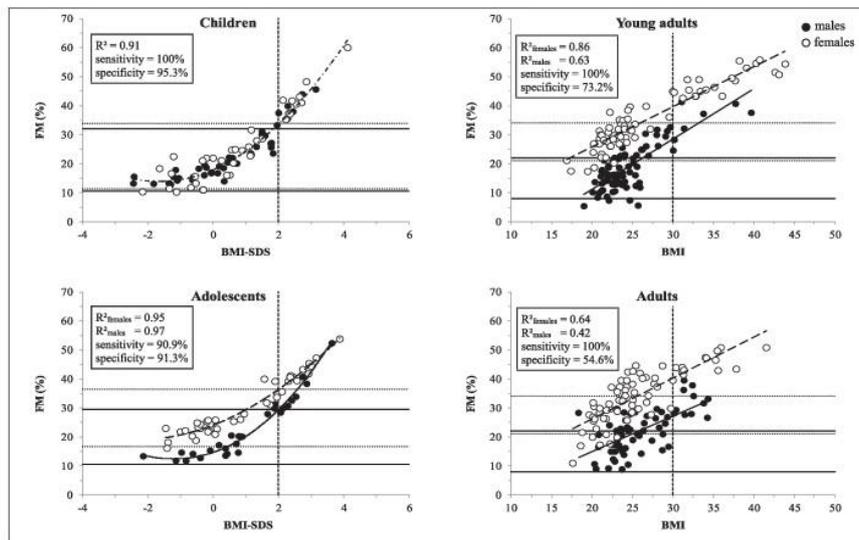


Figure 1 Association between BMI and fat mass (%) in children, adolescents, young adults and adults stratified by gender. Cut-offs of body mass index (BMI) and BMI standard deviation score (SDS) are presented as vertical dashed lines (11,12). Range of normal fat mass (%) is presented as horizontal dashed (females) and solid (males) lines (35,36). Abbreviation: FM, fat mass.

differences became apparent in adolescents and were most obvious in adults with women having higher masses of TAT, SAT_{total} and SAT_{trunk} and men having higher masses of VAT.

The explained variance of all regional fat depots by BMI-SDS and WC-SDS was high in children and adolescents. It decreased in young and older adults. When compared with men in young and older adults, the explained variance of TAT, SAT_{total} and SAT_{trunk} by BMI and WC was higher in women.

The cut-offs of 2.0 SDS in BMI and WC were appropriate to discriminate higher masses of regional fat depots in children and adolescents. In adults, WC cut-offs were less appropriate to discriminate higher masses of regional fat depots.

TAT, SAT_{total}, SAT_{trunk} and VAT at either elevated BMI or WC differed between age and sex groups (Table 2). The corresponding regional fat depots increased from childhood to adulthood. Further, in adolescents and adults, masses of TAT, SAT_{total} and SAT_{trunk} were higher in women with men having higher masses of VAT.

Association between anthropometric traits and masses of regional fat depots with homeostasis model assessment of insulin resistance

Associations between either anthropometric traits or regional fat depots and HOMA-IR in children, adolescents, young adults and adults are shown in Figure 3. In all age

groups, moderate and positive associations were found between either BMI or WC or SAT_{trunk} or VAT and insulin resistance. Accordingly, sex differences were minor. When compared with anthropometric traits and SAT_{trunk}, highest correlations were seen between VAT and insulin resistance with no differences between the different age groups.

Using a HOMA-IR of 2.5 as a cut-off, the corresponding masses of regional fat depots varied between the different age groups (Table 2). Comparing the corresponding masses of TAT, SAT_{total}, SAT_{trunk} and VAT derived from either normative anthropometric traits or HOMA-IR, cut-offs showed lower masses for the latter calculation. These cut-offs are demonstrated as horizontal lines in Figure 2. Each gender-dependent vertical arrow marked the corresponding BMI or WC value. An insulin resistance-derived classification led to lower BMI or WC cut-offs for elevated disease risk.

Discussion

In adults TAT, SAT_{total}, SAT_{trunk} and VAT increased linearly with increasing BMI and WC. When compared with adults, the associations followed a cubic function in children and adolescents. There was a sex difference: Women had higher masses of TAT and SAT with men having higher VAT. Using established BMI or WC cut-offs, TAT, SAT_{total}, SAT_{trunk} and VAT increased from childhood

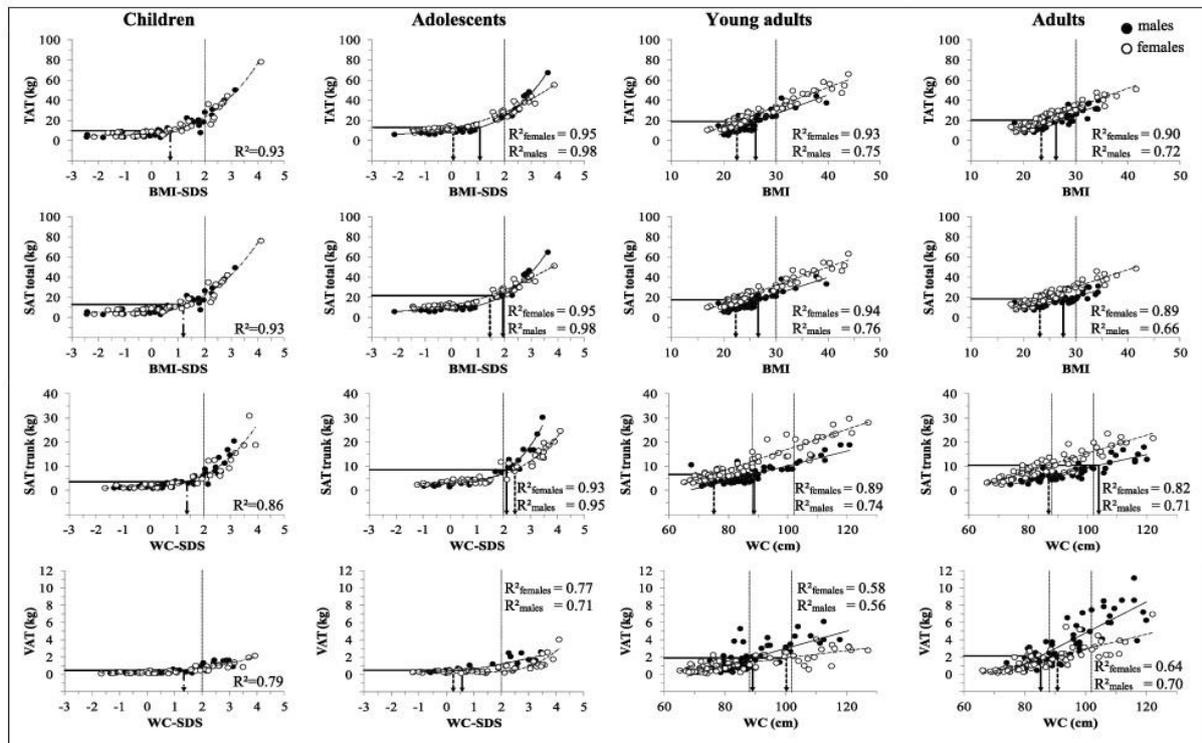


Figure 2 Association between anthropometric traits and regional fat depots in children, adolescents, young adults and adults stratified by gender. Cut-offs of body mass index standard deviation score (BMI-SDS), BMI, waist circumference (WC)-SDS and WC are presented as vertical dashed lines. Cut-offs based on receiver operating characteristics (ROC) curve analyses (Table 2) for predicting elevated homeostasis model assessment of insulin resistance (HOMA-IR) (>2.5) are presented as horizontal solid lines. Accordingly, dashed/dotted (total children), dashed (females) and solid (males) arrows marked the corresponding BMI-SDS, BMI, WC-SDS or WC values. Abbreviations: SAT, subcutaneous adipose tissue; TAT, total adipose tissue; VAT, visceral adipose tissue.

to adulthood. When compared with the normative cut-offs of BMI or WC, insulin resistance-derived cut-offs of regional fat depots were lower in all age groups. To our knowledge, the current study is first to systematically investigate associations between BMI

and WC vs. TAT, SAT_{total}, SAT_{trunk} and VAT by age group and gender as well as to compare masses of regional fat depots at either BMI or WC or insulin resistance derived cut-offs in a greater population of subjects aged 6 to 60 years.

Table 2 Cut-offs of regional fat depots on elevated HOMA, BMI and WC in children, adolescents, young adults and adults¹

	Children ²		Adolescents ²		Young adults ²		Adults ²	
	HOMA-IR	BMI or WC	HOMA-IR	BMI or WC	HOMA-IR	BMI or WC	HOMA-IR	BMI or WC
TAT (kg)	9.8	23.3/23.1 ³	12.9	23.9/27.9	19.0	26.4/33.4	20.3	27.0/32.9
SAT total (kg)	12.8	22.3/22.2	21.6	22.7/27.1	17.7	23.4/31.8	18.4	21.4/30.3
SAT trunk (kg)	3.6	5.7/6.1	8.5	7.7/6.7	6.7	10.8/12.0	10.5	10.0/10.9
VAT (kg)	0.5	0.8/0.6	0.5	1.2/0.3	1.9	3.2/1.4	2.1	5.1/1.9

¹BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin resistance; SAT, subcutaneous adipose tissue; SDS, standard deviation score; TAT, total adipose tissue; VAT, visceral adipose tissue; WC, waist circumference.

²Masses of regional fat depots based on elevated HOMA-IR, BMI (in children and adolescents BMI-SDS) or WC (in children and adolescents WC-SDS).

³Male regional fat mass/female regional fat mass (all such values).

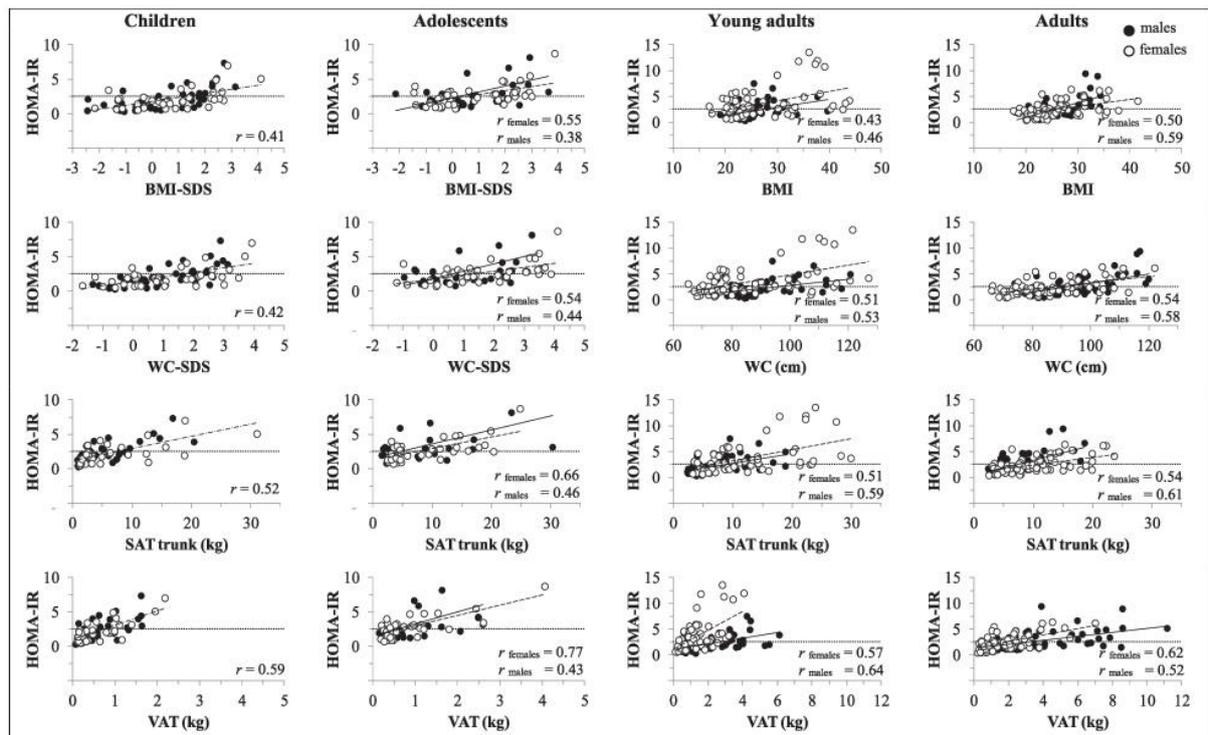


Figure 3 Association between anthropometric traits, regional fat depots and homeostasis model assessment of insulin resistance (HOMA-IR) in children, adolescents, young adults and adults stratified by gender. Cut-off of elevated HOMA-IR (>2.5), which is used in receiver operating characteristics curve analyses, is presented as horizontal dashed lines. All correlations were significant at $P < 0.05$. Abbreviations: SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; WC, waist circumference.

In children and adolescents, FM, TAT, SAT and VAT followed a cubic function with a steep increase at BMI and $WC \geq 1$ SDS (Figure 2). This is contrary to previous studies showing linear relationships between anthropometric traits and regional fat depots in adolescents (10,31,32) whereas VAT increased exponentially with increasing BMI Z-Score or WC (33). The latter data are in line with our results. However, to our knowledge, the cubic association between anthropometric traits and either TAT or SAT_{total} or SAT_{trunk} or VAT has not been previously shown in children and adolescents. Our results indicated that in children and adolescents, masses of regional fat depots started to increase beyond a BMI and $WC \geq 1$ SDS only. Contrary to children and adolescents, in young and older adults, masses of regional fat depots increased linearly with increasing anthropometric traits with women having higher masses of TAT, SAT_{total} and SAT_{trunk} and men having higher masses of VAT. These data confirm with previous findings in adults (9,18,34,35).

The corresponding masses of TAT, SAT_{total}, SAT_{trunk} and VAT using BMI and WC cut-offs differed between age and sex groups and increased from childhood to

adulthood (Table 2). These findings indicated that BMI and WC cut-offs inadequately describe masses of regional fat depots in different age and sex groups. In addition, insulin resistance-derived cut-offs of regional fat depots varied between age groups and also increased from childhood to adulthood. Comparing cut-offs of masses of individual fat depots derived from anthropometric traits and HOMA-IR gave considerably lower values for the latter calculation (Figure 2, Table 2). The data showed only moderate associations between measures of body fat and insulin resistance. Our findings suggested that insulin resistance (as reflected by biomarkers, i.e. HOMA-IR) is not primarily determined by masses of fat depots. In line with our results, previous cross-sectional data also showed only moderate associations between regional fat depots and insulin resistance in children, adolescents and adults (10,36,37). When compared with BMI, recent studies have shown that FM adjusted for height improved the prediction of insulin resistance in adults with no improvements in children and adolescents (38–42). However, insulin resistance results from a multidimensional dynamic process, which

cannot be addressed in a cross-sectional study. Therefore, longitudinal data from birth to adulthood would be conducive. In addition, other determinants of insulin resistance are important to be considered: Adipose tissue remodelling is altered by hyperplasia and or hypertrophy (43). Studies have shown that hypertrophy led to increased secretion of pro-inflammatory cytokines, which were supposed to result in the development of insulin resistance (44–46). Accordingly, most data on hypertrophy and its association to insulin resistance in humans were obtained in obese subjects undergoing bariatric surgery (47,48). A final argument is that HOMA-IR most probably reflects hepatic insulin resistance (49). By contrast, the euglycemic clamp technique, which is considered as the present gold standard in the assessment of insulin sensitivity, measures the stimulation of peripheral, i.e. skeletal muscle glucose uptake (50). Based on our data, we found a correlation of $r = -0.67$ between euglycemic clamp technique and HOMA-IR (51,52). Therefore, the association between FM and insulin sensitivity also depends on the methods used to assess insulin resistance.

To our knowledge, this is the first study to systematically investigate associations between anthropometric traits and regional fat depots in children, adolescents and adults and to compare masses of regional fat depots at either BMI or WC or insulin resistance-derived cut-offs. Regional fat depots were measured by whole-body MRI. This is the gold-standard method of body composition. In addition, metabolically derived cut-offs were chosen to compare the corresponding masses of regional fat depots in different age groups. A limitation of this study is that we have analysed prepubertal and intrapubertal subjects together because of the small number of prepubertal children. Thus, no further differentiation can be performed in this age group. In addition, the associations of HOMA-IR by anthropometric traits and regional fat depots were only moderate. Additional data on muscle mass and ectopic lipids in muscle and liver may add to improve these associations.

Associations between simple anthropometric traits and regional fat depots differed between children and adolescents vs. young and older adults. When compared with the established BMI or WC cut-offs, corresponding masses of regional fat depots varied between age groups and gender. There were positive associations between anthropometric traits and regional fat depots with HOMA-IR. Insulin resistance-derived cut-offs of regional fat depots were lower than the corresponding values based on normative BMI and WC cut-offs. This knowledge may contribute to a better evaluation of nutritional status based on health outcomes instead of normative cut-offs. Our results indicated that using established BMI or WC cut-offs in children, adolescents and adults

partly fails to detect subjects with an elevated risk of insulin resistance. Thus, there is need for new cut-offs of BMI and WC for clinical practice. Because obesity is defined as an excessive fat accumulation, which impairs health (53), thus, in future, BMI and WC should be replaced by measures of body fat and regional fat depots taking into account their associations with metabolic disturbances.

Conflict of Interest Statement

The authors declared no conflict of interest.

Acknowledgements

We thank Britta Jux and the Clinic for Diagnostic Radiology, University Medical Center Schleswig-Holstein, Kiel (Germany), for the help with MRI scanning. We acknowledge financial support by Land Schleswig-Holstein within the funding programme Open Access Publikationsfonds.

Funding

Our own research data presented here have been funded by generous grants from the Federal Ministry of Education and Research (BMBF 01EA1336, 01GI1121A, 01GI1125, 01GI0821), the German Research Foundation (DFG Mü 714/8-3; DFG Bo 3296/1-1) and OMRON, Kyoto, Japan.

References

1. Wabitsch M, Kunze D. (Responsible For Aga) Konsensbasierte (S2) Leitlinie Zur Diagnostik, Therapie Und Prävention Von Übergewicht Und Adipositas Im Kindes- Und Jugendalter (Consensus-based (S2) guideline for the diagnosis, therapy and prevention of overweight and obesity in childhood and adolescence).
2. WHO. *Obesity: Preventing and Managing the Global Epidemic Report of a WHO Consultation*. World Health Organization: Geneva, 2000.
3. Janssen I, Katzmarzyk PT, Srinivasan SR, et al. Utility of childhood BMI in the prediction of adulthood disease: comparison of national and international references. *Obes Res* 2005; **13**: 1106–1115.
4. Freedman DS, Mei Z, Srinivasan SR, Berenson GS, Dietz WH. Cardiovascular risk factors and excess adiposity among overweight children and adolescents: the Bogalusa heart study. *J Pediatr* 2007; **150**: 12–17.E2.
5. Marcus MD, Foster GD, El Ghormli L, et al. Shifts in BMI category and associated cardiometabolic risk: prospective results from healthy study. *Pediatrics* 2012; **129**: E983–E991.
6. Freedman DS, Serdula MK, Srinivasan SR, Berenson GS. Relation of circumferences and skinfold thicknesses to lipid and insulin concentrations in children and adolescents: the Bogalusa heart study. *Am J Clin Nutr* 1999; **69**: 308–317.
7. Wells JCK. *The Evolutionary Biology of Human Body Fatness: Thrift and Control*, 1st edn. Cambridge University Press: Cambridge, 2010.

8. Prentice AM, Jebb SA. Beyond body mass index. *Obes Rev* 2001; **2**: 141–147.
9. Müller MJ, Braun W, Enderle J, Bosity-Westphal A. Beyond BMI: conceptual issues related to overweight and obese patients. *Obes Facts* 2016; **9**: 193–205.
10. Eloi JC, Epifanio M, De Goncalves MM, et al. Quantification of abdominal fat in obese and healthy adolescents using 3 tesla magnetic resonance imaging and free software for image analysis. *PLoS One* 2017; **12**: E0167625.
11. Kromeyer-Hauschild K, Wabitsch M, Kunze D, et al. Perzentile Für Den Body-Mass-Index Für Das Kindes- Und Jugendalter Unter Heranziehung Verschiedener Deutscher Stichproben (Percentiles of body mass index in children and adolescents evaluated from different regional German studies). *Monatsschrift Kinderheilkunde* 2001; **149**: 807–818.
12. Kromeyer-Hauschild K, Gläßer N, Zellner K. Perzentile Für Den Taillenumfang Von Jenaer Kindern Im Alter Von 6 Bis 18 Jahren (Percentiles of waist circumference of Jena children aged 6 to 18 years). *Akt Ernähr Med* 2008; **33**: 116–122.
13. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. *Bmj* 2000; **320**: 1240–1243.
14. Cole TJ, Flegal KM, Nicholls D, Jackson AA. Body mass index cut offs to define thinness in children and adolescents: international survey. *Bmj* 2007; **335**: 194.
15. Hübers M, Geisler C, Plachta-Danielzik S, Müller MJ. Association between individual fat depots and cardio-metabolic traits in normal- and overweight children, adolescents and adults. *Nutr Diabetes* 2017; **7**: E267.
16. Later W, Bosity-Westphal A, Kossel E, Gluer C-C, Heller M, Müller MJ. Is the 1975 reference man still a suitable reference? *Eur J Clin Nutr* 2010; **64**: 1035–1042.
17. Müller MJ, Enderle J, Pourhassan M, et al. Metabolic adaptation to caloric restriction and subsequent refeeding: the Minnesota starvation experiment revisited. *Am J Clin Nutr* 2015; **102**: 807–819.
18. Schautz B, Later W, Heller M, Peters A, Müller MJ, Bosity-Westphal A. Impact of age on leptin and adiponectin independent of adiposity. *Br J Nutr* 2012; **108**: 363–370.
19. Duke PM, Litt IF, Gross RT. Adolescents' self-assessment of sexual maturation. *Pediatrics* 1980; **66**: 918–920.
20. Reinehr T, Wolters B, Knop C, Lass N, Holl RW. Strong effect of pubertal status on metabolic health in obese children: a longitudinal study. *J Clin Endocrinol Metab* 2015; **100**: 301–308.
21. Cole TJ, Green PJ. Smoothing reference centile curves: the LMS method and penalized likelihood. *Stat Med* 1992; **11**: 1305–1319.
22. Neuhauser H. *Referenzperzentile Für Anthropometrische Maßzahlen Und Blutdruck Aus Der Studie Zur Gesundheit Von Kindern Und Jugendlichen In Deutschland (Kiggs) 2003-2006 (Reference Percentiles for Anthropometric Measures and Blood Pressure from the Health Study of Children and Adolescents in Germany (Kiggs) 2003–2006)*: Berlin, 2011.
23. Waist Circumference and Waist-Hip Ratio: Report of a Who Expert Consultation, Geneva, 8–11 December 2008. Geneva: World Health Organization; 2011.
24. Bosity-Westphal A, Danielzik S, Becker C, et al. Need for optimal body composition data analysis using air-displacement plethysmography in children and adolescents. *J Nutr* 2005; **135**: 2257–2262.
25. Siri WE. Body composition from fluid spaces and density: analysis of methods. *Nutrition* 1993 1961; **9**: 480–491. Discussion 480, 492.
26. Bosity-Westphal A, Booke C-A, Blocker T, et al. Measurement site for waist circumference affects its accuracy as an index of visceral and abdominal subcutaneous fat in a Caucasian population. *J Nutr* 2010; **140**: 954–961.
27. Schautz B, Later W, Heller M, Müller MJ, Bosity-Westphal A. Total and regional relationship between lean and fat mass with increasing adiposity – impact for the diagnosis of sarcopenic obesity. *Eur J Clin Nutr* 2012; **66**: 1356–1361.
28. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; **28**: 412–419.
29. de Andrade MIS, Oliveira JS, Leal VS, et al. Identificacao Dos Pontos De Corte Do Indice Homeostatic Model Assessment For Insulin Resistance Em Adolescentes: Revisao Sistemática. *Rev Paul Pediatr* 2016; **34**: 234–242.
30. Gayoso-Diz P, Otero-Gonzalez A, Rodriguez-Alvarez MX, et al. Insulin resistance (HOMA-IR) cut-off values and the metabolic syndrome in a general adult population: effect of gender and age: EPIRCE cross-sectional study. *Bmc Endocr Disord* 2013; **13**: 47.
31. Fox KR, Peters DM, Sharpe P, Bell M. Assessment of abdominal fat development in young adolescents using magnetic resonance imaging. *Int J Obes Relat Metab Disord* 2000; **24**: 1653–1659.
32. Springer F, Ehehalt S, Sommer J, et al. Predicting volumes of metabolically important whole-body adipose tissue compartments in overweight and obese adolescents by different MRI approaches and anthropometry. *Eur J Radiol* 2012; **81**: 1488–1494.
33. Koren D, Ci M, Kim C, et al. Anthropometric predictors of visceral adiposity in normal-weight and obese adolescents. *Pediatr Diabetes* 2013; **14**: 575–584.
34. Barreira TV, Staiano AE, Harrington DM, et al. Anthropometric correlates of total body fat, abdominal adiposity, and cardiovascular disease risk factors in a biracial sample of men and women. *Mayo Clin Proc* 2012; **87**: 452–460.
35. Pouliot MC, Despres JP, Lemieux S, et al. Waist circumference and abdominal sagittal diameter: best simple anthropometric indexes of abdominal visceral adipose tissue accumulation and related cardiovascular risk in men and women. *Am J Cardiol* 1994; **73**: 460–468.
36. Müller MJ, Lagerpusch M, Enderle J, Schautz B, Heller M, Bosity-Westphal A. Beyond the body mass index: tracking body composition in the pathogenesis of obesity and the metabolic syndrome. *Obes Rev* 2012; **13**: 6–13.
37. Kim JA, Park HS. Association of abdominal fat distribution and cardiometabolic risk factors among obese Korean adolescents. *Diabetes Metab* 2008; **34**: 126–130.
38. Kim JY, Oh S, Chang MR, et al. Comparability and utility of body composition measurement vs. anthropometric measurement for assessing obesity related health risks in Korean men. *Int J Clin Pract* 2013; **67**: 73–80.
39. Liu P, Ma F, Lou H, Liu Y. The utility of fat mass index vs. body mass index and percentage of body fat in the screening of metabolic syndrome. *Bmc Public Health* 2013; **13**: 629.
40. Pourshahidi LK, Wallace JMW, Mulhem MS, et al. Indices of adiposity as predictors of cardiometabolic risk and inflammation in young adults. *J Hum Nutr Diet* 2016; **29**: 26–37.
41. Qi Q, Hua S, Perreira KM, et al. Sex differences in associations of adiposity measures and insulin resistance in US Hispanic/Latino youth: the Hispanic community children's health study/study of Latino youth (SOL Youth). *J Clin Endocrinol Metab* 2017; **102**: 185–194.

42. Weber DR, Leonard MB, Shults J, Zemel BS. A comparison of fat and lean body mass index to BMI for the identification of metabolic syndrome in children and adolescents. *J Clin Endocrinol Metab* 2014; **99**: 3208–3216.
43. Choe SS, Huh JY, Hwang UJ, Kim JI, Kim JB. Adipose tissue remodeling: its role in energy metabolism and metabolic disorders. *Front Endocrinol (Lausanne)* 2016; **7**: 30.
44. Yuan M, Konstantopoulos N, Lee J, et al. Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of IKKbeta. *Science* 2001; **293**: 1673–1677.
45. Hirosumi J, Tuncman G, Chang L, et al. A central role for JNK in obesity and insulin resistance. *Nature* 2002; **420**: 333–336.
46. Jernås M, Palming J, Sjöholm K, et al. Separation of human adipocytes by size: hypertrophic fat cells display distinct gene expression. *Faseb J* 2006; **20**: 1540–1542.
47. Andersson DP, Eriksson Hogling D, Thorell A, et al. Changes in subcutaneous fat cell volume and insulin sensitivity after weight loss. *Diabetes Care* 2014; **37**: 1831–1836.
48. Eriksson-Hogling D, Andersson DP, Bäckdahl J, et al. Adipose tissue morphology predicts improved insulin sensitivity following moderate or pronounced weight loss. *Int J Obes (Lond)* 2015; **39**: 893–898.
49. Moura FA, Carvalho LSF, Cintra RMR, et al. Validation of surrogate indexes of insulin sensitivity in acute phase of myocardial infarction based on euglycemic-hyperinsulinemic clamp. *Am J Physiol Endocrinol Metab* 2014; **306**: E399–E403.
50. DeFronzo RA, Ferrannini E, Hendler R, Felig P, Wahren J. Regulation of splanchnic and peripheral glucose uptake by insulin and hyperglycemia in man. *Diabetes* 1983; **32**: 35–45.
51. Lagerpusch M, Enderle J, Eggeling B, et al. Carbohydrate quality and quantity affect glucose and lipid metabolism during weight regain in healthy men. *J Nutr* 2013; **143**: 1593–1601.
52. Bovy-Westphal A, Kossel E, Goele K, et al. Contribution of individual organ mass loss to weight loss-associated decline in resting energy expenditure. *Am J Clin Nutr* 2009; **90**: 993–1001.
53. World Health Organization. (2016) Obesity and overweight: fact sheet updated June 2016. Available From: [Http://www.who.int/mediacentre/factsheets/fs311/en/](http://www.who.int/mediacentre/factsheets/fs311/en/)

Abstract***Objective***

This study aims to determine associations between anthropometric traits, regional fat depots and insulin resistance in children, adolescents and adults to define new cut-offs of body mass index (BMI) or waist circumference (WC).

Design

Cross-sectional data were assessed in 433 children, adolescents and adults [aged: 6-60 years, BMI: 23.6 (21.0-27.7) kg/m²]. Total adipose tissue (TAT), regional subcutaneous adipose tissue (SAT_{total}, SAT_{trunk}) and visceral adipose tissue (VAT) were determined by whole-body magnetic resonance imaging, fat mass (FM) by air displacement plethysmography. Insulin resistance was evaluated by homeostasis model assessment of insulin resistance (HOMA-IR). Bivariate as well as partial correlations and regression analyses were used. Cut-off values of BMI and WC related to regional fat depots and HOMA-IR were analysed by receiver operating characteristics curve.

Results

In adults, TAT, SAT_{total} and SAT_{trunk} increased linearly with increasing BMI and WC, whereas they followed a cubic function in children and adolescents with a steep increase at BMI and WC ≥ 1 standard deviation score, and VAT at WC ≥ 2 standard deviation score. Sex differences were apparent in adults with women having higher masses of TAT and SAT and men having higher VAT. Using established BMI or WC cut-offs, correspondent masses of TAT, SAT_{total}, SAT_{trunk} and VAT increased from childhood to adulthood. In all age groups, there were positive associations between BMI, WC, SAT_{trunk}, VAT and HOMA-IR. When compared with normative cut-offs of BMI or WC, HOMA-IR-derived cut-offs of regional fat depots were lower in all age groups.

Conclusions

Associations between BMI, WC and regional fat depots varied between children, adolescents, young and older adults. When compared with BMI-derived and WC-derived values, an insulin resistance-derived cut-off corresponded to lower masses of regional fat depots. Thus, established BMI and WC cut-offs are not appropriate to assess metabolic disturbances associated with obesity; therefore, new cut-offs of BMI and WC are needed for clinical practice.

Introduction

Body mass index (BMI) and waist circumference (WC) are used to assess obesity and abdominal obesity in children, adolescents and adults (1,2). In epidemiological studies these anthropometric traits are positively associated with cardiometabolic traits (3–6). However, anthropometric traits have considerable limitations. BMI can neither differentiate between fat mass (FM) and fat-free mass (FFM) nor between regional fat depots (7–9). In addition, WC does not allow to distinguish between abdominal subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) (10). In daily practice, health related cut-offs of BMI and WC are established in adult population (2). In children and adolescents BMI and WC \geq 97 age-specific and sex-specific percentiles are used as cut-offs (11,12). The cut-off data had been extrapolated to mortality risk in overweight and obese adults at BMI of 25 and 30 kg/m² (13,14). These references are widely used in clinical and research practice. However, the associations between anthropometric traits and regional fat depots as well as insulin resistance have not been systematically addressed and may vary between children and adolescents compared with adults. Therefore, we questioned the statistical approach based on mortality risk estimates obtained in epidemiological studies.

To go beyond simple anthropometric traits, detailed body composition as assessed by whole-body magnetic resonance imaging (MRI) or computed tomography is used (9). The aims of this study were to determine associations between simple anthropometric traits and regional fat depots in children, adolescents and adults and to compare masses of regional fat depots at either BMI or WC or insulin resistance-derived cut-offs in different age and sex-groups.

Methods

Subjects

This investigation was a secondary analysis. The study sample included pooled data of 433 healthy Caucasian subjects who participated in different studies at the “Reference Center for Body Composition” (Institute of Human Nutrition and Food Science at the University of Kiel, Germany) between 2005 and 2016 as described in detail elsewhere (15–18). Cross-sectional data of 151 children and adolescents [aged: 6-18 years; BMI: 21.0 (18.0-27.6)], 150 young adults [aged: 18-30 years; BMI: 23.5 (22.0-26.7)] and 132 adults [aged: 30-60 years; BMI: 24.4 (22.8-28.5)] were analyzed. The recruitment was conducted by local advertisements and notice board postings. Exclusion criteria were metallic implants, pregnancy, smoking, chronic or acute diseases and medication intake. Each participant underwent examinations of anthropometry and

body composition at the Institute of Human Nutrition (University of Kiel, Germany). Whole-body MRI was performed at the Clinic for Diagnostic Radiology (University Medical Center Schleswig-Holstein, Kiel, Germany). Pubertal stage was self assessed according to the definition of Marshall and Tanner (19). Breast and genital stages were used to categorize pubertal status into three groups (prepubertal: Tanner I; intrapubertal: Tanner II-III, postpubertal: Tanner \geq IV) (20). Because of a low number of prepubertal children ($n = 26$), prepubertal and intrapubertal subjects were grouped together. Before participation, written informed consent was received from each participant and, in state of minority, from its legal guardian. All studies were authorized by the ethical committee of the University Kiel and conducted according to the guidelines laid down in the “Declaration of Helsinki”.

Anthropometric measurements

Body weight was determined to the nearest 0.01 kg using a Tanita scale coupled to the BOD POD[®] Body Composition Tracking System (Life Measurement Instruments, Concord, California, USA) with subjects wearing underwear. Height was assessed without shoes to the nearest 0.5 cm by using a stadiometer (SECA, Modell 220, Hamburg, Germany). BMI was calculated as body weight (kg) / body height (m)². WC was measured with the subjects standing in upright position midway between the lowest rib and the top of the iliac crest. At the end of a normal expiration the measurement was done with a nonelastic plastic tape positioned parallel to the floor. BMI- and WC-values were converted to standard deviation score (SDS) values in children and adolescents using the LMS method from Cole (21). The calculation was based on German reference data for children and adolescents (11,12). Cut-offs for the definition of obesity and abdominal obesity in children and adolescents were BMI \geq 2 SDS and WC \geq 2 SDS (22). In adults, obesity was defined as BMI \geq 30 kg/m², whereas cut-offs for abdominal obesity were WC > 88 cm in women and WC > 102 cm in men (23).

Densitometry

Air displacement plethysmography was performed by BOD POD[®] device. In children and adolescents, specific equations were used to assess fat mass (FM) as in detail described elsewhere (24). The equation by Siri et al. was used to calculate FM in adults (25).

Magnetic resonance imaging

Detailed body composition was performed by using whole-body MRI with a 1.5T scanner (Magnetom Vision or Magnetom Avanto, Siemens Medical Systems, Erlangen, Germany) as

in detail described elsewhere (26,27). Briefly, participants were examined in a supine position with their arms extended above their heads. SAT_{arms} were defined from wrist to humerus heads. SAT_{legs} were defined from femoral heads to ankle. Between humerus and femoral heads volume of SAT_{trunk} was assessed. VAT was defined from the top of the liver to femoral heads. In the present analysis volumes of SAT and VAT were manually segmented by using segmentation software (SliceOmatic 4.3 and 5.0, Tomovision, Montreal, Canada). Tissue volumes were determined from the sum of all areas (cm²) multiplied by slice thickness. Masses of regional SAT and VAT were calculated as tissue volumes multiplied by density (0.923 g/cm³).

Homeostasis model assessment of insulin resistance

Blood samples were taken after an 8-h overnight fast and analyzed by standard procedures. Plasma glucose was determined enzymatically using Konelab-Test-Kit (Thermo Clinical Labsystems, Frankfurt, Germany). Plasma insulin was measured by radioimmunoassay (Adaltis, Freiburg, Germany). Homeostasis model assessment of insulin resistance (HOMA-IR) was used to determine insulin resistance:

HOMA-IR = plasma glucose (mmol/L) x plasma insulin (μU/ml) / 22.5 (28). Because there is no recommended threshold for insulin resistance in children, adolescents and adults, a cut-off of 2.5 was used to separate participants into having normal or elevated HOMA-IR (29,30).

Statistical analysis

All statistical analyses were carried out with SPSS statistical software (SPSS 24.0, Inc., Chicago, Illinois, USA). Data were given as median and interquartil range because of not normally distributed data. Mann-Whitney *U* test and Kruskal-wallis test with bonferroni correction were used to assess differences between groups. To examine the association between BMI and FM bivariate correlations were performed. Age- and gender adjusted partial correlations were used to determine the association between anthropometric traits, regional fat depots and insulin resistance in children, adolescents and adults. Further, regression analyses were done to determine the associations between BMI, WC and regional fat depots in different age groups. Receiver operating characteristics (ROC) curve analyses demonstrated the ability of regional fat depots to discriminate against elevated HOMA-IR. Further, Youden's index was used to obtain the optimal cut-off value for the detection of elevated HOMA-IR. Significance was set at $P < 0.05$ for all tests.

Results

The main characteristics of the study population are shown in **Table 1**. In both genders, BMI, WC, total adipose tissue (TAT), SAT_{trunk} and VAT were significantly higher in young adults when compared with children. When compared to male young adults, WC, FM, TAT and VAT were higher in male adults with no differences in females. In females, HOMA-IR was highest in young adults, whereas in males, HOMA-IR was highest in adolescents. In children, anthropometric traits and regional fat depots did not differ between males and females. By contrast, WC, FM, TAT, SAT_{total}, SAT_{trunk} and VAT differed significantly between female and male adults.

	Children		Adolescents		Young adults		Adults	
	female	male	female	male	female	male	female	male
BMI (kg/m ²)	19.3 ² (16.5 – 25.5)	19.0 (15.7 – 24.9)	21.8 (19.8 – 29.6)	23.4 ³ (20.0 – 31.1)	23.9 ³ (21.7 – 31.8)	23.4 ³ (22.1 – 25.7)	24.3 ³ (21.7 – 27.7)	24.5 ³ (23.2 – 28.7)
WC (cm)	65.5 (57.5 – 83.8)	73.0 (58.0 – 87.5)	75.3 ³ (68.8 – 99.5)	78.3 (72.5 – 99.0)	81.0 ³ (74.0 – 96.8)	85.0 ³ (81.0 – 89.0)	83.0 ³ (77.5 – 94.0)	89.5 ^{3,4,5} (84.0 – 99.0)
FM (%)	22.6 (16.1 – 36.3)	20.1 (15.5 – 27.1)	25.9 (23.0 – 39.5)	20.2 (14.6 – 31.2)	32.0 ³ (27.9 – 43.4)	16.5 (13.2 – 22.9)	34.3 ³ (28.4 – 41.6)	22.1 ⁵ (16.5 – 27.9)
TAT (kg)	9.5 (7.1 – 19.3)	9.0 (4.7 – 18.5)	15.0 (11.9 – 29.1)	11.6 (8.4 – 25.0)	21.5 ³ (17.5 – 34.6)	12.9 ³ (10.8 – 18.6)	22.6 ³ (17.0 – 30.8)	18.2 ^{3,5} (14.0 – 24.5)
SAT _{total} (kg)	9.1 (6.9 – 18.8)	8.7 (4.6 – 17.9)	14.5 (11.5 – 27.9)	11.3 (8.1 – 23.2)	20.0 ³ (17.0 – 33.3)	11.8 (10.2 – 17.2)	21.4 ³ (16.1 – 28.3)	14.8 ³ (11.7 – 18.6)
SAT _{trunk} (kg)	2.9 (2.1 – 6.3)	2.6 (1.3 – 6.7)	4.7 (3.7 – 13.5)	3.8 (2.8 – 10.4)	8.6 ³ (6.2 – 14.3)	4.7 ³ (3.7 – 8.1)	9.1 ³ (6.3 – 13.3)	6.8 ³ (4.9 – 9.0)
VAT (kg)	0.36 (0.22 – 0.87)	0.29 (0.16 – 0.88)	0.47 (0.34 – 0.96)	0.64 (0.37 – 1.30)	1.1 ^{3,4} (0.6 – 1.7)	1.1 ³ (0.7 – 2.1)	1.4 ^{3,4} (0.7 – 2.3)	2.5 ^{3,4,5} (1.7 – 5.2)
HOMA-IR	1.9 (1.0 – 2.4)	1.8 (0.9 – 2.8)	2.5 (1.5 – 3.1)	2.3 (1.5 – 3.1)	2.7 ³ (1.6 – 4.3)	1.6 (1.2 – 2.1)	2.2 (1.5 – 2.8)	2.0 (1.4 – 3.3)

¹BMI, body mass index; FM, fat mass; HOMA-IR, homeostasis model assessment of insulin resistance; SAT, subcutaneous adipose tissue; TAT, total adipose tissue; VAT, visceral adipose tissue; WC, waist circumference.
²Median (IQR) (all such values).
 Median in bold, significantly different from female (Mann-Whitney *U* test, *P* < 0.05).
³significantly different from children for either females or males; ⁴significantly different from adolescents for either females or males; ⁵significantly different from young adults for either females or males (Kruskal-wallis test with bonferroni correction, *P* < 0.05).

Association between body mass index and fat mass in children, adolescents and adults

Associations between BMI and FM in children, adolescents, young adults and adults are shown in **Figure 1**. These associations were strong in children and adolescents but moderate in young and older adults. The explained variance of FM by BMI was highest in children and decreased with increasing age. Sex differences in the BMI - FM - association became apparent in adolescents and were most obvious in adults. Sensitivity of BMI to predict FM was high in all age groups; by contrast, specificity decreased from children to adults.

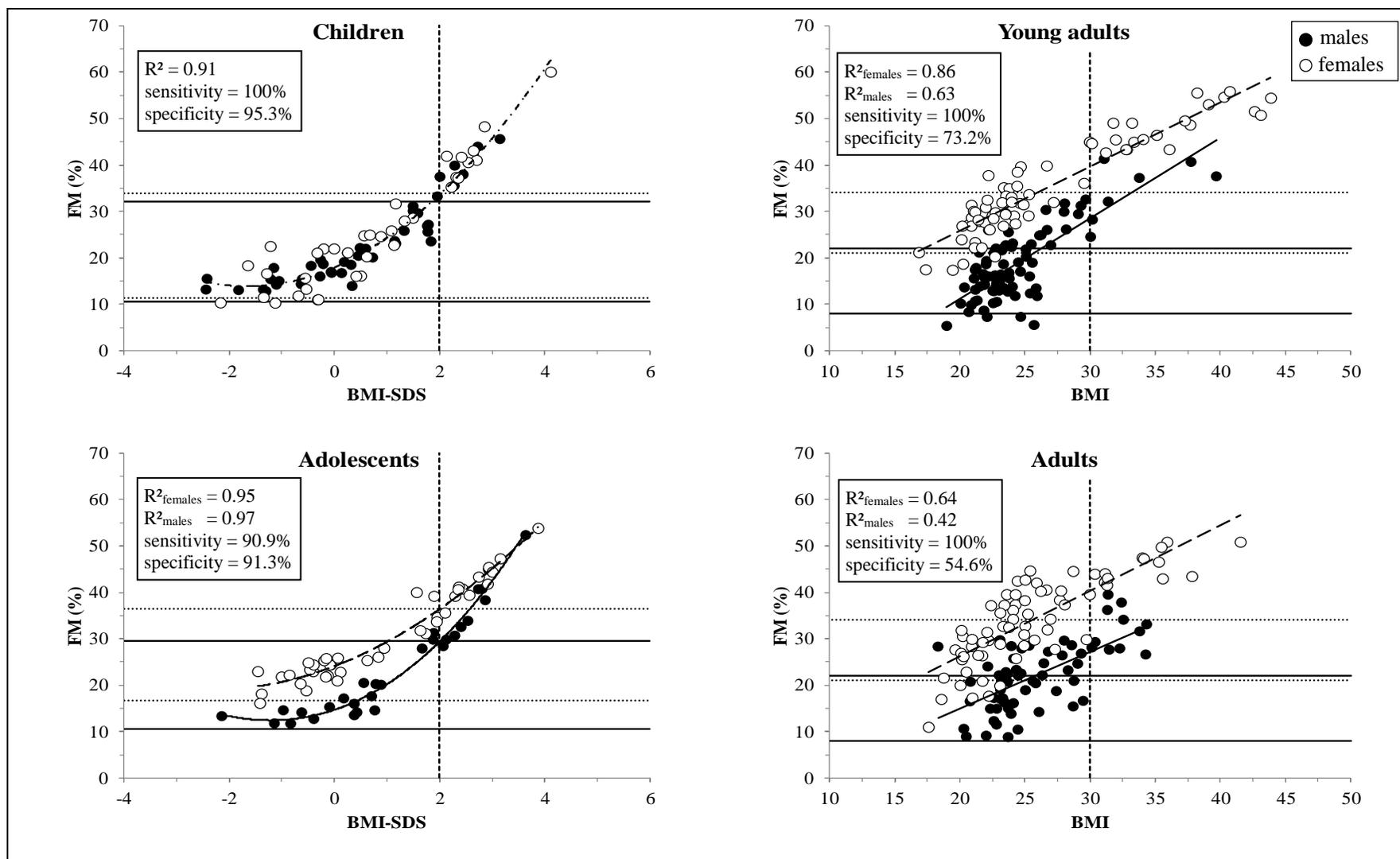


Figure 1. Association between body mass index (BMI) and fat mass (%) in children, adolescents, young adults and adults stratified by gender. Cut-offs of BMI and BMI standard deviation score (SDS) are presented as vertical dashed lines (11, 12). Range of normal fat mass (%) is presented as horizontal dashed (females) and solid (males) lines (35, 36). Abbreviation: FM, fat mass.

Association between anthropometric traits and masses of regional fat depots

Associations between BMI, WC and regional fat depots in children, adolescents, young adults and adults are shown in **Figure 2**. In children and adolescents, associations between either BMI or WC and regional fat depots followed a cubic function. TAT, SAT_{total} and SAT_{trunk} were constant until about 1.0 SDS and started to increase beyond that number. VAT increased with WC > 2 SDS. In contrast to children and adolescents, the associations between either BMI or WC and regional fat depots were linear in young and older adults. TAT, SAT_{total}, SAT_{trunk} and VAT increased with increasing BMI or WC. Sex differences became apparent in adolescents and were most obvious in adults with females having higher masses of TAT, SAT_{total} and SAT_{trunk} and males having higher masses of VAT.

The explained variance of all regional fat depots by BMI-SDS and WC-SDS was high in children and adolescents. It decreased in young and older adults. When compared to males in young and older adults, the explained variance of TAT, SAT_{total} and SAT_{trunk} by BMI and WC was higher in females.

The cut-offs of 2 SDS in BMI and WC were appropriate to discriminate higher masses of regional fat depots in children and adolescents. In adults, WC cut-offs were less appropriate to discriminate higher masses of regional fat depots.

TAT, SAT_{total}, SAT_{trunk} and VAT at either elevated BMI or WC differed between age and sex groups (**Table 2**). The corresponding regional fat depots increased from childhood to adulthood. Further, in adolescents and adults, masses of TAT, SAT_{total} and SAT_{trunk} were higher in females with males having higher masses of VAT.

Association between anthropometric traits and masses of regional fat depots with homeostasis model assessment of insulin resistance

Associations between either anthropometric traits or regional fat depots and HOMA-IR in children, adolescents, young adults and adults are shown in **Figure 3**. In all age groups moderate and positive associations were found between either BMI or WC or SAT_{trunk} or VAT and insulin resistance. Accordingly, sex differences were minor. When compared with anthropometric traits and SAT_{trunk}, highest correlations were seen between VAT and insulin resistance with no differences between the different age groups.

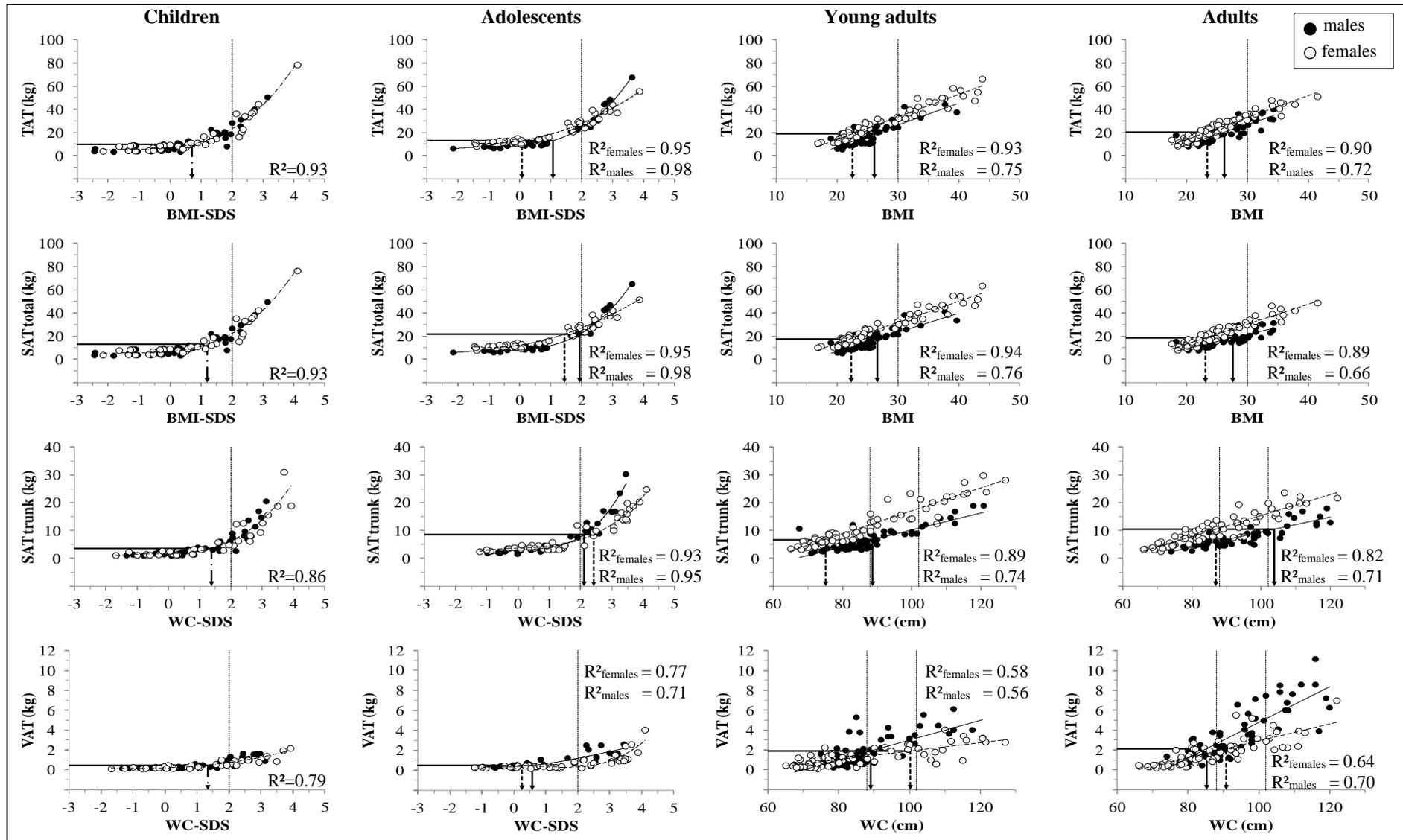


Figure 2. Association between anthropometric traits and regional fat depots in children, adolescents, young adults and adults stratified by gender. Cut-offs of body mass index standard deviation score (BMI-SDS), BMI, waist circumference (WC)-SDS and WC are presented as vertical dashed lines. Cut-offs based on receiver operating characteristics (ROC) curve analyses (Table 2) for predicting elevated HOMA-IR (>2.5) are presented as horizontal solid lines. Accordingly, dashed/dotted (total children), dashed (females) and solid (males) arrows marked the corresponding BMI-SDS, BMI, WC-SDS or WC values. Abbreviations: SAT, subcutaneous adipose tissue; TAT, total adipose tissue; VAT, visceral adipose tissue.

Table 2. Cut-offs of regional fat depots on elevated HOMA, BMI and WC in children, adolescents, young adults and adults¹

	Children ²		Adolescents ²		Young adults ²		Adults ²	
	HOMA-IR	BMI or WC	HOMA-IR	BMI or WC	HOMA-IR	BMI or WC	HOMA-IR	BMI or WC
TAT (kg)	9.8	23.3/23.1 ³	12.9	23.9/27.9	19.0	26.4/33.4	20.3	27.0/32.9
SAT total (kg)	12.8	22.3/22.2	21.6	22.7/27.1	17.7	23.4/31.8	18.4	21.4/30.3
SAT trunk (kg)	3.6	5.7/6.1	8.5	7.7/6.7	6.7	10.8/12.0	10.5	10.0/10.9
VAT (kg)	0.5	0.8/0.6	0.5	1.2/0.3	1.9	3.2/1.4	2.1	5.1/1.9

¹BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin resistance; SAT, subcutaneous adipose tissue; SDS, standard deviation score; TAT, total adipose tissue; VAT, visceral adipose tissue; WC, waist circumference.

²Masses of regional fat depots based on elevated HOMA-IR, BMI (in children and adolescents BMI-SDS) or WC (in children and adolescents WC-SDS).

³Male regional fat mass/female regional fat mass (all such values).

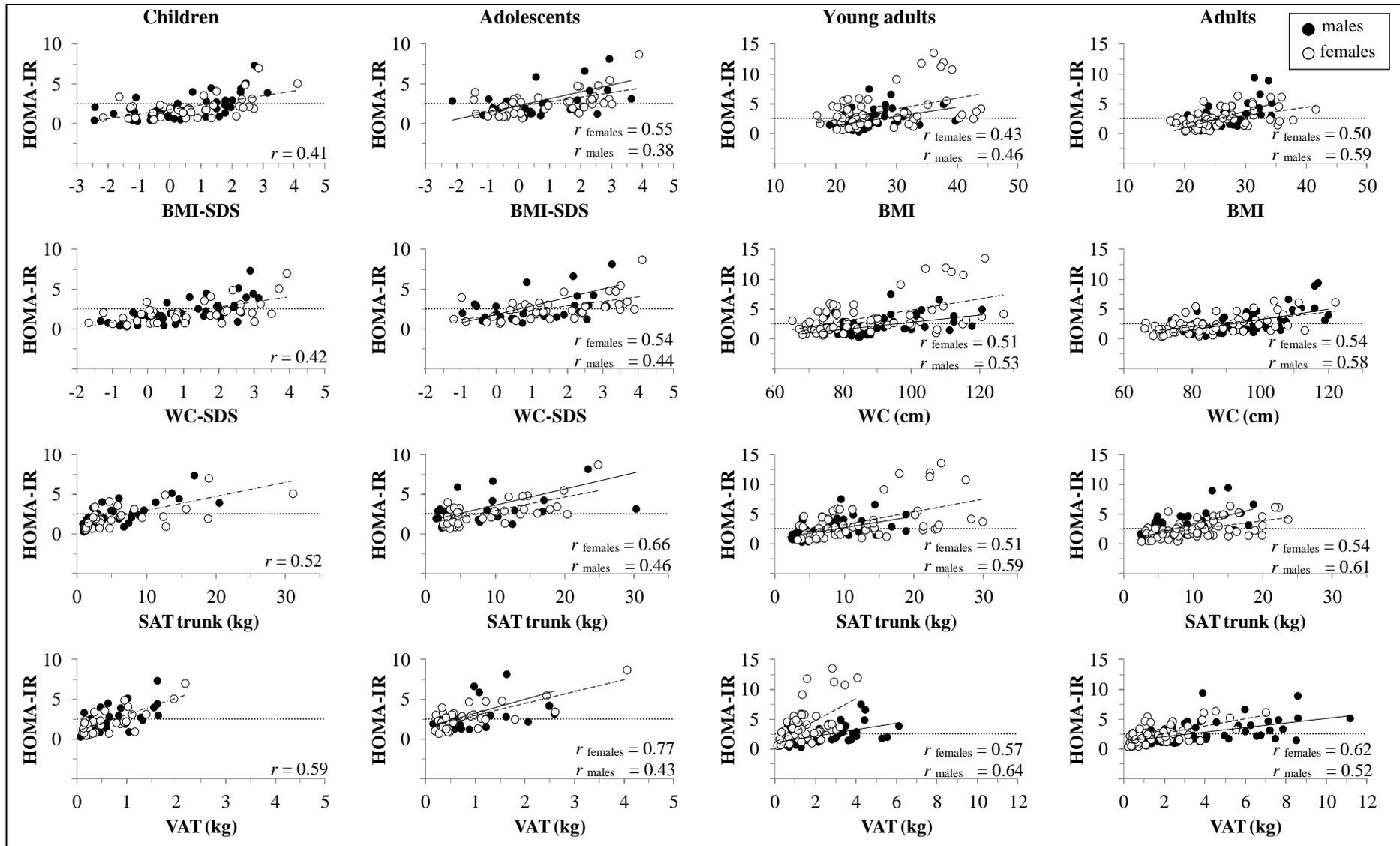


Figure 3. Association between anthropometric traits, regional fat depots and homeostasis model assessment of insulin resistance (HOMA-IR) in children, adolescents, young adults and adults stratified by gender. Cut-off of elevated HOMA-IR (>2.5), which is used in receiver operating characteristics curve analyses, is presented as horizontal dashed lines. All correlations were significant at $P < 0.05$. Abbreviations: SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; WC, waist circumference.

Using a HOMA-IR of 2.5 as a cut-off, the corresponding masses of regional fat depots varied between the different age groups (**Table 2**) Comparing the corresponding masses of TAT, SAT_{total}, SAT_{trunk} and VAT derived from either normative anthropometric traits or HOMA-IR, cut-offs showed lower masses for the latter calculation. These cut-offs are demonstrated as horizontal lines in **Figure 2**. Each gender-dependent vertical arrow marked the corresponding BMI or WC value. An insulin resistance derived classification led to lower BMI or WC cut-offs for elevated disease risk.

Discussion

In adults TAT, SAT_{total}, SAT_{trunk} and VAT increased linearly with increasing BMI and WC. When compared to adults, the associations followed a cubic function in children and adolescents. There was a sex-difference: Females had higher masses of TAT and SAT with males having higher VAT. Using established BMI or WC cut-offs, TAT, SAT_{total}, SAT_{trunk} and VAT increased from childhood to adulthood. When compared to the normative cut-offs of BMI or WC, insulin resistance derived cut-offs of regional fat depots were lower in all age groups. To our knowledge, the current study is first to systematically investigate associations between BMI and WC vs. TAT, SAT_{total}, SAT_{trunk} and VAT by age group and gender as well as to compare masses of regional fat depots at either BMI or WC or insulin resistance derived cut-offs in a greater population of subjects aged 6 to 60 years.

In children and adolescents FM, TAT, SAT and VAT followed a cubic function with a steep increase at BMI and WC ≥ 1 SDS (Figure 2). This is contrary to previous studies showing linear relationships between anthropometric traits and regional fat depots in adolescents (10,31,32) whereas VAT increased exponentially with increasing BMI Z-Score or WC (33). The latter data are in line with our results. However, to our knowledge the cubic association between anthropometric traits and either TAT or SAT_{total} or SAT_{trunk} or VAT has not been previously shown in children and adolescents. Our results indicated that in children and adolescents, masses of regional fat depots started to increase beyond a BMI and WC ≥ 1 SDS only. Contrary to children and adolescents, in young and older adults masses of regional fat depots increased linearly with increasing anthropometric traits with females having higher masses of TAT, SAT_{total} and SAT_{trunk} and males having higher masses of VAT. These data confirm with previous findings in adults (9,18,34,35).

The corresponding masses of TAT, SAT_{total}, SAT_{trunk} and VAT using BMI and WC cut-offs differed between age and sex groups and increased from childhood to adulthood (Table 2). These findings indicated that BMI and WC cut-offs inadequately describe masses of regional

fat depots in different age and sex groups. In addition, insulin resistance derived cut-offs of regional fat depots varied between age groups and also increased from childhood to adulthood. Comparing cut-offs of masses of individual fat depots derived from anthropometric traits and HOMA-IR gave considerably lower values for the latter calculation (Figure 2, Table 2). The data showed only moderate associations between measures of body fat and insulin resistance. Our findings suggested that insulin resistance (as reflected by biomarkers, i.e. HOMA-IR) is not primarily determined by masses of fat depots. In line with our results, previous cross-sectional data also showed only moderate associations between regional fat depots and insulin resistance in children, adolescents and adults (10,36,37). When compared to BMI, recent studies have shown that FM adjusted for height improved the prediction of insulin resistance in adults with no improvements in children and adolescents (38–42). However, insulin resistance results from a multidimensional dynamic process which cannot be addressed in a cross-sectional study. Therefore, longitudinal data from birth to adulthood would be conducive. In addition, other determinants of insulin resistance are important to be considered: Adipose tissue remodeling is altered by hyperplasia and or hypertrophy (43). Studies have shown that hypertrophy led to increased secretion of pro-inflammatory cytokines which were supposed to result in the development of insulin resistance (44–46). Accordingly, most data on hypertrophy and its association to insulin resistance in humans were obtained in obese subjects undergoing bariatric surgery (47,48). A final argument is that HOMA-IR most probably reflects hepatic insulin resistance (49). By contrast, the euglycemic clamp technique, which is considered as the present gold standard in the assessment of insulin sensitivity, measures the stimulation of peripheral, i.e. skeletal muscle glucose uptake (50). Based on our data we found a correlation of $r = -0.67$ between euglycemic clamp technique and HOMA-IR (51,52). Therefore, the association between fat mass and insulin sensitivity also depends on the methods used to assess insulin resistance.

To our knowledge, this is the first study to systematically investigate associations between anthropometric traits and regional fat depots in children, adolescents and adults and to compare masses of regional fat depots at either BMI or WC or insulin resistance derived cut-offs. Regional fat depots were measured by whole-body MRI. This is the gold-standard method of body composition. In addition, metabolically derived cut-offs were chosen to compare the corresponding masses of regional fat depots in different age groups. A limitation of this study is that we have analyzed pre- and intrapubertal subjects together due to the small number of prepubertal children. Thus, no further differentiation can be done in this age group. In addition, the associations of HOMA-IR by anthropometric traits and regional fat depots were only

moderate. Additional data on muscle mass and ectopic lipids in muscle and liver may add to improve these associations.

Associations between simple anthropometric traits and regional fat depots differed between children and adolescents vs. young and older adults. When compared with the established BMI or WC cut-offs corresponding masses of regional fat depots varied between age groups and gender. There were positive associations between anthropometric traits and regional fat depots with HOMA-IR. Insulin resistance derived cut-offs of regional fat depots were lower than the corresponding values based on normative BMI- and WC cut-offs. This knowledge may contribute to a better evaluation of nutritional status based on health outcomes instead of normative cut-offs. Our results indicated that using established BMI or WC cut-offs in children, adolescents and adults partly fails to detect subjects with an elevated risk of insulin resistance. Thus, there is need for new cut-offs of BMI and WC for clinical practice. Since obesity is defined as an excessive fat accumulation which impairs health (53). Thus, in future, BMI and WC should be replaced by measures of body fat and regional fat depots taking into account their associations with metabolic disturbances.

Conflict of Interest: The authors declare no conflict of interest.

Acknowledgements: We thank Britta Jux and the Clinic for Diagnostic Radiology, University Medical Center Schleswig-Holstein, Kiel (Germany) for the help with MRI scanning. We acknowledge financial support by Land Schleswig-Holstein within the funding programme Open Access Publikationsfonds.

Funding: Our own research data presented here have been funded by generous grants from the Federal Ministry of Education and Research (BMBF 01EA1336, 01GI1121A, 01GI1125, 01GI0821), the German Research Foundation (DFG Mü 714/8-3; DFG Bo 3296/1-1) and OMRON Kyoto, Japan.

References

1. Wabitsch M, Kunze D. (responsible for AGA) Konsensbasierte (S2) Leitlinie zur Diagnostik, Therapie und Prävention von Übergewicht und Adipositas im Kindes- und Jugendalter (Consensus-based (S2) guideline for the diagnosis, therapy and prevention of overweight and obesity in childhood and adolescence).

2. WHO. *Obesity: Preventing and managing the global epidemic report of a WHO consultation*. Geneva: World Health Organization; 2000.
3. Janssen I, Katzmarzyk PT, Srinivasan SR, et al. Utility of childhood BMI in the prediction of adulthood disease: comparison of national and international references. *Obes Res* 2005;13:1106–1115.
4. Freedman DS, Mei Z, Srinivasan SR, Berenson GS, Dietz WH. Cardiovascular risk factors and excess adiposity among overweight children and adolescents: the Bogalusa Heart Study. *J Pediatr* 2007;150:12-17.e2.
5. Marcus MD, Foster GD, El Ghormli L, et al. Shifts in BMI category and associated cardiometabolic risk: prospective results from HEALTHY study. *Pediatrics* 2012;129:e983-91.
6. Freedman DS, Serdula MK, Srinivasan SR, Berenson GS. Relation of circumferences and skinfold thicknesses to lipid and insulin concentrations in children and adolescents: the Bogalusa Heart Study. *Am J Clin Nutr* 1999;69:308–317.
7. Wells JCK. *The evolutionary biology of human body fatness: Thrift and control*. 1st ed. Cambridge: Cambridge University Press; 2010.
8. Prentice AM, Jebb SA. Beyond body mass index. *Obes Rev* 2001;2:141–147.
9. Müller MJ, Braun W, Enderle J, Bosy-Westphal A. Beyond BMI: Conceptual Issues Related to Overweight and Obese Patients. *Obes Facts* 2016;9:193–205.
10. Eloi JC, Epifanio M, Goncalves MM de, et al. Quantification of Abdominal Fat in Obese and Healthy Adolescents Using 3 Tesla Magnetic Resonance Imaging and Free Software for Image Analysis. *PLoS One* 2017;12:e0167625.
11. Kromeyer-Hauschild K, Wabitsch M, Kunze D, et al. Perzentile für den Body-mass-Index für das Kindes- und Jugendalter unter Heranziehung verschiedener deutscher Stichproben (Percentiles of body mass index in children and adolescents evaluated from different regional German studies). *Monatsschrift Kinderheilkunde* 2001;149:807–818.
12. Kromeyer-Hauschild K, Gläßer N, Zellner K. Perzentile für den Taillenumfang von Jenaer Kindern im Alter von 6 bis 18 Jahren (Percentiles of waist circumference of Jena children aged 6 to 18 years). *Akt Ernähr Med* 2008;33:116–122.
13. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. *BMJ* 2000;320:1240–1243.

14. Cole TJ, Flegal KM, Nicholls D, Jackson AA. Body mass index cut offs to define thinness in children and adolescents: international survey. *BMJ* 2007;335:194.
15. Hübers M, Geisler C, Plachta-Danielzik S, Müller MJ. Association between individual fat depots and cardio-metabolic traits in normal- and overweight children, adolescents and adults. *Nutr Diabetes* 2017;7:e267.
16. Later W, Bosy-Westphal A, Kossel E, Gluer C-C, Heller M, Müller MJ. Is the 1975 Reference Man still a suitable reference? *Eur J Clin Nutr* 2010;64:1035–1042.
17. Müller MJ, Enderle J, Pourhassan M, et al. Metabolic adaptation to caloric restriction and subsequent refeeding: the Minnesota Starvation Experiment revisited. *Am J Clin Nutr* 2015;102:807–819.
18. Schautz B, Later W, Heller M, Peters A, Müller MJ, Bosy-Westphal A. Impact of age on leptin and adiponectin independent of adiposity. *Br J Nutr* 2012;108:363–370.
19. Duke PM, Litt IF, Gross RT. Adolescents' self-assessment of sexual maturation. *Pediatrics* 1980;66:918–920.
20. Reinehr T, Wolters B, Knop C, Lass N, Holl RW. Strong effect of pubertal status on metabolic health in obese children: a longitudinal study. *J Clin Endocrinol Metab* 2015;100:301–308.
21. Cole TJ, Green PJ. Smoothing reference centile curves: the LMS method and penalized likelihood. *Stat Med* 1992;11:1305–1319.
22. Neuhauser H. *Referenzperzentile für anthropometrische Maßzahlen und Blutdruck aus der Studie zur Gesundheit von Kindern und Jugendlichen in Deutschland (KIGGS) 2003-2006 (Reference percentiles for anthropometric measures and blood pressure from the health study of children and adolescents in Germany (KIGGS) 2003-2006)*. Berlin; 2011.
23. *Waist circumference and waist-hip ratio: Report of a WHO expert consultation, Geneva, 8-11 December 2008*. Geneva: World Health Organization; 2011.
24. Bosy-Westphal A, Danielzik S, Becker C, et al. Need for optimal body composition data analysis using air-displacement plethysmography in children and adolescents. *J Nutr* 2005;135:2257–2262.
25. Siri WE. Body composition from fluid spaces and density: analysis of methods. 1961. *Nutrition* 1993;9:480-91; discussion 480, 492.

26. Bosy-Westphal A, Boone C-A, Blocker T, et al. Measurement site for waist circumference affects its accuracy as an index of visceral and abdominal subcutaneous fat in a Caucasian population. *J Nutr* 2010;140:954–961.
27. Schautz B, Later W, Heller M, Müller MJ, Bosy-Westphal A. Total and regional relationship between lean and fat mass with increasing adiposity--impact for the diagnosis of sarcopenic obesity. *Eur J Clin Nutr* 2012;66:1356–1361.
28. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28:412–419.
29. Andrade MISd, Oliveira JS, Leal VS, et al. Identificacao dos pontos de corte do indice Homeostatic Model Assessment for Insulin Resistance em adolescentes: revisao sistematica. *Rev Paul Pediatr* 2016;34:234–242.
30. Gayoso-Diz P, Otero-Gonzalez A, Rodriguez-Alvarez MX, et al. Insulin resistance (HOMA-IR) cut-off values and the metabolic syndrome in a general adult population: effect of gender and age: EPIRCE cross-sectional study. *BMC Endocr Disord* 2013;13:47.
31. Fox KR, Peters DM, Sharpe P, Bell M. Assessment of abdominal fat development in young adolescents using magnetic resonance imaging. *Int J Obes Relat Metab Disord* 2000;24:1653–1659.
32. Springer F, Eehalt S, Sommer J, et al. Predicting volumes of metabolically important whole-body adipose tissue compartments in overweight and obese adolescents by different MRI approaches and anthropometry. *Eur J Radiol* 2012;81:1488–1494.
33. Koren D, Marcus CL, Kim C, et al. Anthropometric predictors of visceral adiposity in normal-weight and obese adolescents. *Pediatr Diabetes* 2013;14:575–584.
34. Barreira TV, Staiano AE, Harrington DM, et al. Anthropometric correlates of total body fat, abdominal adiposity, and cardiovascular disease risk factors in a biracial sample of men and women. *Mayo Clin Proc* 2012;87:452–460.
35. Pouliot MC, Despres JP, Lemieux S, et al. Waist circumference and abdominal sagittal diameter: best simple anthropometric indexes of abdominal visceral adipose tissue accumulation and related cardiovascular risk in men and women. *Am J Cardiol* 1994;73:460–468.

36. Müller MJ, Lagerpusch M, Enderle J, Schautz B, Heller M, Bosy-Westphal A. Beyond the body mass index: tracking body composition in the pathogenesis of obesity and the metabolic syndrome. *Obes Rev* 2012;13 Suppl 2:6–13.
37. Kim JA, Park HS. Association of abdominal fat distribution and cardiometabolic risk factors among obese Korean adolescents. *Diabetes Metab* 2008;34:126–130.
38. Kim JY, Oh S, Chang MR, et al. Comparability and utility of body composition measurement vs. anthropometric measurement for assessing obesity related health risks in Korean men. *Int J Clin Pract* 2013;67:73–80.
39. Liu P, Ma F, Lou H, Liu Y. The utility of fat mass index vs. body mass index and percentage of body fat in the screening of metabolic syndrome. *BMC Public Health* 2013;13:629.
40. Pourshahidi LK, Wallace JMW, Mulhern MS, et al. Indices of adiposity as predictors of cardiometabolic risk and inflammation in young adults. *J Hum Nutr Diet* 2016;29:26–37.
41. Qi Q, Hua S, Perreira KM, et al. Sex Differences in Associations of Adiposity Measures and Insulin Resistance in US Hispanic/Latino Youth: The Hispanic Community Children's Health Study/Study of Latino Youth (SOL Youth). *J Clin Endocrinol Metab* 2017;102:185–194.
42. Weber DR, Leonard MB, Shults J, Zemel BS. A comparison of fat and lean body mass index to BMI for the identification of metabolic syndrome in children and adolescents. *J Clin Endocrinol Metab* 2014;99:3208–3216.
43. Choe SS, Huh JY, Hwang IJ, Kim JI, Kim JB. Adipose Tissue Remodeling: Its Role in Energy Metabolism and Metabolic Disorders. *Front Endocrinol (Lausanne)* 2016;7:30.
44. Yuan M, Konstantopoulos N, Lee J, et al. Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkbeta. *Science* 2001;293:1673–1677.
45. Hirosumi J, Tuncman G, Chang L, et al. A central role for JNK in obesity and insulin resistance. *Nature* 2002;420:333–336.
46. Jernås M, Palming J, Sjöholm K, et al. Separation of human adipocytes by size: hypertrophic fat cells display distinct gene expression. *FASEB J* 2006;20:1540–1542.
47. Andersson DP, Eriksson Hogling D, Thorell A, et al. Changes in subcutaneous fat cell volume and insulin sensitivity after weight loss. *Diabetes Care* 2014;37:1831–1836.

48. Eriksson-Hogling D, Andersson DP, Bäckdahl J, et al. Adipose tissue morphology predicts improved insulin sensitivity following moderate or pronounced weight loss. *Int J Obes (Lond)* 2015;39:893–898.
49. Moura FA, Carvalho LSF, Cintra RMR, et al. Validation of surrogate indexes of insulin sensitivity in acute phase of myocardial infarction based on euglycemic-hyperinsulinemic clamp. *Am J Physiol Endocrinol Metab* 2014;306:E399-403.
50. DeFronzo RA, Ferrannini E, Hendler R, Felig P, Wahren J. Regulation of splanchnic and peripheral glucose uptake by insulin and hyperglycemia in man. *Diabetes* 1983;32:35–45.
51. Lagerpusch M, Enderle J, Eggeling B, et al. Carbohydrate quality and quantity affect glucose and lipid metabolism during weight regain in healthy men. *J Nutr* 2013;143:1593–1601.
52. Bosy-Westphal A, Kossel E, Goele K, et al. Contribution of individual organ mass loss to weight loss-associated decline in resting energy expenditure. *Am J Clin Nutr* 2009;90:993–1001.
53. World Health Organization. (2016) Obesity and overweight: Fact sheet Updated June 2016. Available from: <http://www.who.int/mediacentre/factsheets/fs311/en/>

CHAPTER IV

Association between fat mass, adipose tissue, fat fraction per adipose tissue and metabolic risks: a cross-sectional study in normal-, overweight and obese adults

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submitted to European Journal of Clinical Nutrition

Abstract

Background/Objectives: We investigated whether fat mass (FM) and total adipose tissue (TAT) can be used interchangeably and FM per TAT adds to metabolic risk assessment.

Subjects/Methods: Cross-sectional data were assessed in 377 adults (aged 18-60 years; 51.2% women). FM was measured by either 4-compartment (4C) model or quantitative magnetic resonance (QMR); total-, subcutaneous- and visceral adipose tissue (TAT, SAT, VAT) and liver fat by whole-body magnetic resonance imaging (MRI); leptin, insulin, homeostasis model assessment of insulin resistance (HOMA-IR), C-reactive protein (CRP) and triglycerides; resting energy expenditure and respiratory quotient by indirect calorimetry were determined. Correlations and stepwise multivariate regression analyses were performed.

Results: FM_{4C} and FM_{QMR} were associated with TAT ($r_{4C} = 0.96$, $r_{QMR} = 0.99$) with a mean FM per TAT of 0.85 and 1.01, respectively. Regardless of adiposity, there was a considerable inter-individual variance of FM/TAT-ratio (FM_{4C}/TAT: 0.77-0.94; FM_{QMR}/TAT: 0.89-1.10). Both, FM_{4C} and TAT were associated with metabolic risks. Further, FM_{4C}/TAT was positively related to leptin but inversely to CRP. There was no association between FM_{4C}/TAT and VAT/SAT or liver fat. FM_{4C}/TAT added to the variance of leptin and CRP.

Conclusions: FM_{4C} and TAT cannot be used interchangeably. Independent of FM or TAT, FM_{4C}/TAT adds to metabolic risk assessment.

Introduction

The 4-compartment (4C) model, quantitative magnetic resonance (QMR) and imaging technologies (magnetic resonance imaging, MRI; computed tomography, CT) are gold standards to assess fat mass (FM) and adipose tissue volume.^{1,2} The 4C-model includes the measurement of body weight, body volume, bone mineral content and body water to assess lipid content of the body, whereas the volume of adipose tissue (either total adipose tissue (TAT) or regional, i.e. visceral and subcutaneous adipose tissue, VAT, SAT) is most accurately quantified by MRI.¹ When compared with the 4C-model (which uses an equation to calculate FM) FM can be directly and precisely assessed with QMR.² Up to now, the results of the different methods have been used interchangeably. The association between FM and TAT and its possible association with metabolic risk has not been addressed in clinical studies.

Based on data obtained by adipose tissue biopsy, FM is assumed to be about 80% of TAT with variations between 62% and 91%.³⁻⁶ While differences in TAT are due to combinations of cellular hyperplasia and hypertrophy, increasing adipose tissue is primarily explained by hypertrophy, i.e. an increased FM fraction per adipocytes.^{7,8} Hypertrophy is then associated with increased secretion of pro-inflammatory cytokines⁹ and is supposed to result in the development of metabolic risks, e.g. insulin resistance.^{10,11} Therefore, the relationship between FM and TAT as a fat fraction per adipose tissue may influence on metabolic risks.

In the present study we have used *in vivo* body composition data on FM (as assessed by either 4C or QMR) and TAT (as assessed by MRI) to assess their associations as well as their relation to metabolic risk in normal-, overweight and obese adults. The aims of this study were (i) to investigate the association between FM and TAT in a large study population *in vivo* and (ii) to analyze possible associations of the FM/TAT ratio to metabolic risks independent of FM and TAT. We hypothesized that due to inter-individual variance in the FM/TAT ratio, FM and TAT cannot be used interchangeably.

Materials and Methods

Subjects

We have analyzed data of 377 healthy Caucasian adults aged 18-60 years [body mass index (BMI): 25.6 (22.8-31.1) kg/m²; women: 51.2%] who had participated in different studies at the “Reference Centre for Body Composition” (Institute of Human Nutrition and Food Science at the University of Kiel, Germany) between 2005 and 2016. Exclusion criteria were pregnancy, acute or chronic diseases, medication intake, smoking and metallic implants. Subjects were recruited by notice board postings and by local advertisements. In children, adolescents and

adults, measurements of anthropometry, FM and metabolic variables were performed at the Institute of Human Nutrition and Food Science (University of Kiel), whereas total and regional adipose tissue were assessed at the MRI-unit of the Clinic of Radiology at the University Hospital Schleswig Holstein on an additional date. In an independent group (48 Caucasian adults aged 20-69 years; BMI: 25.0 (22.6-38.9) kg/m²) we analyzed data on liver fat to investigate the association to fat fraction per adipose tissue. Written informed consent was obtained from each subject before participation. All studies were conducted according to the guidelines laid down in the “Declaration of Helsinki” and approved by the ethical committee of the department of medicine of Christian-Albrechts-University Kiel.

Anthropometric measurements

Body height was determined with participants wearing no shoes to the nearest 0.5 cm by using a stadiometer (SECA, Modell 220, Hamburg, Germany). Body weight was assessed to the nearest 0.01 kg using an electronic scale (Tanita, Tokyo, Japan). Waist circumference (WC) was measured midway between the lowest rib and the top of the iliac crest at the end of a normal expiration with the subjects standing in upright position. The measurement was done with a non-elastic plastic tape positioned parallel to the floor.

4-compartment model

FM and fat-free mass (FFM) were determined using a 4C model as has been described in detail before.¹² Briefly, the model divided the body into lipids, water, mineral and protein content. Body mineral content (BMC) was measured by dual X-ray absorptiometry (DXA), body volume by air-displacement plethysmography (ADP) and total body water (TBW) by deuterium oxide dilution. The following equation was used to determine FM_{4C}:

$$\text{FM}_{4\text{C}} \text{ (kg)} = 2.747 \times \text{body volume}_{\text{ADP}} \text{ (l)} - 0.710 \times \text{TBW} \text{ (l)} + 1.460 \times \text{BMC}_{\text{DXA}} \text{ (kg)} - 2.050 \times \text{body weight} \text{ (kg)}.$$
¹³

FFM_{4C} (kg) was then calculated as body weight (kg) – FM_{4C} (kg). FM_{4C}-Index (FMI_{4C}) was calculated as FM_{4C} (kg) / body height (m)². Further, FM_{4C}/FFM_{4C} ratio was used as a measure of metabolic load related to metabolic capacity.¹⁴

Quantitative magnetic resonance

FM was also determined by non-invasive QMR (EchoMRI; Echo Medical Systems LLC, Houston, TX, USA) as in detail described elsewhere.^{15,16} Briefly, subjects were examined in supine position without wearing any metallic clothing items. Overall, the measurement last for

six minutes and the mean of three measurements was calculated. Quantification of FM_{QMR} is based on Carr-Purcell-Meiboom-Gill-sequence.¹⁷ FFM_{QMR} is then calculated as the difference between body weight (kg) and FM_{QMR} .

Magnetic resonance imaging

Volumes of TAT, SAT and VAT as well as liver fat content were measured by using whole-body MRI with a 1.5T scanner (Magnetom Vision or Magnetom Avanto, Siemens Medical Systems, Erlangen, Germany) as has been described in detail before.¹⁸⁻²¹ Briefly, subjects were examined with their arms extended above their heads in a dorsal position. TAT was defined from wrist to ankle. VAT was assessed from femoral heads to the top of the liver. Volumes of SAT and VAT were segmented by using semi-manual segmentation software (SliceOmatic 4.3, Tomovision, Montreal, Canada). Masses of SAT and VAT were calculated as tissue volumes multiplied by density (0.923 g/cm³). TAT was calculated as SAT + VAT. Further, VAT/SAT ratio was determined as a measure of relative size of the intra-abdominal fat depots. TAT-Index (TATI) was calculated as TAT (l) / body height (m)². Liver fat was determined by a 2-point Dixon method using ImageJ software (US NIH, Bethesda, MD, USA). The quantity of liver fat was assessed as percentage of the total liver core.

Fat fraction per adipose tissue

The fat fraction per adipose tissue was defined as the ratio of FM (kg) / TAT volume (l). We assumed that physiologically the proportion by weight of FM in adipose tissue is approximately 80%.³⁻⁵ We also compared groups differing in FM/TAT (low, normal, high). According to literature-based values, normal FM/TAT was defined as $0.62 \leq FM/TAT \leq 0.91$, low as $FM/TAT < 0.62$, and high as $FM/TAT > 0.91$.³

Resting energy expenditure

As a part of metabolic risk assessment, resting energy expenditure (REE) and respiratory quotient (RQ) were determined by indirect calorimetry using an open-circuit ventilated-hood system (Vmax Spectra 29n, SensorMedics BV, Viasys Healthcare, Bithoven, Netherlands; software V-max version 12-1A) as in detail described elsewhere.²² Briefly, indirect calorimetry was conducted in the morning after an overnight fast with the measurement taking at least 45 min. The first 10 min of each measurement was rejected. The coefficient of variation for repeated measurements was 5.0%.²³ REE was further adjusted for FFM (REE_{FFM}) using regression analysis.²⁴

Metabolic traits

Blood samples were taken at least 8-h postprandially and analyzed by standard procedures. Plasma glucose and TG were determined enzymatically using Konelab-Test-Kit (Thermo Clinical Labsystems, Frankfurt, Germany). Plasma insulin and plasma leptin were measured by radioimmunoassay (Adaltis, Freiburg, Germany; Linco Research, St Charles, MO, USA). C-reactive protein (CRP) was measured by using latex-agglutination (CRP-Dynamik/Hit917, BIOMED Labordiagnostik GmbH Oberschleißheim, Germany). Homeostasis model assessment of insulin resistance (HOMA-IR) was used to determine insulin resistance:

$$\text{HOMA-IR} = \text{plasma glucose (mmol/L)} \times \text{plasma insulin (\mu U/ml)} / 22.5 \text{ (ref. 25)}$$

Statistical analysis

All statistical analyses were performed with SPSS statistical software (SPSS 24.0, Inc., Chicago, Illinois, USA). Data are given as median and inter-quartile range (IQR). Mann-Whitney U test and Kruskal-Wallis test with Bonferroni correction were performed for differences between groups. Further analysis were stratified by gender because of significant differences in body composition and metabolic risks. According to literature-based values of fat fraction per adipose tissue, associations between parameters of body composition and metabolic traits were examined by bivariate correlations and stepwise multivariate regression analyses in men and women within a normal range of FM/TAT (FM/TAT: 0.62-0.91).³ Residuals of leptin, HOMA-IR, CRP and TG regressed on FM_{4C} were calculated by linear regression analysis and related to FM_{4C}/TAT. In gender-specific multivariate regression analyses, leptin, HOMA-IR, CRP and TG were included as dependent variables with either age, FMI_{4C} and FM_{4C}/TAT (Model 1) or age, TATI and FM_{4C}/TAT (Model 2) as independent variables. Regression analyses were corrected for multiple testing using the false discovery rate based on Benjamini and Hochberg procedure at a level of 5%.²⁶ All tests were two-sided and level of significance was set at $P < 0.05$.

Results

The main characteristics of the study population are shown in **Table 1**. 54.4% of women and 54.3% of men were overweight or obese. FM_{4C}, FM_{QMR}, FMI_{4C}, FM_{4C}/FFM_{4C}, TAT, TATI, SAT, leptin, insulin, HOMA-IR and CRP were significantly greater in women than in men. By contrast, body weight, WC, FFM_{4C}, FFM_{QMR}, VAT, VAT/SAT, REE and REE_{FFM} were greater in men.

Table 1. Characteristics of the study population stratified by gender				
	N	Women	N	Men
Age (y)	193	35 ^a (26 – 44)	184	33 (26 – 44)
Body weight (kg)	193	75.6 (64.6 – 90.9)***	184	84.9 (75.3 – 96.2)
BMI \geq 25 (%)	193	54.4	184	54.3
BMI (kg/m ²)	193	25.6 (22.5 – 32.8)	184	25.5 (23.0 – 29.4)
WC (cm)	193	88.0 (77.0 – 102.5)**	184	90.3 (83.3 – 104.3)
FM _{4C} (kg)	193	25.4 (18.7 – 37.6)***	144	20.3 (13.4 – 27.1)
FM _{QMR} (kg)	26	38.5 (24.1 – 52.9)***	72	15.3 (11.1 – 29.7)
FFM _{4C} (kg)	193	48.7 (44.1 – 55.2)***	144	68.0 (61.7 – 72.4)
FFM _{QMR} (kg)	26	50.3 (41.2 – 54.7)***	72	64.7 (59.3 – 69.0)
TBW (l)	193	36.2 (32.7 – 40.8)***	144	49.0 (45.0 – 52.3)
ICW (l)	178	20.3 (17.9 – 23.0)***	132	28.9 (26.6 – 31.1)
ECW (l)	178	14.7 (13.6 – 17.1)***	132	19.1 (17.7 – 20.7)
FMI _{4C} (kg/m ²)	193	8.9 (6.4 – 13.8)***	144	6.2 (4.1 – 8.4)
FM _{4C} /FFM _{4C}	193	0.53 (0.41 – 0.73)***	144	0.30 (0.22 – 0.40)
TAT (l)	193	30.0 (21.8 – 43.4)***	184	20.6 (14.8 – 29.4)
TATI (l/m ²)	193	9.9 (7.0 – 14.5)***	184	6.0 (4.1 – 8.3)
FM _{4C} /TAT (kg/l)	193	0.86 (0.79 – 0.94)	144	0.84 (0.74 – 0.95)
SAT (kg)	193	20.0 (17.2 – 35.4)***	184	14.9 (11.4 – 20.4)
VAT (kg)	192	1.4 (0.7 – 2.3)***	183	2.2 (1.1 – 4.6)
VAT/SAT	192	0.05 (0.04 – 0.08)***	183	0.14 (0.09 – 0.21)
Leptin (ng/ml)	118	17.3 (9.8 – 36.3)***	111	3.5 (2.1 – 6.8)
Insulin (μ U/ml)	181	10.6 (7.0 – 18.5)**	173	8.4 (6.5 – 13.7)
HOMA-IR	181	2.4 (1.6 – 4.4)**	173	1.9 (1.4 – 3.3)
CRP	128	1.2 (0.5 – 2.9)**	99	0.8 (0.3 – 1.6)
Triglycerides (mg/dl)	190	92 (71 – 138)	171	102 (76 – 143)
REE (kcal/d)	190	1500 (1367 – 1666)***	175	1878 (1726 – 2044)
REE _{FFM} (kcal/d)	190	1531 (1444 – 1622)***	136	1895 (1780 – 1998)
RQ	173	0.83 (0.79 – 0.87)	120	0.83 (0.79 – 0.87)

Abbreviations: 4C, four-compartment model; CRP, C-reactive protein; ECW, extracellular water; FFM, fat-free mass; FM, fat mass; FMI, fat mass index; ICW, intercellular water; QMR, quantitative magnetic resonance; REE, resting energy expenditure; REE_{FFM}, resting energy expenditure adjusted for fat-free mass; RQ, respiratory quotient; SAT, subcutaneous adipose tissue; TAT, total adipose tissue; TATI, total adipose tissue index; TBW, total body water; VAT, visceral adipose tissue; WC, waist circumference.

^aMedian (IQR) (all such values).

*women significantly different from men, $P < 0.05$; **women significantly different from men, $P < 0.01$; ***women significantly different from men, $P < 0.001$ (Mann-Whitney U test).

Association between fat mass and total adipose tissue

TAT was closely associated with FM (**Figure 1A and 1B**). This was independent of the method used to assess FM. There was a considerable inter-individual variance in FM/TAT, which was independent of FM as well as the method used to assess FM (**Figure 1C and 1D**). FM_{4C} was closely associated with FM_{QMR} (**Figure S1**). However, differences in FM between these methods were significant (data not shown).

Association of fat mass per adipose tissue with body composition and metabolic traits

Body composition, metabolic traits and REE were characterized in three groups with different FM_{4C}/TAT-ratio (**Table 2**). When compared to men, the proportion of women was greater in the normal and high FM_{4C}/TAT-groups than in the low FM_{4C}/TAT-group. BMI, WC, FM_{4C}, FMI_{4C}, FM_{4C}/FFM_{4C}, TAT, TATI, SAT and leptin were greater in normal- and high FM_{4C}/TAT-groups than in the low FM_{4C}/TAT-group. By contrast, FFM_{4C} was lower in high FM_{4C}/TAT than in low- and normal FM/TAT-groups. Restricting this analysis to normal weight subjects only, the associations of FM_{4C}/TAT to BMI, WC and REE_{FFM} lost significance (**Table S1**).

Associations of FM_{4C}/TAT with FM_{4C}/FFM_{4C}, VAT/SAT and liver fat are shown in **Figure 2**. There was a sex-difference in the associations. FM_{4C}/TAT was stronger associated with FM_{4C}/FFM_{4C} in men than in women (**Figure 2A**). By contrast, FM_{4C}/TAT was not significantly associated with VAT/SAT and liver fat in both genders (**Figure 2B and 2C**).

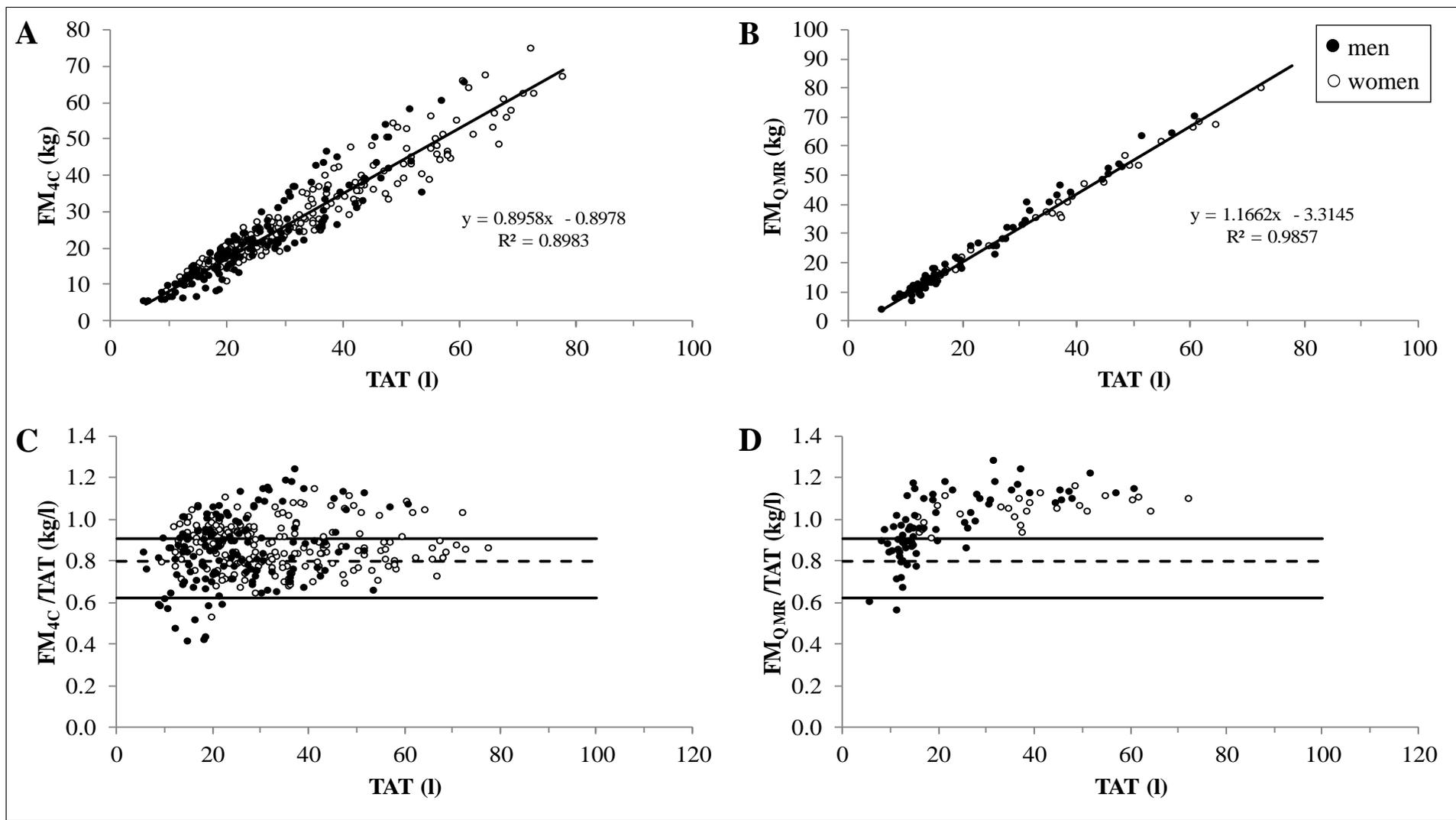


Figure 1. Association between TAT and either FM_{4C} (A) or FM_{QMR} (B) or FM_{4C}/TAT (C) or FM_{QMR}/TAT (D) stratified by gender. Abbreviations: 4C, four-compartment model; FM, fat mass; QMR, quantitative magnetic resonance; TAT, total adipose tissue. solid line (Figure 1A and 1B) = regression line of total population; dashed line (Figure 1C and 1D) = fat content (kg) as 0.8 of adipose tissue (l); solid lines (Figure 1C and 1D) = range of normal fat content (kg) of adipose tissue (l) (3–6)

Table 2. Descriptive characteristic of body composition, metabolic traits and resting energy expenditure stratified by different FM_{4C}/TAT-groups

Groups ^a :	FM/TAT			p-value
	< 0.62	0.62 – 0.91	> 0.91	
N	12	208	117	
Women (%)	8.3	58.7	59.8	
Age (y)	29 ^b (27 – 36)	36 (26 – 43)	37 (28 – 49)	0.085
BMI (kg/m ²)	23.0 (22.1 – 25.2)	26.2 (23.1 – 31.7)	26.8 (23.6 – 34.1)	0.020
WC (cm)	79.5 (75.0 – 86.0)	91.0 (81.0 – 104.9)	94.5 (82.0 – 106.0)	0.010
FM _{4C} (kg)	7.1 (6.1 – 9.6)	21.6 (15.7 – 32.3)	25.5 ^c (19.7 – 37.8)	<0.001
FMI _{4C} (kg/m ²)	2.3 (1.9 – 3.0)	7.1 (5.2 – 10.8)	8.6 (6.5 – 13.4)	<0.001
FFM _{4C} (kg)	67.8 (60.5 – 72.1)	58.0 (48.7 – 68.3)	54.0 ^c (46.1 – 64.8)	0.003
FM _{4C} /FFM _{4C}	0.11 (0.09 – 0.14)	0.39 (0.28 – 0.56)	0.48 ^c (0.38 – 0.70)	<0.001
TBW (l)	49.2 (44.0 – 51.3)	42.5 (36.0 – 49.4)	39.2 (34.4 – 48.0)	0.012
ICW (l)	31.0 (27.6 – 32.9)	24.8 (20.4 – 29.3)	21.9 ^c (18.8 – 27.9)	<0.001
ECW (l)	18.1 (16.2 – 18.4)	16.4 (14.3 – 18.8)	17.7 ^c (14.5 – 20.3)	0.032
FM _{4C} /TAT (kg/l)	0.55 (0.45 – 0.58)	0.80 (0.74 – 0.85)	1.0 (0.94 – 1.06)	<0.001
TAT (l)	15.9 (10.7 – 19.1)	28.6 (10.1 – 40.9)	26.0 (20.4 – 37.4)	<0.001
TATI (l/m ²)	4.7 (3.2 – 5.6)	8.6 (6.1 – 12.9)	8.2 (6.1 – 11.6)	<0.001
SAT _{total} (kg)	11.8 (8.6 – 13.8)	20.4 (14.9 – 30.4)	19.8 (15.8 – 30.1)	<0.001
VAT (kg)	0.85 (0.73 – 2.82)	1.85 (1.02 – 3.74)	1.98 (0.86 – 2.94)	0.282
Leptin (ng/ml)	2.0 (1.3 – 2.9)	11.9 (5.0 – 28.2)	11.2 (7.6 – 19.4)	<0.001
HOMA-IR	1.9 (1.5 – 2.8)	2.4 (1.6 – 4.3)	2.1 (1.4 – 4.0)	0.234
CRP	0.5 (0.1 – 0.8)	0.9 (0.3 – 2.8)	1.1 (0.4 – 1.8)	0.260
Triglyceride (mg/dl)	87 (74 – 116)	95 (77 – 142)	100 (71 – 139)	0.608
REE (kcal/d)	1748 (1616 – 1867)	1638 (1468 – 1891)	1673 (1433 – 1966)	0.533
REE _{FFM} (kcal/d)	1749 (1624 – 1814)	1584 (1464 – 1792)	1668 ^c (1553 – 1969)	0.001
RQ	0.84 (0.80 – 0.85)	0.83 (0.79 – 0.87)	0.83 (0.80 – 0.88)	0.799

Abbreviations: 4C, four-compartment model; CRP, C-reactive protein; ECW, extracellular water; FFM, fat-free mass; FM, fat mass; FMI, fat mass index; ICW, intercellular water; REE, resting energy expenditure; REE_{FFM}, resting energy expenditure adjusted for fat-free mass; RQ, respiratory quotient; SAT, subcutaneous adipose tissue; TAT, total adipose tissue; TATI, total adipose tissue index; TBW, total body water; VAT, visceral adipose tissue; WC, waist circumference.

^alow: FM/TAT < 0.62; normal: 0.62 ≤ FM/TAT ≤ 0.91; high: FM/TAT > 0.91.

^bMedian (IQR) (all such values).

^cp-values, differences between FM/TAT groups (Kruskal-Wallis test with bonferroni correction).

^dsignificant differences between normal- and high FM/TAT-group.

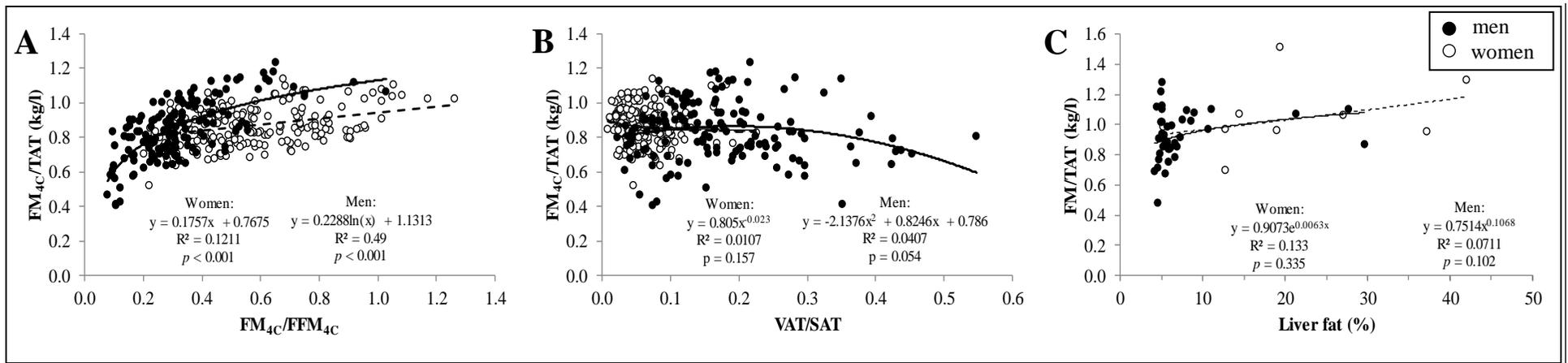


Figure 2. Association between FM_{4C}/TAT and either ratio of FM/FFM (**A**) or VAT/SAT (**B**) or liver fat (**C**) stratified by gender. Liver fat was analysed in an independent group of 48 subjects (women: BMI 46.4 (41.9 – 53.4) kg/m^2 ; men: 23.3 (22.2 – 30.6) kg/m^2). Abbreviations: 4C, four-compartment model; FM, fat mass; FFM, fat-free mass; SAT, subcutaneous adipose tissue; TAT, total adipose tissue; VAT, visceral adipose tissue. Dashed lines = women; solid lines = men

Association between fat mass per adipose tissue and metabolic risk independent of fat mass

Bivariate correlations between FM_{4C} , FMI_{4C} , TAT, TATI, FM_{4C}/TAT and metabolic traits are shown in **Table 3**. In both genders, positive associations were found of FM_{4C} and TAT with leptin, insulin, HOMA-IR, CRP and TG. Similar associations between either FM_{4C} or TAT adjusted for height (FMI_{4C} and TATI) and metabolic traits were found. The associations between FM_{4C}/TAT and leptin and CRP were weaker when compared with either FM_{4C} or FMI_{4C} or TAT or TATI.

Inverse association between FM_{4C}/TAT and residuals of leptin and HOMA-IR regressed on FM_{4C} were seen in men (data not shown). In women, there were no associations between FM_{4C}/TAT and residuals of metabolic traits regressed on FM_{4C} .

Using stepwise multivariate regression analysis, FMI_{4C} and TATI were the major predictors of metabolic risks (**Table 4**). Accordingly, their explained variances of leptin, HOMA-IR, CRP and TG were similar in either women or men. The strongest association was found between FMI_{4C} and plasma leptin. 62.7% or 61.8% of the variance in leptin in women and men, respectively, were explained by FMI_{4C} . In addition, FMI_{4C} or TATI explained up to 39.3% of the variance in HOMA-IR and CRP. Independent of FMI_{4C} or TATI, FM_{4C}/TAT added to the variance of leptin and CRP. Accordingly, greater FM_{4C}/TAT was associated with higher plasma leptin and lower CRP. Using age, whole body extra- and intracellular water as independent variables, did not add to explain the variance in FM_{4C}/TAT (data not shown).

Table 3. Bivariate correlations between FM_{4C}, FMI_{4C}, TAT, TATI, FM_{4C}/TAT and metabolic traits stratified by gender

	Correlation coefficient (<i>r</i>) ^a									
	Women					Men				
	FM _{4C}	FMI _{4C}	TAT	TATI	FM _{4C} /TAT	FM _{4C}	FMI _{4C}	TAT	TATI	FM _{4C} /TAT
Leptin	0.869***	0.864***	0.868***	0.858***	0.206*	0.814***	0.819***	0.838***	0.820***	0.067
Insulin	0.372***	0.357***	0.381***	0.366***	-0.053	0.668***	0.667***	0.684***	0.675***	-0.133
HOMA-IR	0.355***	0.340***	0.367***	0.352***	-0.067	0.680***	0.684***	0.699***	0.693***	-0.150
CRP	0.553***	0.572***	0.579***	0.593***	-0.183	0.433**	0.445***	0.515***	0.524***	-0.317*
TG	0.337***	0.329***	0.337***	0.333***	-0.032	0.438***	0.467***	0.493***	0.510***	-0.214

Abbreviations: 4C, four-compartment model; CRP, C-reactive protein; FM, fat mass; FMI, fat mass index; TAT, total adipose tissue; TATI, total adipose tissue index; TG, triglycerides.

^abased on data with FM/TAT between 0.62 and 0.91 (ref. 3)

*significant correlation, $P < 0.05$; **significant correlation, $P < 0.01$; ***significant correlation, $P < 0.001$.

Table 4. Stepwise multivariate linear regression with leptin, HOMA-IR, CRP and triglycerides as dependent variables

	Leptin ^a			HOMA-IR ^a			CRP ^a			TG ^a		
	R ²	β	p-value	R ²	β	p-value	R ²	β	p-value	R ²	β	p-value
Women^b												
n		101			118			78			120	
Age (y)	1.8	-0.145	0.027	3.1	-0.195	0.032	0.0	-0.190	0.065	0.0	-0.143	0.162
FMI _{4C} (kg/m ²)	62.7	0.815	<0.001	17.3	0.453	<0.001	32.8	0.608	<0.001	8.0	0.296	0.003
FM _{4C} /TAT (kg/l)	0.0	0.069	0.259	0.0	-0.065	0.441	0.0	-0.157	0.092	0.0	-0.060	0.502
Age (y)	1.4	-0.135	0.024	3.1	-0.195	0.032	0.0	-0.190	0.065	0.0	-0.142	0.167
TATI (l/m ²)	61.1	0.800	<0.001	18.0	0.460	<0.001	33.6	0.614	<0.001	7.9	0.294	0.003
FM _{4C} /TAT (kg/l)	3.9	0.201	0.002	0.0	0.012	0.888	0.0	-0.051	0.575	0.0	-0.010	0.913
Men^b												
n		59			82			62			83	
Age (y)	10.4	-0.344	<0.001	0.0	-0.153	0.100	0.0	0.108	0.384	0.0	0.055	0.601
FMI _{4C} (kg/m ²)	61.8	0.894	<0.001	36.7	0.612	<0.001	10.8	0.371	0.006	18.1	0.437	<0.001
FM _{4C} /TAT (kg/l)	0.0	0.059	0.398	0.0	-0.154	0.125	5.9	-0.269	0.038	0.0	-0.128	0.305
Age (y)	10.2	-0.351	<0.001	0.0	-0.162	0.113	0.0	0.116	0.361	0.0	0.053	0.902
TATI (l/m ²)	59.3	0.929	<0.001	39.3	0.633	<0.001	11.7	0.362	0.012	18.4	0.440	<0.001
FM _{4C} /TAT (kg/l)	7.7	0.284	<0.001	0.0	0.001	0.994	0.0	-0.184	0.198	0.0	-0.020	0.843

Abbreviations: 4C, four-compartment model; CRP, C-reactive protein; FMI, fat mass index; TATI, total adipose tissue index; TG, triglycerides.

^abased on data with FM_{4C}/TAT between 0.62 and 0.91 (ref. 3)

^btwo models were conducted: Model 1 includes age, FMI_{4C} and FM_{4C}/TAT as independent variables; Model 2 includes age, TATI and FM_{4C}/TAT as independent variables.

p-value = corrected for multiple testing by controlling the false discovery rate.

Discussion

Either the 4C-model or QMR to determine FM and MRI to determine TAT are the gold-standard methods of body composition analysis. FM is assumed to account for 80% of TAT. However, we found a considerable inter-individual variation in the FM/TAT association. Further, inter-individual variation in the associations between either FM or TAT and metabolic traits were seen.²⁷ The variation in the FM/TAT ratio question the idea that FM and TAT can be used interchangeably to assess body composition associated metabolic risk in a large population of normal-, overweight and obese subjects.

Association between fat mass and total adipose tissue

There was a strong association between FM and TAT (Figure 1A and 1B). This is in line with previous findings.³ However, considerable inter-individual variation of FM/TAT ratio were seen (IQR: FM_{4C}/TAT: 0.77-0.94; FM_{QMR}/TAT: 0.89-1.10) (Figure 1C and 1D). Our data indicated that in this population of adults FM regressed on TAT is neither constant nor dependent on adiposity. This is in line with other biopsy studies showing a wide variability of fat fraction of adipose tissue ranging between 0.62 and 0.91. (refs. 3–6)

Association between fat mass per adipose tissue, body composition and metabolic traits

FM_{4C}/TAT was positively related to BMI, WC, FM_{4C}, FMI_{4C}, FM_{4C}/FFM_{4C} and leptin. By contrast, it was inversely associated with FFM_{4C} (Table 2). FM_{4C}/FFM_{4C} may be used as a measure of metabolic load divided by metabolic capacity with decreasing metabolic capacity as FM/FFM increases.¹⁴ Thus, increasing FM_{4C}/TAT would lead to further decreased metabolic capacity. FM_{4C} was significantly higher in high FM_{4C}/TAT-group than in normal FM_{4C}/TAT-group, whereas TAT was lower in high FM_{4C}/TAT-group than in normal FM_{4C}/TAT-group (Table 2). Accordingly, FM_{4C}/TAT is positively related to age. These findings would suggest that with increasing age a higher amount of FM is deposited in ectopic depots, e.g. liver, pancreas and or skeletal muscle. This is line with the finding that FM_{4C}/TAT was associated with regional fat depots. FM_{4C}/TAT showed a positive tendency to liver fat in both genders (Figure 2C).

Association between fat mass per adipose tissue and metabolic risk independent of fat mass and adipose tissue

FM_{4C}/TAT was associated with plasma leptin and CRP (Table 3). Variances of leptin, HOMA-IR, CRP and TG were similarly explained by FMI_{4C} or TATI (Table 4). This could be due to almost equal associations between either FMI_{4C} or TATI and metabolic traits (Table 3). However, we

found evidence for a FM-independent association of FM_{4C}/TAT with metabolic risk. After adjustment for TATI, FM_{4C}/TAT added to the variance of leptin; greater FM_{4C}/TAT was associated with higher plasma leptin (Table 4). In addition, after adjustment for FMI_{4C}, FM_{4C}/TAT added to the variance of CRP. However, after adjustment for FMI_{4C} or TATI, there were no relationships in leptin or CRP, respectively (Table 4). With regard to fat mass and fat volume, our findings indicated that FM_{4C} and TAT cannot be used interchangeably to assess metabolic risk in normal-, overweight and obese adults. Both variables may add to metabolic risk assessment. It should be mentioned that previous data have shown an FM-independent direct association between adipocyte size and insulin resistance in overweight and obese subjects.^{28,29} Since however, in our studies we were not allowed to do fat biopsies for ethical reason the association between FM_{4C}/TAT and the size of adipocytes remains to be shown.

Study Strengths and Limitations

This study took advantage of access to detailed specific measurement of both FM and TAT in the same individuals allowing investigation of the associations between FM/TAT, body composition and metabolic risk in normal-, overweight and obese subjects. In addition, FM was measured by two independent gold-standard methods (4C-model and QMR) and divided by TAT determined by whole-body MRI. Limitations of the present study include the lack of adipose tissue biopsies, data on liver fat of only a subgroup, and the cross-sectional study design prohibiting assessment of the dynamic relationships between FM/TAT and changes over time in body composition and metabolic risk.

Conclusion

Although FM and TAT were strongly correlated, there is a considerable inter-individual variance in FM/TAT-ratio, which is independent of adiposity measured by FM. Independent of FMI or TATI, FM/TAT added to the variance of leptin and CRP. Therefore, the interchangeable use of FM and TAT to assess metabolic risks is questionable. However, both parameters may complement each other, and their respective physiological roles need to be elucidated.

Conflict of interest:

The authors declare no conflict of interest.

Acknowledgements:

This work was supported by the German Research Foundation (DFG MÜ 714/8-3; DFG BO 3296/1-1), the Federal Ministry of Education and Research (BMBF 01EA1336), OMRON, Kyoto, Japan and Seca GmbH & Co. KG (BCA-01).

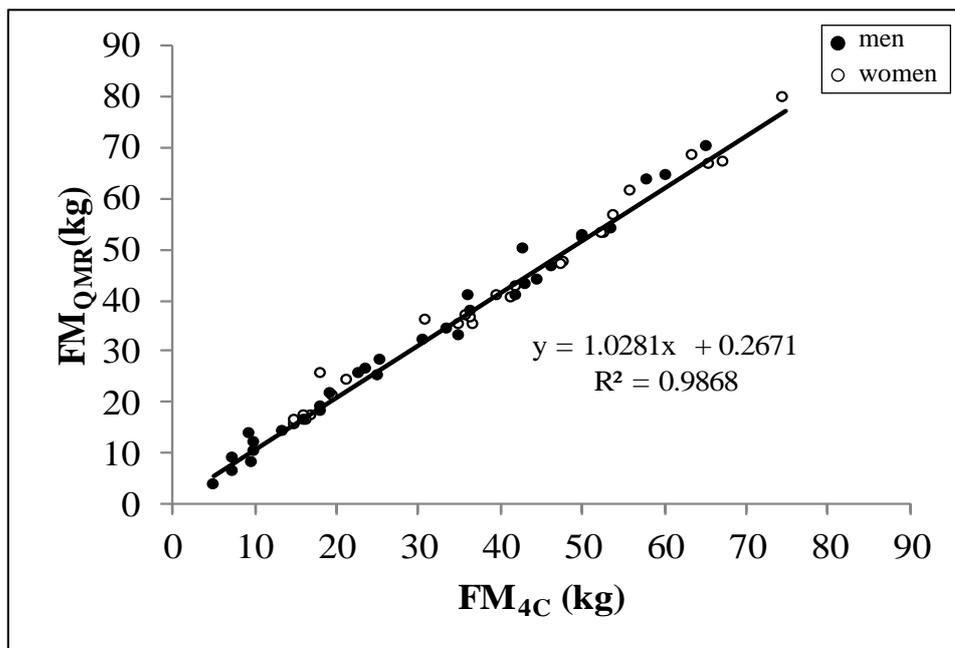


Figure S1. Association between fat mass assessed by four-compartment model and fat mass assessed by quantitative magnetic resonance. Abbreviations: 4C, four-compartment model; QMR, quantitative magnetic resonance

Table S1. Descriptive Characteristic of body composition, metabolic traits and resting energy expenditure stratified by different FM/TAT groups in adults with BMI ≤ 27.5

Groups ^a :	FM/TAT			p-value
	< 0.62	0.62 – 0.91	> 0.91	
N	11	115	66	
Female (%)	9.1	54.8	68.2	
Age (y)	28 ^b (26 – 32)	31 (26 – 42)	39 (28 – 51)	0.003
BMI (kg/m ²)	22.6 (22.0 – 24.7)	23.3 (21.5 – 25.1)	24.0 (22.1 – 25.6)	0.218
WC (cm)	79.0 (75.0 – 86.0)	81.5 (75.0 – 89.0)	83.3 (78.0 – 92.0)	0.112
FM _{4C} (kg)	6.4 (6.0 – 8.5)	16.5 (12.4 – 20.5)	20.5 (17.5 – 23.8)	<0.001
FMI _{4C} (kg/m ²)	2.2 (1.9 – 2.5)	5.4 (4.0 – 6.6)	6.8 (5.5 – 7.7)	<0.001
FFM _{4C} (kg)	67.8 (59.6 – 71.8)	52.8 (46.0 – 63.3)	46.7 (42.1 – 58.8)	<0.001
FM _{4C} /FFM _{4C}	0.11 (0.09 – 0.12)	0.31 (0.22 – 0.41)	0.41 (0.32 – 0.50)	<0.001
TBW (l)	48.0 (43.2 – 51.1)	38.8 (33.5 – 45.5)	35.1 (31.0 – 42.2)	0.001
ICW (l)	29.9 (26.7 – 32.8)	22.8 (19.1 – 27.9)	20.1 (17.1 – 24.6)	<0.001
ECW (l)	18.0 (16.0 – 18.2)	15.5 (13.8 – 17.7)	14.9 (13.6 – 17.9)	0.203
FM _{4C} /TAT (kg/l)	0.52 (0.43 – 0.58)	0.80 (0.75 – 0.85)	0.96 (0.93 – 1.01)	<0.001
TAT (l)	15.1 (10.5 – 18.9)	21.1 (15.5 – 25.4)	21.0 (17.4 – 24.0)	0.002
TATI (l/m ²)	4.5 (3.0 – 5.5)	6.4 (4.7 – 8.0)	6.4 (5.5 – 7.7)	0.002
SAT _{total} (kg)	11.7 (8.6 – 12.7)	16.4 (11.8 – 19.9)	16.1 (13.9 – 19.3)	0.001
VAT (kg)	0.81 (0.71 – 1.86)	1.15 (0.60 – 2.01)	1.13 (0.57 – 2.01)	0.781
Leptin (ng/ml)	1.8 (1.3 – 2.8)	5.7 (3.4 – 12.3)	8.8 (6.7 – 12.2)	0.001
HOMA-IR	1.8 (1.5 – 2.8)	2.0 (1.5 – 3.1)	1.5 (1.1 – 2.1)	0.002
CRP	0.2 (0.1 – 0.8)	0.5 (0.2 – 1.5)	0.9 (0.3 – 1.7)	0.171
Triglyceride (mg/dl)	88 (71 – 127)	85 (71 – 112)	96 (67 – 132)	0.803
REE (kcal/d)	1743 (1597 – 1879)	1497 (1371 – 1735)	1470 (1343 – 1701)	0.042
REE _{FFM} (kcal/d)	1759 (1677 – 1820)	1579 (1452 – 1786)	1566 (1460 – 1826)	0.199
RQ	0.85 (0.80 – 0.85)	0.82 (0.79 – 0.87)	0.83 (0.79 – 0.85)	0.660

Abbreviations: 4C, four-compartment model; CRP, C-reactive protein; ECW, extracellular water; FFM, fat-free mass; FM, fat mass; FMI, fat mass index; ICW, intercellular water; REE, resting energy expenditure; REE_{FFM}, resting energy expenditure adjusted for fat-free mass; RQ, respiratory quotient; SAT, subcutaneous adipose tissue; TAT, total adipose tissue; TATI, total adipose tissue index; TBW, total body water; VAT, visceral adipose tissue; WC, waist circumference.

^alow: FM/TAT < 0.62; normal: 0.62 \leq FM/TAT \leq 0.91; high: FM/TAT > 0.91.

^bMedian (IQR) (all such values).

p-values, differences between different FM/TAT groups (Kruskal-Wallis test with bonferroni correction).

References

1. Lee SY, Gallagher D. Assessment methods in human body composition. *Curr Opin Clin Nutr Metab Care* 2008; **11**(5): 566–72.
2. Bosy-Westphal A, Müller MJ. Assessment of fat and lean mass by quantitative magnetic resonance: a future technology of body composition research? *Curr Opin Clin Nutr Metab Care* 2015; **18**(5): 446–51.
3. Report of the task group on reference man ICRP Publication 23 (1975). *Annals of the ICRP* 1980; **4**(3-4): III-III.
4. Sohlstrom A, Wahlund LO, Forsum E. Adipose tissue distribution as assessed by magnetic resonance imaging and total body fat by magnetic resonance imaging, underwater weighing, and body-water dilution in healthy women. *Am J Clin Nutr* 1993; **58**(6): 830–8.
5. Wang Z, Zhu S, Wang J, Pierson RN JR, Heymsfield SB. Whole-body skeletal muscle mass: development and validation of total-body potassium prediction models. *Am J Clin Nutr* 2003; **77**(1): 76–82.
6. Woodard HQ, White DR. The composition of body tissues. *Br J Radiol* 1986; **59**(708): 1209–18.
7. Choe SS, Huh JY, Hwang IJ, Kim JI, Kim JB. Adipose Tissue Remodeling: Its Role in Energy Metabolism and Metabolic Disorders. *Front Endocrinol (Lausanne)* 2016; **7**: 30.
8. Spalding KL, Arner E, Westermark PO, Bernard S, Buchholz BA, Bergmann O et al. Dynamics of fat cell turnover in humans. *Nature* 2008; **453**(7196): 783–7.
9. Jernas M, Palming J, Sjöholm K, Jennische E, Svensson P-A, Gabrielsson BG et al. Separation of human adipocytes by size: hypertrophic fat cells display distinct gene expression. *FASEB J* 2006; **20**(9): 1540–2.

10. Yuan M, Konstantopoulos N, Lee J, Hansen L, Li ZW, Karin M et al. Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkbeta. *Science* 2001; **293**(5535): 1673–7.
11. Hirosumi J, Tuncman G, Chang L, Gorgun CZ, Uysal KT, Maeda K et al. A central role for JNK in obesity and insulin resistance. *Nature* 2002; **420**(6913): 333–6.
12. Pourhassan M, Schautz B, Braun W, Gluer C-C, Bosy-Westphal A, Müller MJ. Impact of body-composition methodology on the composition of weight loss and weight gain. *Eur J Clin Nutr* 2013; **67**(5): 446–54.
13. Fuller NJ, Jebb SA, Laskey MA, Coward WA, Elia M. Four-component model for the assessment of body composition in humans: Comparison with alternative methods, and evaluation of the density and hydration of fat-free mass. *Clin. Sci.* 1992; **82**(6): 687–93.
14. Siervo M, Prado CM, Mire E, Broyles S, Wells JCK, Heymsfield S et al. Body composition indices of a load-capacity model: gender- and BMI-specific reference curves. *Public Health Nutr* 2015; **18**(7): 1245–54.
15. Müller MJ, Bosy-Westphal A, Lagerpusch M, Heymsfield SB. Use of balance methods for assessment of short-term changes in body composition. *Obesity (Silver Spring)* 2012; **20**(4): 701–7.
16. Müller MJ, Enderle J, Pourhassan M, Braun W, Eggeling B, Lagerpusch M et al. Metabolic adaptation to caloric restriction and subsequent refeeding: the Minnesota Starvation Experiment revisited. *Am J Clin Nutr* 2015; **102**(4): 807–19.
17. Gallagher D, Thornton JC, He Q, Wang J, Yu W, Bradstreet TE et al. Quantitative magnetic resonance fat measurements in humans correlate with established methods but are biased. *Obesity (Silver Spring)* 2010; **18**(10): 2047–54.
18. Bosy-Westphal A, Booke C-A, Blocker T, Kossel E, Goele K, Later W et al. Measurement site for waist circumference affects its accuracy as an index of visceral and abdominal subcutaneous fat in a Caucasian population. *J Nutr* 2010; **140**(5): 954–61.

19. Schautz B, Later W, Heller M, Müller MJ, Bosy-Westphal A. Total and regional relationship between lean and fat mass with increasing adiposity--impact for the diagnosis of sarcopenic obesity. *Eur J Clin Nutr* 2012; **66**(12): 1356–61.
20. Lagerpusch M, Enderle J, Eggeling B, Braun W, Johannsen M, Pape D et al. Carbohydrate quality and quantity affect glucose and lipid metabolism during weight regain in healthy men. *J Nutr* 2013; **143**(10): 1593–601.
21. Ma J. Dixon techniques for water and fat imaging. *J Magn Reson Imaging* 2008; **28**(3): 543–58.
22. Bosy-Westphal A, Kossel E, Goele K, Later W, Hitze B, Settler U et al. Contribution of individual organ mass loss to weight loss-associated decline in resting energy expenditure. *Am J Clin Nutr* 2009; **90**(4): 993–1001.
23. Bader N, Bosy-Westphal A, Dilba B, Müller MJ. Intra- and interindividual variability of resting energy expenditure in healthy male subjects -- biological and methodological variability of resting energy expenditure. *Br J Nutr* 2005; **94**(5): 843–9.
24. Ravussin E, Bogardus C. Relationship of genetics, age, and physical fitness to daily energy expenditure and fuel utilization. *Am J Clin Nutr* 1989; **49**(5 Suppl): 968–75.
25. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; **28**(7): 412–9.
26. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society. Series B* 1995; (Vol. 57, No. 1): 289–300.
27. Sims EA. Are there persons who are obese, but metabolically healthy? *Metabolism* 2001; **50**(12): 1499–504.

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28. Lundgren M, Svensson M, Lindmark S, Renstrom F, Ruge T, Eriksson JW. Fat cell enlargement is an independent marker of insulin resistance and 'hyperleptinaemia'. *Diabetologia* 2007; **50**(3): 625–33.
29. Ryden M, Andersson DP, Bergstrom IB, Arner P. Adipose tissue and metabolic alterations: regional differences in fat cell size and number matter, but differently: a cross-sectional study. *J Clin Endocrinol Metab* 2014; **99**(10): E1870-6.

CHAPTER V

General discussion

Summary of study rationale and objectives

During the last decades, the prevalence of overweight and obesity increased in all age- and sex groups. However, these numbers depend on an appropriate definition of overweight and obesity. In children, adolescents and adults, overweight and obesity are defined by BMI only. In adults, BMI *cut-offs* are associated with morbidity and mortality. By contrast, in children and adolescents the respective BMI *cut-offs* relate to normative analyses without a strict association with any health outcome. Thus, strictly spoken, the comparison of overweight and obesity between children, adolescents and adults is not possible.

Since, (i) overweight and obesity are characterized by excessive fat accumulation which again (ii) is associated with cardiometabolic diseases [1], associations between anthropometric traits, detailed body composition and cardiometabolic risks have to be characterized in more detail and, particularly, there is need of a systematic analysis in children, adolescents and adults using identical methods, outcomes and statistical analyses. Accordingly, individuals with a risk-related unfavorable body composition could be identified at an early stage.

Our present knowledge about the relationship between detailed body composition and cardiometabolic risks is mainly based on studies on adults. By contrast, there is not much evidence about the associations between detailed body composition and cardiometabolic risks in children and adolescents.

Therefore, the present thesis examined the associations between regional fat depots and cardiometabolic risks in normal- and overweight children, adolescents and adults (Chapter II). Further, detailed body composition based on either normative- or metabolic derived *cut-offs* were compared in different age groups (Chapter III). Taking different fat fraction per adipose tissue into account, we focused on FM and TAT, which are interchangeably used for metabolic risk assessment in clinical and research practice (Chapter IV).

Regional fat depots and associated cardiometabolic risks in children, adolescents and adults

Main findings and new issues of this thesis

Whereas FM and SAT increased from childhood to adulthood, VAT remained low in children and adolescents with a considerable increase in young adults (Chapter II, Table 1). Comparing different age groups, associations between regional fat depots and most cardiometabolic risks became evident after puberty only (Chapter II, Table 2 and Table 3). When compared with FM adjusted for height, insulin resistance had a stronger association with SAT_{trunk} and VAT

(Chapter II, Table 2 and Table 3). Addressing the BMI-independent role of regional fat depots on insulin resistance, the impact of SAT became evident after adolescence with VAT being the most important body fat depot in older adults only (Chapter II, Table 4). This is the first study, which systematically investigate age-related associations between regional fat depots, measured by the gold standard method whole-body MRI, and cardiometabolic risks in children and adolescents compared with adults.

State of research

Up to now, only limited data on the development of regional fat depots and their association with cardiometabolic risks in normal- and overweight children, adolescents and adults are available [2–5].

DIFFERENCES IN REGIONAL FAT DEPOTS. Shen and colleagues were first to focus on detailed adipose tissue distribution in 5 to 88 years old subjects determined by whole-body MRI [5]. They observed that children had lower TAT, SAT_{total}, and VAT than adults. In addition, females had higher volumes of TAT and SAT than males in both age groups. By contrast, males had higher VAT volumes in adults only. However, due to changes in body composition and body fat distribution during pubertal development as well as during aging in adulthood [6–9], detailed body composition should be differentially investigated. We could confirm that regional fat depots were higher in adulthood than in childhood. Nonetheless, our results indicated that differences in regional fat depots between children and adolescents as well as young and older adults became apparent (Chapter II, Table 1).

REGIONAL FAT DEPOTS ASSOCIATED WITH CARDIOMETABOLIC RISKS. Previous studies reported a decreased cardiometabolic risk with higher proportion of SAT_{legs} in children, adolescents and adults [10–12]. This is contrary to our findings (Chapter II, Table 2). The discrepancy could be explained by body fat adjustment. Whereas Staiano and colleagues investigated the association between cardiometabolic risks and leg fat in relation to total body fat [12], we examined these associations using masses of SAT_{legs} without adjustment.

Previous studies in children reported that FM and SAT_{total} had a stronger relationship to insulin resistance than VAT [13,14]. Although VAT was low in children and adolescents (Chapter II, Table 1) the explained variance of HOMA-IR by abdominal fat depots were moderately increased in children, adolescents, young and older adults when compared with FMI (Chapter II, Table 3). Samouda and colleagues also observed that adding either trunk/leg fat mass index or FM_{trunk} to total FM increased the explained variance of insulin resistance in children and adolescents [15]. However, in contrast to other authors, they did not differentiate between

SAT_{trunk} and VAT [15–17]. Accordingly, SAT_{trunk} and VAT were both associated with insulin resistance. This was independent of body fat or BMI percentile [16,17]. These findings are contrary to our results. Differences in regional fat depots on insulin resistance, independent of BMI were seen in young and older adults only (Chapter II, Table 4). Nonetheless, we used whole-body MRI to assess SAT_{trunk} and VAT, whereas other authors determined VAT and SAT_{trunk} by DXA or single slice MRI using appropriate modelling [16,17]. In line with our results, VAT independently influenced insulin resistance in adults, with no significant influence in children and adolescents [2].

Outlook

Our results indicated that associations between cardiometabolic risks and regional fat depots should be separately investigated in children, adolescents as well as young and older adults. When compared with FMI, SAT_{trunk} and VAT increased the explained variance of insulin resistance. Most studies on regional fat depots have identified SAT_{trunk} and VAT as independent predictors of insulin resistance [2,12,15–17]. Nevertheless, the role of VAT on insulin resistance, independent of BMI, is apparent in adults only and therefore may describe a potential dynamic process of VAT being increasingly essential of insulin resistance during aging. However, an investigation on 100 obese children determined truncal fat to significantly affected the development of non-alcoholic fatty liver disease [18]. Thus, the measurement of regional fat depots, especially SAT_{trunk} and VAT, are important in clinical practice for cardiometabolic risk assessment in all age groups. For a better understanding of the dynamic process of VAT and insulin resistance, longitudinal studies from childhood to adulthood would be preferable.

Detailed body composition based on normative- and metabolic derived cut-offs

Main findings and new issues of this thesis

Sensitivity of BMI to predict FM was high in all age groups, whereas specificity decreased from children to adults (Chapter III, Figure 1). With increasing BMI and WC, TAT, SAT_{total}, SAT_{trunk} and VAT increased cubically in children and adolescents. By contrast, these associations were linear in young and older adults (Chapter III, Figure 2). In addition, TAT, SAT_{total}, SAT_{trunk} and VAT increased from childhood to adulthood based on normative BMI or WC *cut-offs* (Chapter III, Table 2). When compared with the normative *cut-offs*, insulin resistance derived *cut-offs* of regional fat depots were lower in all age groups (Chapter III, Figure 2, Table 2). This is the first study to analyze and compare regional fat depots based on normative- and equal insulin resistance derived *cut-offs* in children, adolescents as well as young and older adults.

State of research

Up to now, data on a systematic investigation on associations between anthropometric traits and regional fat depots as assessed by whole-body MRI in normal- and overweight children, adolescents and adults are lacking. Further, this is the first study to compare masses of regional fat depots at either BMI- or WC- or insulin resistance derived *cut-offs*. Thus, based on the associations between regional fat depots and insulin resistance, normative *cut-offs* can be evaluated.

SENSITIVITY AND SPECIFICITY. Zimmermann and colleagues examined the capacity of BMI for FM prediction in 6-12 years old children [19]. In line with our results, specificity of BMI to predict FM was high. However, they determined a lower sensitivity, which is contrary to our findings (Chapter III, Figure 1). In addition, another study in adults found a low sensitivity and a high specificity [20]. Although our results partly differ from these studies, it can commonly be concluded that BMI is not an appropriate predictor of FM.

ANTHROPOMETRIC TRAITS AND REGIONAL FAT DEPOTS. Previous studies examined the associations between anthropometric traits and regional fat depots in children, adolescents and adults separately [21–28]. A study in preschool children have found moderate associations between VAT and either BMI or WC ($r = 0.39$; $r = 0.43$ respectively) [21]. Moreover, moderate associations between anthropometric traits and VAT were seen in adolescents, whereas BMI and WC were stronger associated with TAT, SAT_{total} and SAT_{trunk} [22–24]. Nevertheless, reported associations between anthropometric traits and regional fat depots were linear in children and adolescents. This is contrary to our findings showing that TAT, SAT_{total}, SAT_{trunk} and VAT followed a cubic function with a steep increase at BMI and WC ≥ 1 SDS (Chapter III, Figure 2). In line with our results, Koren and colleagues reported that VAT increased exponentially with increasing BMI or WC [25]. In adults, associations between anthropometric traits and either TAT or SAT_{total} or SAT_{trunk} were stronger than with VAT [26,27]. Supporting our findings (Chapter III, Figure 2), these associations were linear in adulthood.

ANTHROPOMETRIC TRAITS, REGIONAL FAT DEPOTS AND ASSOCIATED INSULIN RESISTANCE. In clinical practice, quick and easy to use methods for metabolic risk assessment are preferable. Therefore, associations between anthropometric traits, regional fat depots and insulin resistance were compared in previous studies, which have shown only moderate associations between regional fat depots and insulin resistance in all age groups [22,29,30]. Further, when compared with BMI, FMI improved the prediction of insulin resistance in adults with no improvements in children and adolescents [31–35]. Overall, strongest associations between anthropometric

traits, regional fat depots and insulin resistance were observed for VAT in children, adolescents and adults [2,22,36]. This is in line with our findings (Chapter III, Figure 3). However, our results indicated that insulin resistance, as assessed by HOMA-IR is not primarily determined by masses of regional fat depots. A possible explanation is that HOMA-IR most probably reflects hepatic insulin resistance [37], whereas the gold standard of insulin sensitivity, the euglycemic clamp technique, reflects peripheral glucose uptake [38]. Associations between body composition, HOMA-IR and euglycemic clamp technique are shown in Figure D1.

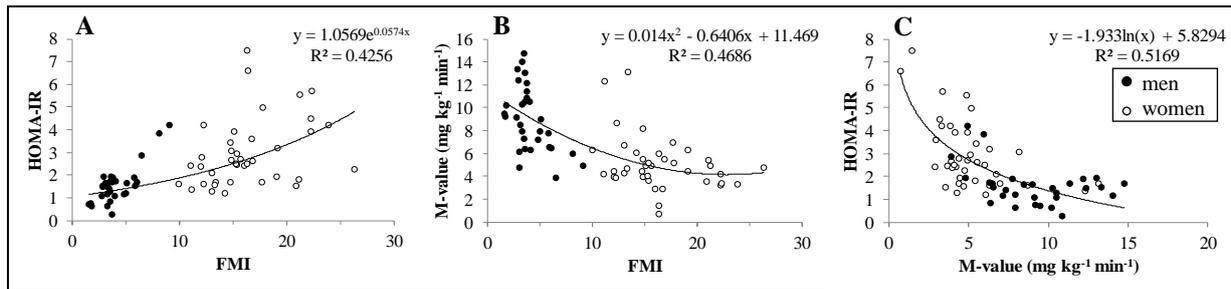


Figure D1. Associations between FMI, HOMA-IR and M-value by euglycemic clamp technique in 20-41 years old adults. Data obtained from German Reference Centre for Body Composition, Kiel [39,40]. Abbreviations: HOMA-IR, homeostasis model assessment of insulin resistance; FMI, fat mass index.

FMI equally explained the variance of HOMA-IR and M-value by euglycemic clamp technique (Figure D1 A and B). However, as seen in Figure D1 C and supported by previous studies, the association between HOMA-IR and M-value by euglycemic clamp technique is moderate [37,39,40]. Thus, the association between fat mass and insulin sensitivity also depends on the method used to assess insulin resistance.

REGIONAL FAT DEPOTS ON NORMATIVE AND INSULIN RESISTANCE DERIVED *CUT-OFFS*. In clinical practice, optimal *cut-offs* for anthropometric traits that predict cardiometabolic risks are preferable. Therefore, previous studies focused on BMI and WC in regard to metabolic syndrome and cardiovascular diseases [41–43]. Performing a ROC analysis with elevated HOMA-IR (> 2.5) as the state variable and either BMI or WC as the test variable, we determined different *cut-offs* for BMI and WC in children, adolescents, young and older adults stratified by gender (Table D1). BMI and WC *cut-offs* differ between age- and sex groups and increased from childhood to adulthood. Comparing young and older adults, WC *cut-offs* differ strongly. However, in line with previous studies, optimal *cut-offs* of BMI and WC for elevated cardiometabolic risk prediction were lower than established BMI- and WC *cut-offs* for obesity and abdominal obesity.

Table D1. Optimal *cut-offs* for BMI and WC for predicting elevated HOMA-IR (> 2.5) in children, adolescents, young adults and adults stratified by gender¹

	Children		Adolescents		Young adults		Adults	
	males	females	males	females	males	females	males	females
BMI (kg/m ²)	21.7	21.8	22.8	20.9	25.1	24.6	25.7	25.8
WC (cm)	73.9	71.0	76.1	87.2	87.5	88.4	95.5	90.5

Abbreviations: BMI, body mass index; WC, waist circumference; HOMA-IR, homeostasis model assessment of insulin resistance.

¹Based on data of subjects (6-60 y) of the German Reference Centre for Body Composition, Kiel [44].

By contrast, we developed an approach based on regional fat depots. Masses of TAT, SAT_{total}, SAT_{trunk} and VAT, using BMI- and WC *cut-offs*, differed between age- and sex groups and increased from childhood to adulthood (Chapter III, Table 2). In addition, comparing *cut-offs* of masses of regional fat depots derived from anthropometric traits and insulin resistance gave considerably lower values for the latter calculation (Chapter III, Table 2).

What do the new cut-offs mean for the population issue of childhood overweight?

To answer this question, we used cross-sectional data of the Kiel Obesity Prevention Study (KOPS) [45]. Accordingly, we compared the prevalence of overweight and obesity as well as abdominal overweight and obesity taking into account normative-, FM- and metabolic derived *cut-offs* in children and adolescents (Table D2). FM derived *cut-offs* are based on the association between FM and either BMI-SDS (Chapter III, Figure 1) or WC-SDS. With regard to the metabolic derived *cut-off*, we applied the calculated BMI and WC *cut-offs* based on the associations between regional fat depots (TAT, VAT) and insulin resistance (Chapter III, Figure 2).

Table D2. Prevalence of overweight and obesity using cross-sectional data of Kiel Obesity Prevention Study (KOPS) based on normative-, fat mass- and metabolic (HOMA-IR > 2.5) derived *cut-offs* stratified by age group¹

	Prevalence					
	Children			Adolescents		
	normative ²	FM ³	metabolic ⁴	normative ²	FM ³	metabolic ⁴
OW (OB) (%)	13.3 (5.2)	33.6 (6.5)	26.7	13.5 (4.6)	51.2 (17.5)	40.4
Abd. OW/OB (%)	37.5 (12.6)	51.2 (17.5)	27.2	34.4 (9.3)	49.5 (19.3)	60.4

Abbreviations: FM, fat mass; OW, overweight; OB, obese; Abd., abdominal; BMI, body mass index; SDS, standard deviation score; WC, waist circumference; HOMA-IR, homeostasis model assessment of insulin resistance.

¹Based on cross-sectional data of the Kiel Obesity Prevention Study [45].

²Based on BMI-SDS [46] and WC-SDS [47] *cut-offs* for overweight and obesity.

³Based on FM derived *cut-offs* for overweight and obesity.

⁴No differentiation between overweight and obesity because BMI and WC were related to elevated HOMA-IR only

When compared with normative- and FM derived *cut-offs*, prevalence of obesity were substantially higher according to the metabolic derived *cut-off*. This is alarming, because subjects with insulin resistance would not be entirely detected by using either the normative- or FM derived approach.

Outlook

Obesity is defined as an excessive fat accumulation which impairs health [48]. Our results indicated that established BMI- or WC *cut-offs* in children, adolescents and adults inadequately define subjects with an elevated risk of insulin resistance. Therefore, anthropometric *cut-offs* being used in clinical practice should be revised with regard to metabolic health outcomes or even replaced by body fat measurements and regional fat depots taking into account their associations with metabolic disturbances. However, insulin resistance results from a multidimensional and dynamic process. Therefore, longitudinal data from birth to adulthood are needed to address this complexity.

Fat fraction per adipose tissue

Main findings and new issues of this thesis

FM measured by either 4C-Model or QMR were strongly associated with TAT as assessed by MRI (Chapter IV, Figure 1 A and B). However, substantial inter-individual variance of FM/TAT-ratio was seen (Chapter IV, Figure 1 C and D). FM/TAT showed a positive tendency to liver fat (Chapter IV, Figure 2 C). Further, FM and TAT were equally associated with metabolic risks (Chapter IV, Table 3). Independent of FM or TAT, FM/TAT added to the variance of leptin and CRP (Chapter IV, Table 4).

State of research

Up to now, FM and TAT have been used interchangeably with regard to metabolic risk assessment. This is the first study, which used *in vivo* body composition data to assess the associations between FM, TAT, FM/TAT and metabolic risks in normal-, overweight and obese adults.

FAT FRACTION PER ADIPOSE TISSUE. In line with others [49,50], FM was strongly associated with TAT (Chapter IV, Figure 1 A and B). However, using adipose tissue biopsies, several studies have shown that fat fraction per adipose tissue differed strongly between individuals as well as between individuals' fat depots [49–51]. Measured by *in vivo* body composition data on FM and TAT, our findings confirmed the inter-individual variance in fat fraction per adipose tissue

(Chapter IV, Figure 1 C and D). Moreover, FM/TAT was independent of adiposity. This is contrary to previous findings, showing a linear relationship between fat fraction per adipose tissue and body fatness [50]. These differences may be explained by the fact that they examined six male cadavers only.

FAT FRACTION PER ADIPOSE TISSUE RELATED TO REGIONAL FAT DEPOTS. The fat fraction in adipocytes can be increased by G0/G1 switch regulatory protein 2 [52]. This promotes adipocyte hypertrophy due to restriction of triglycerides turnover. Hypertrophy in turn can cause fat accumulation in ectopic regions such as the liver and muscle [53,54]. This is in line with our results showing a positive tendency between FM/TAT and liver fat (Chapter IV, Figure 2 C). However, our results were not significant, which can be due to the relatively small number of subjects with data on liver fat (n=48).

ASSOCIATIONS BETWEEN FAT MASS, TOTAL ADIPOSE TISSUE, FAT FRACTION PER ADIPOSE TISSUE AND METABOLIC RISKS. Both, FM and TAT were equally associated with metabolic risks (Chapter IV, Table 3). Our results are in line with previous findings showing moderate to strong associations [2,9,55]. Further, significant associations between FM/TAT and either leptin or CRP were observed (Chapter IV, Table 3) and partly supported by previous findings according to FM/TAT and leptin [9]. However, associations between FM/TAT and either insulin or HOMA-IR or CRP or TG have not been investigated yet. Thus, further studies are needed to confirm our results.

FAT FRACTION PER ADIPOSE TISSUE IS INDEPENDENTLY ASSOCIATED WITH METABOLIC RISKS. Increased fat fraction per adipose tissue is accompanied by adipose tissue remodeling, i.e. hypertrophy [52]. Several studies indicated that hypertrophy leads to cardiometabolic diseases, which can also be seen in early childhood [56–60]. Independent of FM or TAT, FM/TAT as a measure of fat fraction per adipose tissue added to the variance of leptin and CRP (Chapter IV, Table 4). Therefore, FM/TAT may be used as measure of adipocyte size to additionally predict metabolic risks in children, adolescents and adults without undergoing adipose tissue biopsy. Since, we were not allowed to do fat biopsies for ethical reason the association between FM/TAT as a measure of adipocyte size and metabolic risks remains to be shown.

Outlook

FM and TAT cannot be used interchangeably to assess metabolic risks, because fat fraction per adipose tissue differed strongly between individuals regardless of adiposity. However, both parameters may complement each other. In future studies, associations between FM/TAT and

adipocyte size should be investigated to verify if adipose tissue biopsy could be replaced through *in vivo* body composition analysis.

Conclusion

In children, adolescents and adults, overweight and obesity are defined by BMI only. In adults, BMI *cut-offs* are associated with mortality risk. By contrast, respective BMI *cut-offs* are not strictly associated with any health outcome in children and adolescents. Therefore, overweight and obesity cannot be compared between different age groups. Moreover, overweight and obesity are characterized by excessive fat accumulation, which in turn is related to cardiometabolic diseases. Consequently, there is need of a systematic investigation of anthropometric traits and detailed body composition related to cardiometabolic diseases in children, adolescents and adults by using identical methods, outcomes and analyses. This is the only approach to compare results in different age groups.

Overall, the results of the present thesis show the limited value of anthropometric traits in children, adolescents and adults. This can be proved by means of detailed body composition and the associations between regional fat depots and cardiometabolic risks.

Our findings demonstrate that associations between regional fat depots and most cardiometabolic risks became evident after puberty. Whereas VAT was the strongest predictor of insulin resistance in all age groups, insulin resistance was associated with VAT, independent of BMI, in adults only.

Besides, associations between anthropometric traits and regional fat depots varied between children, adolescents and adults. When compared with insulin resistance derived *cut-offs*, BMI- and WC derived values led to higher masses of regional fat depots. Therefore, BMI- and WC *cut-offs* are not appropriate to determine metabolic risks. Instead, our new *cut-offs* of BMI and WC could be used. These are related to metabolic disorders in children, adolescents and adults equally. Otherwise, *cut-offs* of regional fat depots, which are directly associated with insulin resistance should be established.

With regard to the final chapter, FM and TAT cannot be used interchangeably. Substantial inter-individual variances in FM/TAT were seen. Independent of FM or TAT, FM/TAT added to the variance of metabolic risks. In future studies measurements of FM and TAT should be performed together because both parameters could complement each other.

In summary, associations between regional fat depots and cardiometabolic risks differ between children, adolescents as well as young and older adults. In addition, established *cut-offs* of

anthropometric traits are not appropriate for metabolic risk assessment. Considering anthropometric traits, regional fat depots and HOMA-IR together, this would lead to a better characterization of treatable subjects. Further, FM and TAT cannot be used interchangeably.

Nevertheless cardiovascular diseases result from a multidimensional dynamic process, which cannot be addressed in a cross-sectional study. Focusing on the dramatic increase in childhood obesity and its related diseases, longitudinal studies from early childhood to older adulthood are crucial.

References

1. Abarca-Gómez, L.; Abdeen, Z.A.; Hamid, Z.A.; Abu-Rmeileh, N.M.; Acosta-Cazares, B.; Acuin, C.; Adams, R.J.; Aekplakorn, W.; Afsana, K.; Aguilar-Salinas, C.A.; *et al.* Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: A pooled analysis of 2416 population-based measurement studies in 128·9 million children, adolescents, and adults. *The Lancet*.
2. Ali, O.; Cerjak, D.; Kent, J.W.; James, R.; Blangero, J.; Zhang, Y. Obesity, central adiposity and cardiometabolic risk factors in children and adolescents: a family-based study. *Pediatric obesity* **2014**, *9*, e58-e62.
3. Huang, T.T.; Johnson, M.S.; Figueroa-Colon, R.; Dwyer, J.H.; Goran, M.I. Growth of visceral fat, subcutaneous abdominal fat, and total body fat in children. *Obesity research* **2001**, *9*, 283–289.
4. Johnson, M.S.; Figueroa-Colon, R.; Huang, T.T.; Dwyer, J.H.; Goran, M.I. Longitudinal changes in body fat in African American and Caucasian children: influence of fasting insulin and insulin sensitivity. *The Journal of clinical endocrinology and metabolism* **2001**, *86*, 3182–3187.
5. Shen, W.; Punyanitya, M.; Silva, A.M.; Chen, J.; Gallagher, D.; Sardinha, L.B.; Allison, D.B.; Heymsfield, S.B. Sexual dimorphism of adipose tissue distribution across the lifespan: a cross-sectional whole-body magnetic resonance imaging study. *Nutrition & metabolism* **2009**, *6*, 17.
6. Taylor, R.W.; Grant, A.M.; Williams, S.M.; Goulding, A. Sex differences in regional body fat distribution from pre- to postpuberty. *Obesity (Silver Spring, Md.)* **2010**, *18*, 1410–1416.

7. Geisler, C.; Braun, W.; Pourhassan, M.; Schweitzer, L.; Glüer, C.-C.; Bosity-Westphal, A.; Müller, M.J. Gender-Specific Associations in Age-Related Changes in Resting Energy Expenditure (REE) and MRI Measured Body Composition in Healthy Caucasians. *The journals of gerontology. Series A, Biological sciences and medical sciences* **2016**, *71*, 941–946.
8. Schautz, B.; Later, W.; Heller, M.; Müller, M.J.; Bosity-Westphal, A. Total and regional relationship between lean and fat mass with increasing adiposity--impact for the diagnosis of sarcopenic obesity. *European journal of clinical nutrition* **2012**, *66*, 1356–1361.
9. Schautz, B.; Later, W.; Heller, M.; Peters, A.; Müller, M.J.; Bosity-Westphal, A. Impact of age on leptin and adiponectin independent of adiposity. *The British journal of nutrition* **2012**, *108*, 363–370.
10. Schautz, B.; Later, W.; Heller, M.; Müller, M.J.; Bosity-Westphal, A. Associations between breast adipose tissue, body fat distribution and cardiometabolic risk in women: cross-sectional data and weight-loss intervention. *European journal of clinical nutrition* **2011**, *65*, 784–790.
11. Snijder, M.B.; Dekker, J.M.; Visser, M.; Bouter, L.M.; Stehouwer, C.D.A.; Yudkin, J.S.; Heine, R.J.; Nijpels, G.; Seidell, J.C. Trunk fat and leg fat have independent and opposite associations with fasting and postload glucose levels: the Hoorn study. *Diabetes care* **2004**, *27*, 372–377.
12. Staiano, A.E.; Gupta, A.K.; Katzmarzyk, P.T. Cardiometabolic risk factors and fat distribution in children and adolescents. *The Journal of pediatrics* **2014**, *164*, 560–565.
13. Maffeis, C.; Manfredi, R.; Trombetta, M.; Sordelli, S.; Storti, M.; Benuzzi, T.; Bonadonna, R.C. Insulin sensitivity is correlated with subcutaneous but not visceral body fat in overweight and obese prepubertal children. *The Journal of clinical endocrinology and metabolism* **2008**, *93*, 2122–2128.
14. Goran, M.I.; Bergman, R.N.; Gower, B.A. Influence of total vs. visceral fat on insulin action and secretion in African American and white children. *Obesity research* **2001**, *9*, 423–431.
15. Samouda, H.; Beaufort, C. de; Stranges, S.; Guinhouya, B.C.; Gilson, G.; Hirsch, M.; Jacobs, J.; Leite, S.; Vaillant, M.; Dadoun, F. Adding anthropometric measures of regional adiposity to BMI improves prediction of cardiometabolic, inflammatory and adipokines profiles in youths: a cross-sectional study. *BMC pediatrics* **2015**, *15*, 168.

16. Kelly, A.S.; Dengel, D.R.; Hodges, J.; Zhang, L.; Moran, A.; Chow, L.; Sinaiko, A.R.; Steinberger, J. The relative contributions of the abdominal visceral and subcutaneous fat depots to cardiometabolic risk in youth. *Clinical obesity* **2014**, *4*, 101–107.
17. He, F.; Rodriguez-Colon, S.; Fernandez-Mendoza, J.; Vgontzas, A.N.; Bixler, E.O.; Berg, A.; Imamura Kawasawa, Y.; Sawyer, M.D.; Liao, D. Abdominal obesity and metabolic syndrome burden in adolescents--Penn State Children Cohort study. *Journal of clinical densitometry : the official journal of the International Society for Clinical Densitometry* **2015**, *18*, 30–36.
18. Yang, H.R.; Chang, E.J. Insulin resistance, body composition, and fat distribution in obese children with nonalcoholic fatty liver disease. *Asia Pacific journal of clinical nutrition* **2016**, *25*, 126–133.
19. Zimmermann, M.B.; Gübeli, C.; Püntener, C.; Molinari, L. Detection of overweight and obesity in a national sample of 6-12-y-old Swiss children: accuracy and validity of reference values for body mass index from the US Centers for Disease Control and Prevention and the International Obesity Task Force. *The American journal of clinical nutrition* **2004**, *79*, 838–843.
20. Donini, L.M.; Poggiogalle, E.; Del Balzo, V.; Lubrano, C.; Faliva, M.; Opizzi, A.; Perna, S.; Pinto, A.; Rondanelli, M. How to Estimate Fat Mass in Overweight and Obese Subjects. *International journal of endocrinology* **2013**, *2013*.
21. Karlsson, A.-K.; Kullberg, J.; Stokland, E.; Allvin, K.; Gronowitz, E.; Svensson, P.-A.; Dahlgren, J. Measurements of total and regional body composition in preschool children: A comparison of MRI, DXA, and anthropometric data. *Obesity (Silver Spring, Md.)* **2013**, *21*, 1018–1024.
22. Eloi, J.C.; Epifanio, M.; Gonçalves, M.M. de; Pelliccioli, A.; Vieira, P.F.G.; Dias, H.B.; Bruscato, N.; Soder, R.B.; Santana, J.C.B.; Mouzaki, M.; *et al.* Quantification of Abdominal Fat in Obese and Healthy Adolescents Using 3 Tesla Magnetic Resonance Imaging and Free Software for Image Analysis. *PloS one* **2017**, *12*, e0167625.
23. Springer, F.; Ehehalt, S.; Sommer, J.; Ballweg, V.; Machann, J.; Binder, G.; Claussen, C.D.; Schick, F. Predicting volumes of metabolically important whole-body adipose tissue compartments in overweight and obese adolescents by different MRI approaches and anthropometry. *European journal of radiology* **2012**, *81*, 1488–1494.

24. Laddu, D.R.; Lee, V.R.; Blew, R.M.; Sato, T.; Lohman, T.G.; Going, S.B. Predicting visceral adipose tissue by MRI using DXA and anthropometry in adolescents and young adults. *International journal of body composition research* **2012**, *10*, 93–100.
25. Koren, D.; Marcus, C.L.; Kim, C.; Gallagher, P.R.; Schwab, R.; Bradford, R.M.; Zemel, B.S. Anthropometric predictors of visceral adiposity in normal-weight and obese adolescents. *Pediatric diabetes* **2013**, *14*, 575–584.
26. Ludescher, B.; Machann, J.; Eschweiler, G.W.; Vanhöfen, S.; Maenz, C.; Thamer, C.; Claussen, C.D.; Schick, F. Correlation of fat distribution in whole body MRI with generally used anthropometric data. *Investigative radiology* **2009**, *44*, 712–719.
27. Thomas, E.L.; Parkinson, J.R.; Frost, G.S.; Goldstone, A.P.; Doré, C.J.; McCarthy, J.P.; Collins, A.L.; Fitzpatrick, J.A.; Durighel, G.; Taylor-Robinson, S.D.; *et al.* The missing risk: MRI and MRS phenotyping of abdominal adiposity and ectopic fat. *Obesity (Silver Spring, Md.)* **2012**, *20*, 76–87.
28. Müller, M.J.; Braun, W.; Enderle, J.; Bosy-Westphal, A. Beyond BMI: Conceptual Issues Related to Overweight and Obese Patients. *Obesity facts* **2016**, *9*, 193–205.
29. Müller, M.J.; Lagerpusch, M.; Enderle, J.; Schautz, B.; Heller, M.; Bosy-Westphal, A. Beyond the body mass index: tracking body composition in the pathogenesis of obesity and the metabolic syndrome. *Obesity reviews : an official journal of the International Association for the Study of Obesity* **2012**, *13 Suppl 2*, 6–13.
30. Kim, J.A.; Park, H.S. Association of abdominal fat distribution and cardiometabolic risk factors among obese Korean adolescents. *Diabetes & metabolism* **2008**, *34*, 126–130.
31. Liu, P.; Ma, F.; Lou, H.; Liu, Y. The utility of fat mass index vs. body mass index and percentage of body fat in the screening of metabolic syndrome. *BMC public health* **2013**, *13*, 629.
32. Kim, J.Y.; Oh, S.; Chang, M.R.; Cho, Y.G.; Park, K.H.; Paek, Y.J.; Yoo, S.H.; Cho, J.J.; Caterson, I.D.; Song, H.J. Comparability and utility of body composition measurement vs. anthropometric measurement for assessing obesity related health risks in Korean men. *International journal of clinical practice* **2013**, *67*, 73–80.

33. Qi, Q.; Hua, S.; Ferreira, K.M.; Cai, J.; van Horn, L.; Schneiderman, N.; Thyagarajan, B.; Delamater, A.M.; Kaplan, R.C.; Isasi, C.R. Sex Differences in Associations of Adiposity Measures and Insulin Resistance in US Hispanic/Latino Youth: The Hispanic Community Children's Health Study/Study of Latino Youth (SOL Youth). *The Journal of clinical endocrinology and metabolism* **2017**, *102*, 185–194.
34. Weber, D.R.; Leonard, M.B.; Shults, J.; Zemel, B.S. A comparison of fat and lean body mass index to BMI for the identification of metabolic syndrome in children and adolescents. *The Journal of clinical endocrinology and metabolism* **2014**, *99*, 3208–3216.
35. Pourshahidi, L.K.; Wallace, J.M.W.; Mulhern, M.S.; Horigan, G.; Strain, J.J.; McSorley, E.M.; Magee, P.J.; Bonham, M.P.; Livingstone, M.B.E. Indices of adiposity as predictors of cardiometabolic risk and inflammation in young adults. *Journal of human nutrition and dietetics : the official journal of the British Dietetic Association* **2016**, *29*, 26–37.
36. Elffers, T.W.; Mutsert, R. de; Lamb, H.J.; Roos, A. de; van Willems Dijk, K.; Rosendaal, F.R.; Jukema, J.W.; Trompet, S. Body fat distribution, in particular visceral fat, is associated with cardiometabolic risk factors in obese women. *PloS one* **2017**, *12*, e0185403.
37. Moura, F.A.; Carvalho, L.S.F.; Cintra, R.M.R.; Martins, N.V.; Figueiredo, V.N.; Quinaglia e Silva, J.C.; Almeida, O.L.R.; Coelho, O.R.; Sposito, A.C. Validation of surrogate indexes of insulin sensitivity in acute phase of myocardial infarction based on euglycemic-hyperinsulinemic clamp. *American journal of physiology. Endocrinology and metabolism* **2014**, *306*, E399-403.
38. DeFronzo, R.A.; Ferrannini, E.; Hendler, R.; Felig, P.; Wahren, J. Regulation of splanchnic and peripheral glucose uptake by insulin and hyperglycemia in man. *Diabetes* **1983**, *32*, 35–45.
39. Bosy-Westphal, A.; Kossel, E.; Goele, K.; Later, W.; Hitze, B.; Settler, U.; Heller, M.; Glüer, C.-C.; Heymsfield, S.B.; Müller, M.J. Contribution of individual organ mass loss to weight loss-associated decline in resting energy expenditure. *The American journal of clinical nutrition* **2009**, *90*, 993–1001.
40. Lagerpusch, M.; Enderle, J.; Eggeling, B.; Braun, W.; Johannsen, M.; Pape, D.; Müller, M.J.; Bosy-Westphal, A. Carbohydrate quality and quantity affect glucose and lipid metabolism during weight regain in healthy men. *The Journal of nutrition* **2013**, *143*, 1593–1601.

41. Yu, J.; Tao, Y.; Tao, Y.; Yang, S.; Yu, Y.; Li, B.; Jin, L. Optimal cut-off of obesity indices to predict cardiovascular disease risk factors and metabolic syndrome among adults in Northeast China. *BMC public health* **2016**, *16*, 1079.
42. Xu, T.; Liu, J.; Liu, J.; Zhu, G.; Han, S. Relation between metabolic syndrome and body compositions among Chinese adolescents and adults from a large-scale population survey. *BMC public health* **2017**, *17*, 337.
43. Bennet, L.; Stenkula, K.; Cushman, S.W.; Brismar, K. BMI and waist circumference cut-offs for corresponding levels of insulin sensitivity in a Middle Eastern immigrant versus a native Swedish population - the MEDIM population based study. *BMC public health* **2016**, *16*, 1242.
44. Hübers, M.; Pourhassan, M.; Braun, W.; Geisler, C.; Müller, M.J. Definition of new cut-offs of BMI and waist circumference based on body composition and insulin resistance: Differences between children, adolescents and adults. *Obesity Science & Practice* **2017**, *3*, 272–281.
45. Plachta-Danielzik, S.; Landsberg, B.; Lange, D.; Langnäse, K.; Müller, M.J. 15 Jahre Kieler Adipositas-Präventionsstudie (KOPS). Ergebnisse sowie deren Einordnung und Bedeutung für die Prävention von Adipositas bei Kindern und Jugendlichen. *Bundesgesundheitsblatt, Gesundheitsforschung, Gesundheitsschutz* **2011**, *54*, 304–312.
46. Kromeyer-Hauschild, K.; Wabitsch, M.; Kunze, D.; Geller, F.; Geiß, H.C.; Hesse, V.; Hippel, A. von; Jaeger, U.; Johnsen, D.; Korte, W.; *et al.* Perzentile für den Body-mass-Index für das Kindes- und Jugendalter unter Heranziehung verschiedener deutscher Stichproben. *Monatsschrift Kinderheilkunde* **2001**, *149*, 807–818.
47. Kromeyer-Hauschild, K.; Gläßer, N.; Zellner, K. Perzentile für den Taillenumfang von Jenaer Kindern im Alter von 6 bis 18 Jahren. *Akt Ernähr Med* **2008**, *33*, 116–122.
48. World Health Organization. *Obesity and overweight: Fact sheet Updated October 2017*; 2017. Available online: <http://www.who.int/mediacentre/factsheets/fs311/en/> (accessed on 25 October, 2017).
49. Report of the task group on reference man ICRP Publication 23 (1975). *Annals of the ICRP* **1980**, *4*, III-III.

50. Martin, A.D.; Daniel, M.Z.; Drinkwater, D.T.; Clarys, J.P. Adipose tissue density, estimated adipose lipid fraction and whole body adiposity in male cadavers. *International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity* **1994**, *18*, 79–83.
51. Fang, L.; Guo, F.; Zhou, L.; Stahl, R.; Grams, J. The cell size and distribution of adipocytes from subcutaneous and visceral fat is associated with type 2 diabetes mellitus in humans. *Adipocyte* **2015**, *4*, 273–279.
52. Engin, A. Fat Cell and Fatty Acid Turnover in Obesity. *Advances in experimental medicine and biology* **2017**, *960*, 135–160.
53. Rutkowski, J.M.; Stern, J.H.; Scherer, P.E. The cell biology of fat expansion. *The Journal of cell biology* **2015**, *208*, 501–512.
54. Choe, S.S.; Huh, J.Y.; Hwang, I.J.; Kim, J.I.; Kim, J.B. Adipose Tissue Remodeling: Its Role in Energy Metabolism and Metabolic Disorders. *Frontiers in endocrinology* **2016**, *7*, 30.
55. Genske, F.; Kühn, J.-P.; Pietzner, M.; Homuth, G.; Rathmann, W.; Grabe, H.J.; Völzke, H.; Wallaschofski, H.; Friedrich, N. Abdominal fat deposits determined by magnetic resonance imaging in relation to leptin and vaspin levels as well as insulin resistance in the general adult population. *International journal of obesity (2005)* **2017**.
56. Landgraf, K.; Rockstroh, D.; Wagner, I.V.; Weise, S.; Tauscher, R.; Schwartz, J.T.; Löffler, D.; Bühligen, U.; Wojan, M.; Till, H.; *et al.* Evidence of early alterations in adipose tissue biology and function and its association with obesity-related inflammation and insulin resistance in children. *Diabetes* **2015**, *64*, 1249–1261.
57. Kursawe, R.; Eszlinger, M.; Narayan, D.; Liu, T.; Bazuine, M.; Cali, A.M.G.; D'Adamo, E.; Shaw, M.; Pierpont, B.; Shulman, G.I.; *et al.* Cellularity and adipogenic profile of the abdominal subcutaneous adipose tissue from obese adolescents: Association with insulin resistance and hepatic steatosis. *Diabetes* **2010**, *59*, 2288–2296.
58. Jernås, M.; Palming, J.; Sjöholm, K.; Jennische, E.; Svensson, P.-A.; Gabrielsson, B.G.; Levin, M.; Sjögren, A.; Rudemo, M.; Lystig, T.C.; *et al.* Separation of human adipocytes by size: Hypertrophic fat cells display distinct gene expression. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **2006**, *20*, 1540–1542.

59. Cotillard, A.; Poitou, C.; Torcivia, A.; Bouillot, J.-L.; Dietrich, A.; Klöting, N.; Grégoire, C.; Lolmede, K.; Blüher, M.; Clément, K. Adipocyte size threshold matters: Link with risk of type 2 diabetes and improved insulin resistance after gastric bypass. *The Journal of clinical endocrinology and metabolism* **2014**, *99*, E1466-70.
60. Kim, J.I.; Huh, J.Y.; Sohn, J.H.; Choe, S.S.; Lee, Y.S.; Lim, C.Y.; Jo, A.; Park, S.B.; Han, W.; Kim, J.B. Lipid-overloaded enlarged adipocytes provoke insulin resistance independent of inflammation. *Molecular and cellular biology* **2015**, *35*, 1686–1699.

SUMMARY

In children, adolescents and adults, overweight and obesity are defined by body mass index (BMI) only. In adults, respective BMI *cut-offs* are associated with mortality risk. By contrast, in children and adolescents, established *cut-offs* are based on statistical normative analyses and are not directly related to any health outcome.

Overweight and obesity are characterized by excessive fat mass (FM), which again is associated with cardiometabolic diseases. This association is not accurately represented by BMI. Accordingly, BMI can neither differentiate between FM and fat-free mass (FFM) nor give detailed information about body fat distribution. In addition, waist circumference (WC) does not distinguish between visceral and subcutaneous adipose tissue (VAT, SAT). Finally, there are differences in the association between BMI and morbidity between age groups.

Within this thesis, the associations between anthropometric traits, detailed body composition and cardiometabolic risks in children, adolescents and adults are investigated and systematically compared. The results show the limited value of anthropometric traits in relation to regional fat depots and cardiometabolic risks.

Within this thesis we investigated whether (i) associations between regional fat depots and cardiometabolic risks differ in normal- and overweight children, adolescents and adults, (ii) normative *cut-offs* of BMI and WC are appropriate for metabolic risk assessment when compared with directly measured regional fat depots and metabolic derived *cut-offs* and (iii) fat fraction per adipose tissue adds to metabolic risk assessment.

The main results of this thesis are:

- (i) Associations between regional fat depots and cardiometabolic risks became evident after puberty only. Insulin resistance was strongest correlated with VAT. Independent of BMI, VAT was associated with insulin resistance in older adults only.
- (ii) When compared with metabolic derived values, normative *cut-offs* are not appropriate to define children, adolescents and adults with insulin resistance.
- (iii) FM and TAT were strongly correlated. However, substantial inter-individual variances in fat fraction per adipose tissue were found. Regarding this, fat fraction per adipose tissue added value to metabolic risk assessment independent of FM or TAT.

These findings demonstrate the need for future differentiated and metabolic disorders orientated (e.g. insulin resistance) *cut-offs* for metabolic risk assessment of obese people. For the first time, the *cut-offs* reported here allow a risk assessment of obesity that is comparable for

different age groups. It also becomes apparent that the health problem of "being overweight" is greater than previously assumed based on statistical normative data.

ZUSAMMENFASSUNG

Übergewicht und Adipositas werden bei Kindern, Jugendlichen und Erwachsenen ausschließlich anhand des Body Mass Index (BMI) definiert. Die Grenzwerte des BMI sind bei Erwachsenen zum Mortalitätsrisiko assoziiert. Hingegen beruhen die Grenzwerte bei Kindern und Jugendlichen auf statistisch normativen Analysen und nicht direkt auf gesundheitlichen Folgen.

Übergewicht und Adipositas sind durch eine hohe Fettmasse (FM) gekennzeichnet, die wiederum mit kardio-metabolischen Erkrankungen einhergeht. Diese Beziehung wird durch den BMI nicht präzise abgebildet. Der BMI kann weder zwischen FM und fettfreier Masse (FFM) unterscheiden noch erlaubt er eine detaillierte Beschreibung der Körperfettverteilung. Auch der Taillenumfang (TU) ermöglicht keine Aussage zum Verhältnis von viszeralem und subkutanem Fettgewebe (VAT, SAT). Letztendlich bestehen zwischen den verschiedenen Altersgruppen Unterschiede in der Beziehung zwischen BMI und der Morbidität.

In dieser Arbeit werden die Beziehungen zwischen anthropometrischen Parametern, detaillierter Körperzusammensetzung und kardio-metabolischen Risiken bei Kindern, Jugendlichen und Erwachsenen untersucht und systematisch miteinander verglichen. Die Ergebnisse zeigen den begrenzten Wert anthropometrischer Parameter in Bezug auf die Erfassung von regionalen Fettdepots und kardio-metabolischen Risiken.

In dieser Arbeit wurde untersucht, ob (i) sich die Zusammenhänge zwischen regionalen Fettdepots und kardio-metabolischen Risiken bei normal- und übergewichtigen Kindern, Jugendlichen und Erwachsenen unterscheiden, (ii) normative Grenzwerte von BMI und TU, verglichen zu anhand der direkten Messung individueller Fettdepots und metabolisch definierten Grenzwerten, für die gesundheitliche Bewertung geeignet sind und (iii) FM pro Fettgewebe (FG) zur Beurteilung des metabolischen Risikos einen additiven Nutzen hat.

Die wesentlichen Ergebnisse dieser Arbeit sind:

(i) Die Zusammenhänge zwischen regionalen Fettdepots und kardio-metabolischen Risiken wurden erst nach der Pubertät evident. Die Insulinresistenz war am stärksten mit VAT korreliert. Unabhängig vom BMI, war die Insulinresistenz mit VAT ausschließlich im späteren Erwachsenenalter assoziiert.

(ii) Verglichen zum metabolisch definierten Grenzwert sind normative Grenzwerte für die Bestimmung der Insulinresistenz bei Kindern, Jugendlichen und Erwachsenen nicht angemessen.

(iii) FM und FG korrelierten stark. Es bestanden jedoch erhebliche inter-individuelle Unterschiede in der FM pro FG. Unabhängig von FM oder FG hat der Fettanteil pro Fettgewebe zur metabolischen Risikobewertung beigetragen.

Diese Ergebnisse belegen die Notwendigkeit von zukünftig differenzierten und an möglichen Stoffwechselstörung (wie der Insulinresistenz) orientierten Grenzwerten für die Beurteilung des gesundheitlichen Risikos übergewichtiger Menschen. Die hier mitgeteilten Grenzwerte erlauben erstmalig eine für die verschiedenen Altersgruppen vergleichbare Risikoeinschätzung von Übergewicht. Es wird auch offensichtlich, dass das gesundheitliche Problem "Übergewicht" größer ist, als es bisher anhand von statistisch-normativen Daten angenommen wurde.

APPENDIX

Ethical approvals: Titles and reference numbers

Declaration of co-authorship Chapter II

Declaration of co-authorship Chapter III

Declaration of co-authorship Chapter IV

Danksagung

Lebenslauf

Ethical approvals: Titles and reference numbers

AZ: A 122/07

Einfluss der Körperzusammensetzung auf die Varianz des Ruheenergieverbrauchs und das metabolische Risiko bei Kindern und Jugendlichen im Alter von 6-19 Jahren.

AZ: 167/08

Einfluß einer Gewichtsreduktion auf die Körperzusammensetzung, den Energieverbrauch sowie auf das kardiometabolische Risiko bei Jugendlichen.

AZ: A 111/96

Variabilität des Energieverbrauchs: Zur Bedeutung der Körperzusammensetzung.

AZ: A 138/09

Metabolisch-funktionelle Phänotypen der adaptiven Thermogenese bei kontrollierter Über- und Unterernährung.

AZ: A126/09

Variabilität des Energieverbrauchs: Zur Bedeutung der Körperzusammensetzung.

AZ: A 155/10

Untersuchungen zum langfristigen Erhalt einer diätetischen Gewichtsreduktion bei adipösen Erwachsenen.

AZ: A 103/11

Einfluss des Glykämischen Indexes auf die metabolische Anpassung bei kontrollierter Über- und Unterernährung.

AZ: A 111/11

Generation of prediction equations to analyze body composition of adults based on Bioelectrical Impedance analysis (BIA).

AZ: A101/13

Einfluss von kontrollierter Über- und Unterernährung auf die adaptive Thermogenese.

AZ: A 36/95

Prävention der Adipositas bei Kindern und Jugendlichen

Declaration of co-authorship Chapter II

C	A	U	Christian-Albrechts-Universität zu Kiel	Agrar- und Ernährungs- wissenschaftliche Fakultät
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Declaration of co-authorship

If a dissertation is based on already published or submitted co-authored articles, a declaration from each of the authors regarding the part of the work done by the doctoral candidate must be enclosed when submitting the dissertation.

1. Doctoral candidate

Name: Mark Hübers

2. This co-author declaration applies to the following article:

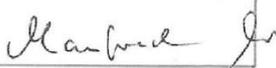
Association between individual fat depots and cardio-metabolic traits in normal- and overweight children, adolescents and adults

The extent of the doctoral candidat's contribution to the article is assessed on the following scale:

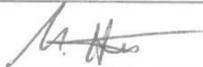
- A. Has contributed to the work (0-33%)
- B. Has made a substantial contribution (34-66%)
- C. Did the majority of the work independently (67-100%)

3. Declaration on the individual phases of the scientific work (A,B,C)	Extent
Concept: Formulation of the basic scientific problem based on theoretical questions which require clarification, including a summery of the general questions which, it is assumed, will be answerable via analyses or concrete experiments/investigations	A
Planning: Planning of experiments/analyses and formulation of investigative methodology, including choice of method and independent methodological development, in such a way that the scientific questions asked can be expected to be answered	B
Execution: Involvement in the analysis or the concrete experiments/investgation	C
Manuscript preparation: Presentation, interpretation and discussion of the results obtained in article form	C

4. Signature of all co-authors

Date	Name	Signature
14.11.17	Corinna Geisler	
10.11.17	Sandra Plachta-Danielzik	
14.11.17	Manfred J. Müller	

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Date	Name	Signature
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Declaration of co-authorship Chapter III

C	A	U	Christian-Albrechts-Universität zu Kiel	Agrar- und Ernährungs- wissenschaftliche Fakultät
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Declaration of co-authorship

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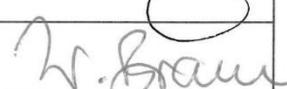
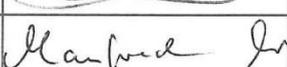
Definition of new cut-offs of BMI and waist circumference based on body composition and insulin resistance: differences between children, adolescents and adults

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- B. Has made a substantial contribution (34-66%)
- C. Did the majority of the work independently (67-100%)

3. Declaration on the individual phases of the scientific work (A,B,C)	Extent
Concept: Formulation of the basic scientific problem based on theoretical questions which require clarification, including a summary of the general questions which, it is assumed, will be answerable via analyses or concrete experiments/investigations	A
Planning: Planning of experiments/analyses and formulation of investigative methodology, including choice of method and independent methodological development, in such a way that the scientific questions asked can be expected to be answered	B
Execution: Involvement in the analysis or the concrete experiments/investigation	C
Manuscript preparation: Presentation, interpretation and discussion of the results obtained in article form	C

4. Signature of all co-authors

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Date	Name	Signature
14.11.17	Mark Hübers	

Declaration of co-authorship Chapter IV

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Declaration of co-authorship

If a dissertation is based on already published or submitted co-authored articles, a declaration from each of the authors regarding the part of the work done by the doctoral candidate must be enclosed when submitting the dissertation.

1. Doctoral candidate

Name: Mark Hübers

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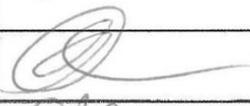
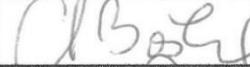
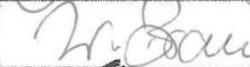
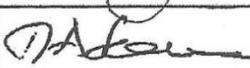
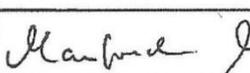
Association between fat mass, adipose tissue, fat fraction per adipose tissue and metabolic risks: a cross-sectional study in normal-, overweight and obese adults

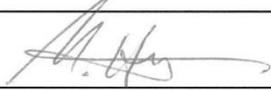
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Manuscript preparation: Presentation, interpretation and discussion of the results obtained in article form	B

4. Signature of all co-authors

Date	Name	Signature
16.11.17	Corinna Geisler	
16.11.17	Anja Bosy-Westphal	
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14.11.2017	Manfred J. Müller	

5. Signature of doctoral candidate		
Date	Name	Signature
15.11.17	Mark Hübers	

DANKSAGUNG

Meinem Doktorvater Herrn Prof. Dr. Manfred J. Müller gilt ein ganz außerordentlicher Dank. Sie haben mir nicht nur die Möglichkeit einer Promotion am Institut für Humanernährung und Lebensmittelkunde eingeräumt und damit mein Interesse an der Wissenschaft gefördert, sondern haben mich zu jeder Zeit unterstützt, meine Arbeitsschwerpunkte einer breiten wissenschaftlichen Öffentlichkeit zu präsentieren. Dadurch haben Sie neben meiner fachlichen Weiterbildung auf dem Gebiet der Körperzusammensetzung durch Diskussion und Kritik, stark zu meiner persönlichen Entwicklung beigetragen. Zusammenarbeit, Teamgeist und Durchsetzungswille sind bedeutend in Gesell- und Wissenschaft. Ich möchte mich Bedanken bei Ihnen für die vielen bedeutenden Erfahrungen, die ich während meiner Promotionszeit gewinnen durfte.

Zudem möchte ich mich bei der Graduiertenförderung der Christian-Albrechts-Universität zu Kiel bedanken, die mich während meiner Promotion finanziell unterstützt hat. Ferner gilt ein Dank an die Heinz-Wüstenberg-Stiftung, die mir einen Auslandsaufenthalt am Pennington Biomedical Research Center in Baton Rouge, Louisiana, USA ermöglicht. Ohne die von Ihnen gewährten Stipendien wäre die Aufnahme des Promotionsvorhabens, sowie eine tiefgreifende wissenschaftliche Fortbildung nicht möglich.

Ein weiterer besonderer Dank gilt Frau PD Dr. Sandra Plachta-Danielzik. Durch deine freundliche und unterstützende Art, unsere statistisch intensiven Diskussionen, sowie deinen fachlichen Hilfestellungen hast du sehr zum Gelingen der Doktorarbeit beigetragen.

Weiterhin möchte ich Frau Dr. Corinna Geisler für Ihren fachlichen Austausch danken. Diese haben meine Arbeit sehr vorangebracht. Darüber hinaus hatte ich durch das MaNuEl-Projekt (Malnutrition in the Elderly Knowledge Hub) eine finanzielle Sicherheit, meine Promotionsarbeit abzuschließen.

Danken möchte ich außerdem Frau Prof. Dr. Dr. Anja Bosy-Westphal für die fachlichen Diskussionen sowie Ihre kompetenten Anregungen.

Liebe Kolleginnen und Kollegen. Euch gilt ein ganz besonderer Dank. Jedem einzelnen von Euch! Ihr habt mit euren Persönlichkeiten dafür gesorgt, dass ich am Institut einen wunderbaren und aufregenden Lebensabschnitt verbringen durfte. Es gab so viele tolle Momente, die ich mit euch erleben konnte. Daher habt ihr maßgeblich an meiner persönlichen Entwicklung beigetragen.

Ein spezieller Dank gilt Frau Daniela Liesegang sowie allen Hiwis. Ihr habt dafür gesorgt, dass ich bei technisch und organisatorischen Problemen nie allein gelassen wurde. Jedes dieser Probleme habt ihr gelöst.

Außerdem möchte ich mich bei meiner Familie und meinen Freunden bedanken. Zu jeder Zeit, egal ob in positiven oder in weniger positiven Phasen, habe ich bei Euch ein offenes Ohr gefunden. Ihr habt mich unterstützt, bestärkt und in schwierigen Zeiten aufgebaut.

Mona, die Zeit meiner Promotion war spannend, positiv, lehrreich und auch aufreibend. Du warst in allen Momenten an meiner Seite. Wie lässt sich so eine Unterstützung in Worte fassen? – Ich bekam am ersten Tag meiner Promotion eine Karte von dir geschenkt „*Good thing needs while*“. Grammatikalisch bewusst inkorrekt hast du damit inhaltlich recht behalten. Meine Karte für dich lautet: *Good thing needs while and a beloved person with to live.*

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SCHULBILDUNG

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BERUFSAUSBILDUNG

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AKADEMISCHER WERDEGANG

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B.Sc. in Ökotrophologie Fachrichtung Ernährungswissenschaften
 Thema der Bachelorarbeit: Bewertung des Erfolgs des Programms „Fördekids XXL“
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04/2013 – 01/2015 Christian-Albrechts-Universität zu Kiel
M.Sc. in Ökotrophologie Fachrichtung Ernährungs- und Lebensmittelwissenschaften
 Thema der Masterarbeit: Diagnose und Prognose des gesundheitlichen Risikos von Kindern und Jugendlichen anhand des BMI sowie des Verhältnisses von Fettmasse und fettfreier Masse.
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BERUFSERFAHRUNGEN

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Freiberufliche Tätigkeit im Bereich Bewegungstraining für Seelenpflege-bedürftige Kinder

09/2011 – heute	THW Kiel e.V. Freiberufliche Tätigkeit im Bereich Bewegungstraining für übergewichtige Kinder und Jugendliche
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02/2015 – 06/2015	Leibniz-Institut für die Pädagogik der Naturwissenschaften und Mathematik in Kiel Technischer Angestellter im Projekt National Educational Panel Study

 STIPENDIEN

07/2015 – 10/2016	Graduiertenzentrum , Promotionsstipendium der Christian-Albrechts-Universität zu Kiel
06/2016	European Association for the Study of Obesity (EASO) , Reisetstipendium für den European Obesity Summit 2016 in Göteborg, Schweden
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 ORIGINALARBEITEN

Hübers, M.; Geisler, C.; Plachta-Danielzik, S.; Müller, M.J. Association between individual fat depots and cardio-metabolic traits in normal- and overweight children, adolescents and adults. *Nutrition & diabetes* 2017, 7, e267.

Hübers, M.; Pourhassan, M.; Braun, W.; Geisler, C.; Müller, M.J. Definition of new cut-offs of BMI and waist circumference based on body composition and insulin resistance: Differences between children, adolescents and adults. *Obesity science & practice* 2017, 3, 272–281.

Hübers, M.; Geisler, C.; Bosy-Westphal, A.; Braun, W.; Pourhassan, M.; Sørensen, T.I.A.; Müller, M.J. Association between fat mass, adipose tissue, fat fraction per adipose tissue, and metabolic risks: a cross-sectional study in normal, overweight, and obese adults. *European Journal of Clinical Nutrition* 2018, [Epub ahead of print].

Geisler, C.; **Hübers, M.**; Granert, O.; Müller, M.J. Contribution of structural brain phenotypes to the variance in resting energy expenditure in healthy Caucasian subjects. *Journal of applied physiology* 2017, ja00690.2017.

 KONGRESS- UND TAGUNGSBEITRÄGE

Hübers, M.; Daâs, B.; Claaß, A.; Plachta-Danielzik, S.; Müller, M.J. Bewertung des Schulungsprogramms Fördekids XXL. Vortrag im Rahmen der 29. Jahrestagung der Deutschen Adipositas-Gesellschaft in Hannover, 03.-05. Oktober 2013. In: *Adipositas* 2013 Vol. 7 Heft 3: A40.

Hübers, M.; Kehden, B.; Müller, M.J.; Plachta-Danielzik, S. Kann die Beurteilung des gesundheitlichen Risikos bei Kindern und Jugendlichen mittels BMI durch Hinzunahme des Verhältnisses von Fett- zu fettfreier Masse verbessert werden? Vortrag im Rahmen der 31. Jahrestagung der Deutschen Adipositas-Gesellschaft in Berlin, 15.-17. Oktober 2015. In: Adipositas 2015 Vol. 9 Heft 3: A11.

Hübers, M.; Pourhassan, M.; Humeida, H.; Braun, W.; Geisler, C.; Glüer, C.C.; Plachta-Danielzik, S.; Müller, M.J. Metabolic Syndrome in obese children and adolescents – influence of the degree of obesity, age, gender and puberty. Postervortrag im Rahmen des 1. European Obesity Summit in Göteborg, 01.-04. Juni 2016. In: Obesity Facts 2016 Vol. 9 (Supplement 1): 104.

Hübers, M.; Pourhassan, M.; Humeida, H.; Braun, W.; Geisler, C.; Glüer, C.C.; Plachta-Danielzik, S.; Müller, M.J. Metabolisches Syndrom bei adipösen Kindern und Jugendlichen – Einfluss von Adipositasausmaß, Alter, Geschlecht und Pubertät. Postervortrag im Rahmen der 112. Jahrestagung der Deutschen Gesellschaft für Kinder- und Jugendmedizin in Hamburg, 14.-17. September 2016.

Hübers, M.; Müller, M.J. Linking Body Fat to Disease Risk. Vortrag im Rahmen der Tagung *The Power of Programming 2016 - Developmental Origins of Adiposity and Long-term Health* in München, 13.-15. Oktober 2016.

Hübers, M.; Pourhassan, M.; Humeida, H.; Geisler, C.; Plachta-Danielzik, S.; Müller, M.J. Ist die Diagnose des Metabolischen Syndroms bei Kindern und Jugendlichen sinnvoll? Vortrag und Poster im Rahmen der 32. Jahrestagung der Deutschen Adipositas-Gesellschaft in Frankfurt, 17.-19. November 2016. In: Adipositas 2016 Vol. 10 Heft 4: A22.

Hübers, M.; Pourhassan, M.; Humeida, H.; Geisler, C.; Plachta-Danielzik, S.; Müller, M.J. Der Taillenumfang ist als indirektes Maß der viszeralen Fettmasse bei Kindern und Jugendlichen nicht geeignet. Vortrag im Rahmen des 54. Wissenschaftlichen Kongresses der Deutschen Gesellschaft für Ernährung in Kiel, 01.-03. März 2017. In: Proceedings of the German Nutrition Society 2017 Vol. 23: 28.

Hübers, M.; Pourhassan, M.; Braun, W.; Geisler, C.; Müller, M.J. Beziehung von regionalen Fettdepots zu BMI und Taillenumfang bei Kindern, Jugendlichen und Erwachsenen: Definition neuer Grenzwerte von BMI und Taillenumfang basierend auf Körperzusammensetzung und Insulinresistenz. Vortrag im Rahmen der 33. Jahrestagung der Deutschen Adipositas-Gesellschaft in Potsdam, 28.-30. September 2017. In: Adipositas 2017 Vol. 11 Heft 3:A2.