Original Article
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Obesity

Relationships of Body Composition and Liver Fat Content with Insulin Resistance in Obesity-Matched Adolescents and Adults

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Objective: While in adults not total body- or visceral fat mass, but liver fat content was found to independently determine insulin resistance, it is unclear whether these relationships are already present in obese adolescents.

Methods: Thirty-nine overweight/obese adolescents were matched for sex and BMI with 39 adults. To compare the age- and sex-specific BMI values of adolescents and adults, the percentile value of each adolescent was projected to the age of 18. Body fat depots were quantified by whole-body magnetic resonance (MR) imaging. Liver fat content was measured with ^{1}H-MR spectroscopy. Insulin resistance was estimated from the homeostasis model assessment of insulin resistance (HOMA-IR).

Results: Compared to overweight and obese adults, adolescents had higher HOMA-IR ($P < 0.001$) and lower lean body mass ($P = 0.002$). Furthermore, they had higher total body- ($P = 0.02$), but lower visceral- ($P < 0.001$) fat mass, while liver fat content was not significantly different between the groups ($P = 0.16$). In both groups liver fat content (both $P \leq 0.007$), but not total body- or visceral fat mass (all $P \geq 0.64$) was an independent predictor of insulin resistance.

Conclusions: Having lower visceral fat mass, overweight and obese adolescents are more insulin resistant than sex- and BMI-matched adults. Liver fat content, but not total body- or visceral fat mass, is an independent determinant of insulin resistance in adolescents.

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Introduction

Human studies recently provided convincing evidence that increased total body- and visceral fat mass as well as ectopic fat deposition in the liver and the skeletal muscle determine insulin resistance in adults (1-4). Of these fat storage compartments, which strongly correlate with each other, high liver fat content, but not total body- or visceral fat mass, was found to be an independent determinant of insulin resistance in cross-sectional and longitudinal studies in adults (2,4,5). Thus, although the causes and consequences of hepatic steatosis with respect to insulin resistance are still not fully understood, increased accumulation of lipids in the liver is thought to considerably contribute to the pathogenesis of insulin resistance (6-9).

In children and adolescents, in whom obesity is recognized as an early major health threat, elevated total body- and visceral fat mass, as well as high liver fat content were also found to strongly determine insulin resistance and hyperglycemia (10-17). However, in childhood and adolescence there is little information about the independent impact of the body fat compartments and liver fat content on the determination of insulin resistance. D'Adamo et al. showed that in a group of adolescents with high liver fat content and insulin resistance, total body-, visceral-, and subcutaneous fat mass, as well

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as lean body mass and intra- and extramyocellular lipids did not differ from those of a group of adolescents with low liver fat content and high insulin sensitivity (14). On the other hand, it was shown that liver fat content and visceral fat mass, which both strongly determine insulin resistance in adolescents, also strongly correlate with each other (15,18).

To better understand the impact of expanded fat mass versus high liver fat content on insulin resistance in adolescents, it is now important to perform a matched comparison of these factors to investigate which of them are independent determinants of this condition. In addition, because of the phenomenon of physiological insulin resistance in puberty, it is unclear whether the body fat distribution pattern and the ectopic fat accumulation in the liver determine insulin resistance in children and adolescents as strongly as in adults.

In the present study, therefore, we asked firstly: how do these relationships in adolescents compare to the data in adults with a similar degree of obesity, and secondly: which of these parameters most strongly determine insulin resistance in adolescence? For this purpose we matched obese adolescents and adults who underwent the same phenotyping procedures to precisely quantify lean body mass, body fat mass, body fat distribution, and liver fat content.

Methods

Study population

Data from adolescents and adults, who participated in two studies in Tübingen, Germany, were included in this analysis. The adolescents participated in the Tübingen DISKUS study in which subjects were enrolled when having either a body mass index (BMI) $>$ 99.5th percentile or $>$ 90th percentile and at least one of the following metabolically relevant conditions: family history of type 2 diabetes, impaired glucose tolerance, or acanthosis nigricans (19). Adults participated in the Tübingen Lifestyle Intervention Program (TULIP), which was designed as a longitudinal intervention study for adult subjects at risk of developing insulin resistance and type 2 diabetes. In the TULIP study, the inclusion criteria were as follows: BMI $>$ 27 kg/m^2, family history of type 2 diabetes, impaired glucose tolerance and/or history of gestational diabetes in females (2). Both studies had been approved by the local institutional review board. Written informed consent was obtained from all participants and, in case of the DISKUS study, additionally from their legal guardians, prior to the study.

Anthropometry

All anthropometric measures were taken by trained healthcare professionals with standardized equipment. BMI was evaluated as a continuous measure (weight [kg]/height^2 [m^2]). For adolescents BMI data were referenced to age- and sex-specific percentiles. To compare these age- and sex-specific BMI values to the data of the adult population, and to identify adults having comparable BMI, the percentile value of each adolescent was projected to the age of 18 with the use of up-to-date BMI charts (20).

In the adolescents, pubertal maturity was assessed by a pediatric endocrinologist and rated using Tanner staging. The characteristics of the different maturation stages have been described and shown in photographs in their original papers by Marshall and Tanner (21,22). Tanner stage II occurs at start of puberty, while Tanner stage V is the final stage of puberty. This staging is the only accepted international standard to judge and communicate the clinical degree of pubertal maturity.

Metabolic data

Fasting glucose levels and fasting insulin levels were measured in the same laboratory. Blood glucose was determined using a bedside glucose analyzer (YSI, Yellow Springs, CO). Plasma insulin was determined on an ADVIA Centaur XP and all other routine parameters (e.g., liver enzymes) on an ADVIA 1800 clinical chemistry system (Siemens Healthcare systems, Erlangen, Germany). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated to obtain quantitative estimates of insulin sensitivity (23). Impaired fasting glucose [glucose levels 100 mg/dl (5.6 mmol/l) to 125 mg/dl (6.9 mmol/l)] was diagnosed according to the criteria of the Expert Committee on Diagnosis and Classification of Diabetes Mellitus (24).

MRI protocol whole-body fat distribution

For evaluation of fat distribution in both adolescents and adults, MR examinations were performed on the same 1.5 Tesla whole-body MR scanner (Magnetom Sonata, Siemens Healthcare, Erlangen, Germany). Whole-body fat distribution was evaluated using a 2D axial T1-weighted fast spin-echo sequence with an echo train length of 7 and the following parameters: echo time (TE) 12 ms, repetition time (TR) 490 ms, slice thickness 10 mm, 10 mm inter-slice gap, 5 slices per breath-hold (12 s acquisition time per each 5-slice stack (21,25). Field of view was adjusted to the body extension from 450 to 530 mm with a matrix size of 256 $\times$ 178. Volunteers were placed in a prone position with the arms extended over the head. After each breath-hold the table was shifted by 100 mm to acquire one adjacent stack of slices after the other, covering the complete body from toes to finger tips. Since the total table feed of the MR unit was limited to 110 cm, one rearrangement of each study subject was necessary (first half of examination from iliac crest to finger tips, second half from iliac crest to toes). To ensure identical slice positions after repositioning, participants were marked at the iliac crest. In this way, the complete body could be covered in about 100-130 axially oriented slices depending on each subject's body height. Total examination time was between 20 and 25 min. For all measurements the body coil was used as a combined transmit/receive coil in order to excite the proton spins homogenously and prevent inhomogeneous signal intensity of adipose tissue.

Post-processing of whole-body data

All recorded images were post-processed by a semi-automatic segmentation program using customized MATLAB routines (Version 6.5, MathWorks, Matick, MA). In principle, the routine identifies adipose tissue because of its higher signal intensity compared to other tissues on T1-weighted fast spin-echo images. Applying a suitable signal intensity threshold, adipose tissue can be accurately separated from non-adipose tissue, air containing structures or background noise as previously published. Whole-body volumes of different adipose tissue compartments were quantified from data with complete body coverage: total adipose tissue and visceral adipose tissue were evaluated. Volumes of adipose tissue were

TABLE 1 Subject characteristics

	Adolescents (n = 39)	Adults (n = 39)	P value
Sex (f/m)	18/21	18/21	
Age (years)	14.0 (13.6, 14.4)	42.6 (40.0, 45.2)	<0.001
Weight (kg)	91.8 (84.7, 98.9)	98.6 (92.8, 104.4)	0.09
Height (cm)	166.7 (163.3, 170.2)	172.7 (169.7, 175.6)	0.01
BMI (kg/m^2)	32.9 (31.6, 34.2)	32.8 (31.6, 34.1)	0.98
Lean body mass (kg)	47.3 (43.7, 51.0)	56.0 (52.1, 60.0)	0.002
Total body fat mass (kg)	38.1 (33.9, 42.4)	31.7 (28.9, 34.5)	0.02
Visceral fat mass (kg)	2.2 (1.9, 2.5)	3.9 (3.2, 4.6)	<0.001
Total subc. fat mass (kg)	36.0 (31.9, 40.0)	27.8 (25.0, 30.6)	0.003
Abdominal subc. fat mass (kg)	17.1 (14.9, 19.2)	14.9 (13.4, 16.3)	0.25
Liver fat content (%)	8.1 (6.2, 10.0)	7.2 (4.7, 9.8)	0.16
Fatty liver[a]	n = 25/39	n = 16/39	0.04
Fast. glucose (mmol/l)	5.0 (4.9, 5.2)	5.3 (5.1, 5.4)	0.13
Fast. ins. (pmol/l)	187 (149, 225)	79 (57, 101)	<0.001
HOMA-IR	7.0 (5.5, 8.4)	3.2 (2.2, 4.2)	<0.001
Impaired fast. glycemia[a]	n = 3/39	n = 11/39	0.02

Data are means (95% confidence intervals); f: female; m: male; BMI: body mass index; HOMA-IR: homeostasis model assessment of insulin resistance;
[a]χ^2-test.

calculated by multiplying the corresponding number of segmented pixels by in-plane pixel dimensions and slice thickness. Volumes of adipose tissue compartments were referenced to individual body height and weight before different study groups were compared. Total lean body mass was derived by separating the total body adipose tissue from the total body volume.

Proton magnetic resonance spectroscopy of intrahepatic lipids

In vivo proton magnetic resonance spectroscopy (MRS) was performed on the identical whole-body MR scanner (Magnetom Sonata, Siemens Healthcare, Erlangen, Germany) within the same imaging session as the measurement of whole-body fat distribution. Participants were placed on the 6-channel spine array receiver coil of the manufacturer in supine position. A conventional T1-weighted gradient echo scouting sequence was used to identify liver parenchyma and correctly set the volume-of-interest (VOI) for MRS.

A single-voxel stimulated-echo technique (STEAM) was applied in order to acquire spectroscopic data. The VOI was placed in the posterior part of liver segment 7 (according to the Couinaud classification). The size of the VOI was set to 3.0 × 3.0 × 2.0 cm^3 to be able to avoid macroscopic visible vessels on the one hand, but also to obtain a sufficiently high signal-to-noise ratio on the other. Measurement parameters were chosen as follows: TR = 4 s; TE = 10 ms, mixed time (TM) = 15 ms. Automatic shimming of the VOI was performed prior to data acquisition during which the participants were requested to breathe only lightly. Participants were told to breathe in and out between each radio frequency (RF) excitation (~3 s) and hold breath in mild expiration during each data acquisition (~1 s). For training purposes the first eight excitations were used as dummy excitations, after these eight dummy excitations 32 spectroscopic data acquisitions were obtained for averaging and subsequent data evaluation. This MRS protocol resulted in a total acquisition time of 2 min 40 s in addition to the time needed for the automatic shimming procedure. For quantification of intrahepatic lipid content, the signal integrals of water (at 4.7 ppm) and lipids (combined integral of methyl and methylene at 1.3 ppm and 0.95 ppm) were evaluated. Integrals (Int) of the spectral peaks were evaluated in fixed frequency intervals (water: 3.1-6.2 ppm; lipids: 0.5-1.8 ppm). Intrahepatic lipids (IHL) was then calculated as the ratio of Int(lipids) over Int(lipids+water). Fatty liver was defined as liver fat content > 5.56% (26).

Statistics

Data are presented as means and 95% confidence intervals. Variables were tested for normality using the Shapiro–Wilk test, and equality of variances was assessed using Bartlett's test. Where possible, non-normally distributed variables were log-transformed prior to parametric data analysis. Otherwise, the non-parametric Wilcoxon rank-sum test was used to evaluate differences between groups (e.g., total body- and visceral adipose tissue). The strength of relationship between different measures and the HOMA-IR was estimated using the Pearson correlation coefficient. Multiple *post-hoc* pairwise comparisons were performed using the Tukey–Kramer method. The independent relationship between HOMA-IR and age, sex, and body composition factors was estimated using multiple linear regression. The significance level was set at 5%, thus, a P-value ≤0.05 indicates a statistically significant result. All data of all volunteers were analyzed using the JMP 10.0 statistical software package (SAS Institute, Cary, NC).

Results

Characteristics of adolescents and adults

The matching process resulted in an identical sex and an almost identical BMI distribution (P = 0.98) between the groups of adolescents and adults. As expected, body weight was not statistically different between the groups, and adolescents had a lower mean height

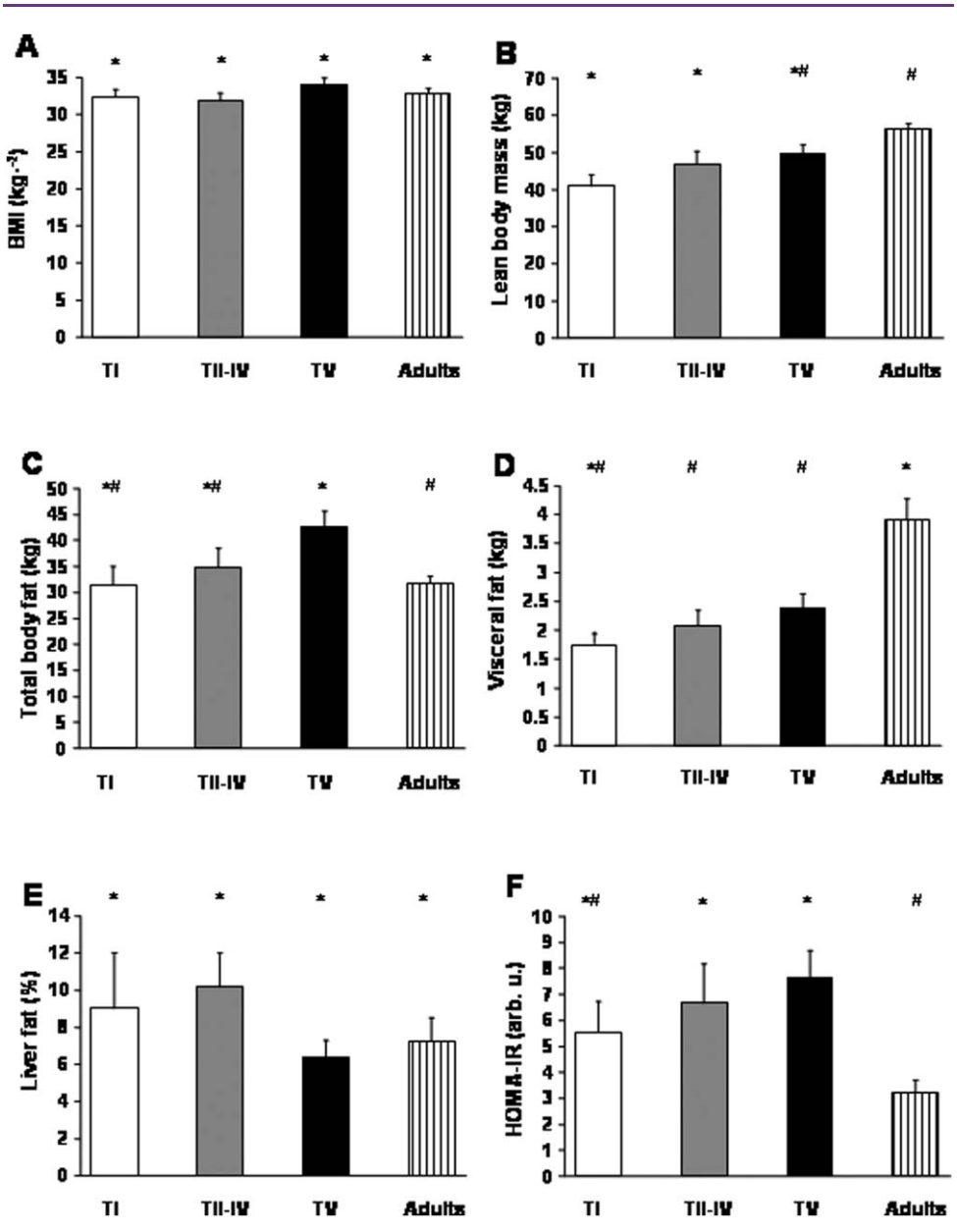

Figure 1 Body fat content, body fat distribution, lean body mass, liver fat content, and HOMA-IR of the 39 adolescents according to Tanner stage and of the 39 adults [TI: $n = 6$, age 12.3 years (upper, lower 95% mean 11.6, 13.0); TII-IV: $n = 14$, age 13.5 years (13.0, 14.1); TV: $n = 19$, age 14.9 years (14.4, 15.3). Values (means and SEM) that are not connected by the same symbol are statistically different from each other at $P < 0.05$ after correction for multiple comparisons (Tukey–Kramer test).

compared to adults. With respect to body composition, adolescents had a lower lean body mass, but a higher total fat mass. The visceral fat mass was lower than in adults. Furthermore, while mean liver fat content was not significantly higher in adolescents, they had a higher prevalence of fatty liver (Table 1).

While the mean fasting glucose level was also not statistically different between the groups, in the adolescent group fewer subjects had impaired fasting glycemia. In contrast, fasting insulin levels were higher in adolescents. Accordingly, adolescents also had a higher HOMA-IR (Table 1).

When the adolescents were grouped according to the Tanner stages, those in Tanner stage V had a similar BMI, lean body mass, and liver fat content as the adults (Figure 1, panels A, B, and E). Yet, they differed from them strongly with respect to total body- and visceral fat mass and to the HOMA-IR (Figure 1, panels C, D, and F).

Next, we investigated which of the anthropometric measures, that were different between the groups, could explain the differences in HOMA-IR between the adolescents and the adults. For this purpose, the following variables were included into multiple regression models: age, sex, lean body mass, total body-, visceral fat mass, and liver fat content.

TABLE 2 Univariate relationships of anthropometrics and glucose and insulin levels with HOMA-IR

	Adolescents		Adults	
	r	*P*	*r*	*P*
Age	0.31	0.05	−0.10	0.53
Weight	0.48	0.002	0.43	0.007
Height	0.37	0.02	0.05	0.75
BMI	0.33	0.03	0.62	<0.001
Fast. glucose	0.31	0.05	0.64	<0.001
Fast. insulin	0.99	<0.001	0.99	<0.001
Lean body mass	0.47	0.003	0.36	0.02
Total body fat mass	0.32	0.05	0.38	0.02
Visceral fat mass	0.38	0.02	0.34	0.03
Total subc. fat mass	0.31	0.06	0.29	0.07
Abdominal subc. fat mass	0.46	0.004	0.47	<0.001
Liver fat content	0.53	<0.001	0.54	<0.001
	z	*P*	*z*	*P*
Sex[a]	−0.13	0.88	−1.23	0.22
Fatty liver[a]	−3.18	0.001	2.68	0.007
Impaired fast. Glycemia[a]	−0.18	0.83	2.68	0.007

[a]Wilcoxon rank-sum test (*z* statistics); BMI: body mass index; HOMA-IR: homeostasis model assessment of insulin resistance.

Univariate relationships of the HOMA-IR with anthropometrics, body fat mass and distribution, liver fat content, and metabolic factors within each group

We then studied the relationships of these factors with the HOMA-IR within each group. In the adolescents, HOMA-IR correlated positively with age, height, and BMI. It also correlated positively with lean body mass, total body-, abdominal subcutaneous-, and visceral fat mass as well as with liver fat content. Irrespective of fasting insulinemia, HOMA-IR correlated strongest with liver fat content (Table 2).

Similar relationships were seen for most factors in adults. However, no clear correlation of HOMA-IR with age and height was observed in this group and, except for fasting insulinemia, HOMA-IR correlated most strongly with fasting glycemia and BMI (Table 2).

Independent relationships of the HOMA-IR with anthropometrics, body fat mass and distribution, liver fat content, and metabolic parameters within each group

In the adolescents, among age, sex, lean body mass, total body-, and visceral fat mass, and liver fat content, only sex, lean body mass, and liver fat content were confirmed as independent determinants of the HOMA-IR in multiple regression. Within the group of adolescents the strongest relationship was found for liver fat content in the model (Table 3). In the adults only liver fat content, but not the other factors, was found as an independent determinant of the HOMA-IR (Table 3).

To better depict the relationships of visceral fat mass and liver fat content with insulin resistance the groups of adolescents and adults were each divided into tertiles of the HOMA-IR. Both, in adolescents and in adults, a stronger increase in liver fat content than in visceral fat mass was found with increasing HOMA-IR classes (Figure 2).

Relationships of the HOMA-IR with anthropometrics, body fat mass and distribution, liver fat content, and metabolic parameters within each group by sex

In the adolescent females, from the anthropometrics and fat compartments studied, only liver fat content correlated strongly with the HOMA-IR, while in adolescent males also weight, height, BMI, lean body mass, and total body-, and visceral fat mass were significantly associated with the HOMA-IR (Supporting Information Table 1).

Very similar relationships as in both adolescent sexes were found in the adult females and in the males, respectively (Supporting Information Table 1).

Discussion

With respect to the effects of obesity on metabolism, the induction of insulin resistance is an early pathogenic condition that is involved

TABLE 3 Independent determinants of HOMA-IR in multivariate regression models

	Adolescents (r^2 = 0.48)			Adults (r^2 = 0.47)		
	Estimate	SEM	p value	Estimate	SEM	p value
Intercept	−0.39	2.36	0.10	−4.63	5.10	0.37
Age	0.18	1.13	0.88	−0.49	0.63	0.44
Sex (female)	0.22	0.11	0.04	0.35	0.28	0.22
Lean body mass	1.36	0.62	0.04	1.03	1.15	0.38
Total body fat mass	−0.16	0.36	0.66	0.73	0.39	0.07
Visceral fat mass	0.11	0.27	0.68	0.10	0.33	0.77
Liver fat content	0.30	0.11	0.007	0.42	0.13	0.004

SEM: standard error of the mean.

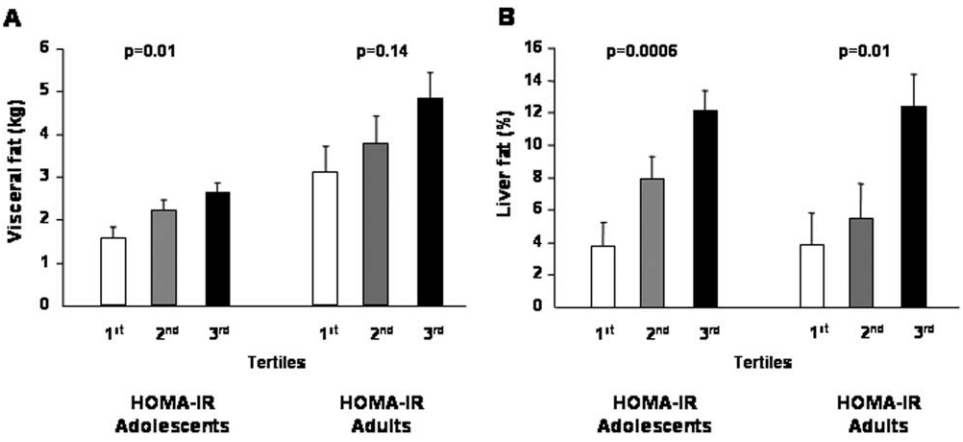

Figure 2 Relationships of the visceral fat mass and the liver fat content with tertiles of the HOMA-IR in adolescents and adults. *P* for statistical significance in the ANOVA; data are means and SEM.

in many metabolic diseases, such as type 2 diabetes, cardiovascular disease, and certain types of cancer (27). Because of the increasing prevalence of obesity in children and adolescents and the putative resulting health threats for them, more effort needs to be undertaken to understand the pathomechanisms of the consequences of this epidemic for these children and adolescents. So far most of the data in humans concerning these relationships originate from studies in adults, and just in the recent years important studies in children and adolescents helped to understand whether these pathomechanisms may also be applicable to them (14-21,28-34). However, to our knowledge, no study investigated such relationships in children, adolescents, and adults in the very same phenotyping setting. This is important as body composition imaging techniques often largely differ between studies including children and adults. Particularly, the problem that the measurement of insulin is not standardized makes the comparison of data between studies difficult. In the present study, we therefore set out to investigate differences in the body fat distribution pattern and the ectopic deposition of fat in the liver in adolescents and adults, and studied whether the relationships, which were found in adults, are also determinants of insulin resistance in adolescents.

We found higher levels of fasting insulin and insulin resistance in overweight and obese adolescents compared to BMI-matched adults, which presumably reflects, at least in part, the physiological insulin resistance that is often present in adolescents (30-34). As expected, adolescents had a lower lean body mass and a higher total body fat mass, but they only had about half of the visceral fat mass of the adults. Moreover, the total subcutaneous body fat mass, which is considered as protection from insulin resistance (1), was higher in the adolescents.

But what explains the higher insulin resistance in adolescents? The fact that the glucose levels tended to be lower in adolescents and the lower prevalence of impaired fasting glycemia in adolescents support the hypothesis that the observed insulin resistance in adolescents may not result from a dysregulated adipokine, cytokine, myokine, and hepatokine secretion that is typically seen in most, but not all obese adults (2-4,35-37). Rather than the body fat mass, the body fat distribution, or the liver fat content, the altered sex-hormone levels and sex-hormone sensitivity, and the increased growth hormone signaling may result in the hyperinsulinemia. This is supported by our data showing that age, but not the other aforementioned factors, explained part of the high HOMA-IR in the adolescents. Furthermore, the fact that in our study the HOMA-IR was highest in the adolescents in the higher Tanner stages, where the adolescents experience the largest alterations in these hormonal signalling properties, is another support for this hypothesis. However, we cannot exclude that the moderately higher total body fat mass and the larger percentage of girls, who are more insulin resistant than boys, in the highest Tanner stage (63% vs 42% in Tanner stages II-IV) may explain the somewhat unexpected higher HOMA-IR in Tanner stage V compared to the Tanner stages II-IV in our study.

It is important to determine whether expanded and disproportional adipose tissue, and high liver fat content independently determine insulin resistance in adolescents, despite their perceived lesser importance in regulating insulin resistance in this age group. Furthermore, it is unclear whether these relationships are similar to an equally obese adult population. We found that in adolescents, similarly as in adults, high liver fat content, but not visceral obesity, was an independent determinant of insulin resistance. Although the causal relationships between hepatic steatosis and insulin resistance are not fully understood (7-11), and the fact that a metabolically benign fatty liver exists in some individuals (38), these data further support that it is necessary to better understand the relationships between fatty liver and insulin resistance in these adolescents.

In addition, in our present study, a high lean body mass correlated positively, and independently of other factors, with the HOMA-IR in the adolescents. This is in agreement with a large study of more than five hundred Caucasian adolescents where lean body mass, which was measured by DEXA, was also independently and positively associated with insulin resistance that was estimated from fasting glucose and insulin levels (39). Likewise, the finding that this relationship was only present in males, but not in females (39), was in agreement with our study. Putatively the high lean body mass, which was associated with insulin resistance in males, reflects the age-related steep rise of serum testosterone (and growth hormone) and, as its consequence, the increase in muscle mass and

body height. This is supported by the fact that lean body mass continues to increase in parallel to the increase in the Tanner stages and further up to the age of 20 years in males, but not in females (39,40). Compared to the data in adults, in whom increased lean body mass predominantly reflects a physical exercise-induced increase in skeletal muscle mass, these findings in adolescents are unexpected. Thus, the relationships of the body composition with metabolic traits present in adults as well as the resulting findings cannot be easily transferred to the adolescents.

A weakness of our study is the relatively small sample size. Furthermore, we cannot provide information about molecular mechanisms regulating body fat distribution and insulin resistance, which may differ between adolescents and adults. In contrast, strength of our study is the fact that, as far as we are aware of, this is the first report about a comparison of two well-matched and precisely phenotyped groups of adolescents and adults in respect to body fat distribution and liver fat content. These findings may be particularly relevant for future studies aiming at understanding the role of sex-hormone levels and sex-hormone sensitivity, as well as growth hormone signaling in the determination of body fat distribution and glucose metabolism in adolescents.

In conclusion, by comparing obese adolescents and adults who underwent the same phenotyping procedures, we provide novel information, showing that although having a lower visceral fat mass, overweight and obese adolescents are more insulin resistant than sex- and BMI-matched adults. Furthermore, already in adolescents liver fat content, but not total body- or visceral fat mass, is an independent determinant of insulin resistance.

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