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Abdominal subcutaneous adipose tissue accumulation is positively correlated with hepatic steatosis in Sprague-Dawley rats

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The precise roles of visceral (VAT) or subcutaneous adipose tissue (SAT) on hepatic fat accumulation have not been fully elucidated. In this report, we examined the correlation between VAT or SAT volume and severity of hepatic fat accumulation. In the present study, Sprague-Dawley (SD) rats were fed a standard diet containing 10% fat or a high-fat diet containing 45% or 60% fat for 16 weeks. Abdominal VAT and SAT volume, as well as fat percentage of the liver were measured by computed tomography (CT). Hepatic triglyceride (TG) content and histopathological findings of hepatic steatosis were also examined. Abdominal SAT weight/body weight ratio was positively and strongly correlated with abdominal VAT weight/body weight ratio. Fat percentage of the liver by CT evaluation, hepatic TG content, and hepatic steatosis score by histopathological evaluation showed positive correlations with one another. Fat percentage of the liver by CT evaluation and hepatic TG content was positively correlated with both the abdominal VAT weight/body weight ratio and SAT weight/body weight ratio, respectively. Furthermore, hepatic TG content was negatively correlated with the abdominal VAT weight/SAT weight ratio. Our data suggest that abdominal SAT accumulation is positively correlated with hepatic steatosis in SD rats, rather than abdominal VAT accumulation. Further investigations are needed in order to clarify the precise mechanisms of SAT and VAT effects on the development of hepatic fat accumulation.

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Key words: hepatic steatosis, subcutaneous adipose tissue, visceral adipose tissue, computed tomography, Sprague-Dawley (SD) rats

Introduction

Nonalcoholic fatty liver disease (NAFLD) is considered one of the phenotypes of metabolic syndrome, or an additional feature of metabolic syndrome.[1,2] Visceral fat accumulation has been reported to be a more important risk factor for the development of metabolic syndrome than subcutaneous fat,[3] and therefore, hepatic fat infiltration in NAFLD is correlated with visceral fat accumulation.[2,4,5] In fact, Koda et al. reported that abdominal subcutaneous fat thickness was not different among hepatic steatosis scores, as evaluated semi-quantitatively by ultrasonography, and visceral fat was the most important factor for the development of hepatic steatosis.[6] However, Koda et al. also demonstrated that the change in abdominal subcutaneous fat thickness was weakly correlated with changes in hepatic fat deposits.[6] Furthermore, Choudhary et al. reported that subcutaneous adipose tissue (SAT) volume defined as fat superficial to the abdominal and back muscles was correlated with hepatic steatosis and severity of liver disease, whereas visceral adipose tissue (VAT) volume was not correlated with severity of liver disease in Indian patients with NAFLD.[7] Thus, abdominal SAT may be an important adipose tissue compartment that should not be overlooked.

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The aim of the present study was to elucidate the correlation between abdominal VAT or SAT volume and severity of hepatic fat accumulation by computed tomographic (CT), biochemical, or histopathological evaluation in Sprague-Dawley (SD) rats fed a normal or high-fat diet.

Materials and Methods

Animals and experimental design

Five-week-old male SD rats (n=19) were purchased from Japan SLC Inc. (Hamamatsu, Japan). Rats were housed in individual cages that were kept in a room maintained at 22-24℃ with 50-60% relative humidity and a 12-hour light/dark cycle. All rats were acclimatized for 7 days, during which they had access to standard diet containing 10% fat (5.5% soybean oil and 4.4% lard, kcal) (D12450B; Research Diets Inc., New Brunswick, NJ, USA) and water ad libitum. After acclimation, rats were randomly divided into 3 groups: a control group (C group, n=6) fed a standard diet (D12450B; Research Diets); a middle-high fat diet group (M group, n=6) fed a diet containing 45% fat (5.5% soybean oil and 39.4% lard, kcal) (D12451; Research Diets); and a high-fat diet group (H group, n=7) fed a diet containing 60% fat (5.5% soybean oil and 54.4% lard, kcal) (D12492; Research Diets). The proximate compositions of the diets fed to rats are shown in Table 1. Daily energy intake and body weights were monitored during the study.

Table 1. Dietary compositions by grams and kilocalories.

Component	Control (D12450B*)		Middle-high fat (D12451*)		High-fat (D12492*)	
	gram	kcal	gram	kcal	gram	kcal
Casein	200	800	200	800	200	800
L-cystine	3	12	3	12	3	12
Cornstarch	315	1260	72.8	291	0	0
Maltodextrin	35	140	100	400	125	500
Sucrose	350	1400	172.8	691	68.8	272
Cellulose	50	0	50	0	50	0
Soybean oil	25	225	25	225	25	225
Lard	20	180	177.5	1598	245	2205
Mineral mix	10	0	10	0	10	0
Dicalcium phosphate	13	0	13	0	13	0
Calcium carbonate	5.5	0	5.5	0	5.5	0
Potassium citrate, $1H_2O$	16.5	0	16.5	0	16.5	0
Vitamin mix	10	40	10	40	10	40
Choline bitartrate	2	0	2	0	2	0
Yellow, red, or blue dye	0.05	0	0.05	0	0.05	0
Total	1055.05	4057	858.15	4057	773.85	4057
Protein (%)	19	20	24	20	26	20
Carbohydrate (%)	67	70	41	35	26	20
Fat (%)	4	10	24	45	35	60
Total		100		100		100
Energy (kcal/gram)	3.8		4.7		5.2	

*This number was provided by Research Diets Inc. (New Brunswick, NJ, USA).

At 22 weeks of age, rats underwent CT measurement, and were sacrificed under anesthesia (pentobarbital sodium) after 12-hour fasting. Blood samples were taken from the inferior vena cava or the heart for analysis of biochemical parameters. Serum samples were kept at −20℃ until analysis. Livers were removed, washed in cold saline, and weighed. Liver portions (approximately 5g) were fixed in 10% neutral buffered formalin for histopathological examination. Other liver portions (0.5g) were immediately frozen in liquid nitrogen and were stored at −80℃ for hepatic total cholesterol (TC) and triglyceride (TG) measurement.

All procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals (eighth edition, National Research Council of the National Academies), and were approved by the Animal Usage Committee of the University of Nagasaki, Japan.

Computed tomography (CT) imaging

Computed tomographic (CT) images were obtained using an X-ray CT system (Latheta LCT-100 Lite; Hitachi Aloka Medical Ltd., Tokyo, Japan) for imaging experimental animals according to the manufacturer's protocol. Rats at 22 weeks of age were anesthetized with isoflurane before CT scanning, and were then mounted on a holder and placed in the X-ray CT system. In the present study, CT imaging from the upper end of the diaphragm to the root of the tail was evaluated. Quantitative assessment of the lesion area was performed with Latheta software version 3.00 (Hitachi Aloka Medical). Abdominal weight of the muscle, VAT, and SAT were calculated from cumulative area measurement and mean CT values (Hounsfield unit, HU) of each CT slice. Abdominal VAT weight/body weight ratio, abdominal SAT weight/body weight ratio, and abdominal VAT weight/SAT weight ratio were also calculated. Fat percentage of the liver (%) was calculated as follows: (mean CT value of muscle – mean CT value of liver) / (mean CT value of muscle – mean CT value of fat) x 100, in accordance with the manufacturer's information, which was modified from a previously reported formula.[8]

Serum biochemical analysis

Serum TC and TG levels were measured using the cholesterol oxidase DAOS method (Cholesterol E test Wako, Wako Pure Chemical Industries Ltd., Osaka, Japan) and the GPO DAOS glycerol method (Triglyceride E test Wako, Wako Pure Chemical Industries), respectively. Serum high-density lipoprotein cholesterol (HDL-C) and free fatty acid (FFA) levels were measured using the phosphotungstic acid magnesium chloride precipitation method (HDL-cholesterol E test Wako; Wako Pure Chemical Industries), and the ACS ACOD method (NEFA C test Wako; Wako Pure Chemical Industries), respectively. Serum glucose levels were measured using the mutarotase and glucose oxidase method (Glucose C II test Wako; Wako Pure Chemical Industries). Serum insulin levels were measured with a rat insulin enzyme-linked immunosorbent assay (ELISA) kit (Morinaga Institute of Biological Science Inc., Yokohama, Japan). Serum adiponectin levels were measured with a mouse/rat ELISA kit (Otsuka Pharmaceuticals Co., Ltd., Tokyo, Japan). Serum leptin levels were measured with a Leptin ELISA kit (Morinaga Institute of Biological Science). Because the units of measurement in the present study were not same as in the previously reported homeostasis model assessment-insulin resistance (HOMA-IR) and adipose tissue insulin resistance (Adipo-IR),[9,10] "HOMA-IR index" was calculated as the product of fasting serum glucose (mg/dL) × fasting serum insulin (ng/mL) concentration, while "Adipo-IR index" was calculated as the product of fasting serum FFA (mEq/L) × fasting serum insulin (ng/mL) concentration.

Hepatic TC and TG concentrations

Lipids in the livers were extracted from frozen livers (0.5g) with the Bligh-Dyer extraction method,[11] and extracted TC and TG were measured using individual assay kits (Cholesterol E test Wako and triglyceride E test Wako, respectively) as described above.

Histopathological examination

After fixation in neutral-buffered formalin, liver tissues were embedded in paraffin, sectioned, and processed for hematoxylin-eosin (HE) staining for histopathological examination. All histopathological examinations were performed by a pathologist (K.T.) who was blinded to the experimental and serological data. Histopathological findings were scored using the NASH Clinical Research Network Scoring System based on four semi-quantitative factors, as described previously:[12] steatosis (0-3); lobular inflammation (0-3); hepatocyte ballooning (0-2); and fibrosis (0-4). In the present study, scores for steatosis, lobular inflammation, and hepatocyte ballooning were further classified as follows: score 0.5, the feature was between scores 0 and 1; score 1.5, between scores 1 and 2; and score 2.5, between scores 2 and 3.NAFLD activity score (NAS) was defined as the unweighted sum of the scores for steatosis, lobular inflammation, and

hepatocyte ballooning; thus, scores ranged from 0 to 8. A NAS of 0 to 2 was not considered to be diagnostic of steatohepatitis, and scores of 5 or greater were considered to be diagnostic of steatohepatitis.[12]

Statistical analysis

All values were expressed as mean ± standard error (SE). Differences between the three groups were tested for statistical significance using one-way analysis of variance (ANOVA), followed by Bonferroni multiple comparison test, chi-squared test, or Fisher's exact probability test. Correlations between two variables were determined by Spearman's rank correlation coefficient. All analyses were performed using IBM SPSS statistics software program, version 21 (IBM Co., Somers, NY, USA) on a Windows computer. A *p* value of less than 0.05 was considered to be statistically significant.

Results

Food intake, body weight, and liver weight/body weight ratio

Cumulative energy intake during the 16-week study period was not significantly different among the groups, but body weight at 22 weeks of age and weight gain during the 16-week study period was significantly larger in the M group than that in the C group (p=0.036 and 0.027, respectively). Liver weight/body weight ratio was significantly larger in the C group than in the H group (p=0.041, Table 2).

Computed tomography (CT) parameters

Representative CT imaging for quantitative assessment of the lesion area is shown in Figure 1. Abdominal VAT weight/body weight ratio and SAT weight/body weight ratio were larger in the M group and H group than those in the C group (p<0.001 and 0.003, respectively). Abdominal VAT weight/SAT weight ratios were not significantly different among the groups. Body weight at 22 weeks of age and weight gain during the 16-week study period were not correlated with abdominal VAT weight/SAT weight ratios (p=0.424 and 0.258, respectively). Mean CT value of the liver was highest in the C group (74.4 ± 2.5 HU), followed by the M group (56.4 ± 3.8 HU) and the H group (42.5 ± 3.8 HU, p<0.001). The calculated fat percentage of the liver was lowest in the C group, followed by the M group and the H group (p<0.001, Table 2).

Serum biochemical parameters

Serum TC and HDL-C levels were significantly higher in the M group than in the H group (p=0.017 and 0.014, respectively). Serum TG and FFA levels were significantly higher in the C group than in the H group (p=0.001 and 0.026, respectively). There were no significant differences in serum levels of glucose, insulin, adiponectin, leptin, HOMA-IR index, and Adipo-IR index among the groups (Table 2).

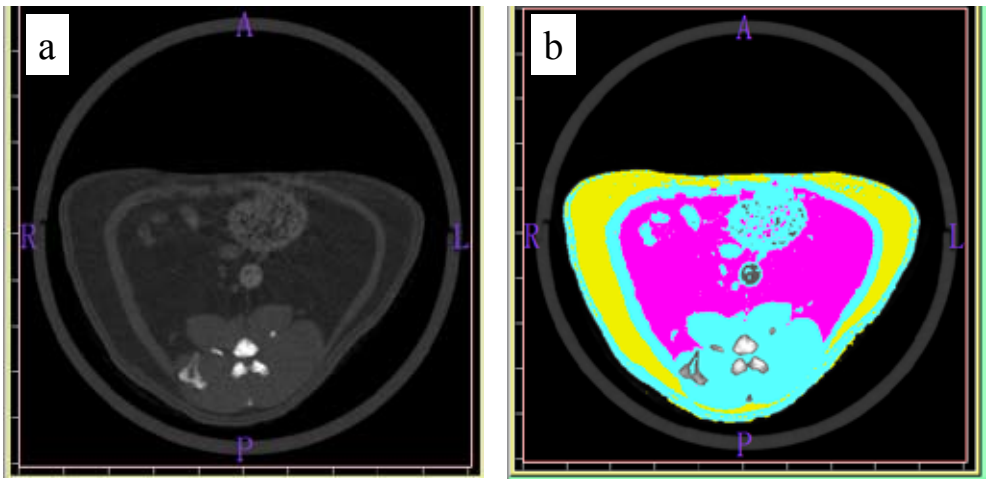

Figure 1. Representative CT imaging for quantitative assessment of lesion area. (a): Raw gray scale scan slice. (b): Selected areas of abdominal visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) are indicated in pink and yellow, respectively. A, anterior; P, posterior; R, right; L, left.

Table 2. Cumulative energy intake, body weight, liver weight/body weight ratio, CT parameters, serum biochemical parameters, hepatic lipid contents, and histopathological findings in rats at 22 weeks of age.

Parameter	C group (n=6)	M group (n=6)	H group (n=7)
Cumulative energy intake (kcal)	9822 ± 272	11207 ± 272	10976 ± 216
Final body weight (g)	547 ± 13^{a}	598 ± 10^{b}	579 ± 14^{ab}
Weight gain (g)	382 ± 14^{c}	437 ± 10^{d}	416 ± 13^{cd}
Liver weight/BW ratio (%)	2.8 ± 0.1^{e}	2.7 ± 0.1^{ef}	2.5 ± 0.1^{f}
CT parameters			
Abdominal VAT weight / BW ratio (%)	7.8 ± 0.3^{g}	11.7 ± 0.5^{h}	11.6 ± 0.4^{h}
Abdominal SAT weight / BW ratio (%)	3.4 ± 0.3^{i}	5.9 ± 0.4^{j}	5.9 ± 0.5^{j}
Abdominal VAT weight / SAT weight ratio (%)	234.1 ± 14.0	201.0 ± 13.6	207.1 ± 16.3
Fat percentage of liver (%)	2.4 ± 0.7^{k}	8.1 ± 1.3^{l}	12.8 ± 1.3^{m}
Serum biochemical parameters			
Serum TC (mg/dL)	110 ± 4no	119 ± 13^{n}	79 ± 8^{o}
Serum TG (mg/dL)	195 ± 17^{p}	144 ± 15^{pq}	108 ± 7^{q}
Serum HDL-C (mg/dL)	59 ± 1^{rs}	65 ± 6^{r}	41 ± 3^{s}
Serum FFA (mEq/L)	0.64 ± 0.06^{t}	0.55 ± 0.03^{tu}	0.43 ± 0.05^{u}
Serum glucose (mg/dL)	190 ± 10	170 ± 10	183 ± 7
Serum insulin (ng/mL)	4.7 ± 0.2	5.1 ± 1.0	5.3 ± 0.6
Adiponectin (μg/mL)	3.5 ± 0.2	5.0 ± 0.5	4.3 ± 0.8
Leptin (ng/mL)	14.5 ± 1.2	20.7 ± 1.4	19.4 ± 1.8
HOMA-IR index (glucose x insulin)	2.22 ± 0.16	2.17 ± 0.44	2.42 ± 0.30
Adipo-IR index (FFA x insulin)	2.97 ± 0.22	2.85 ± 0.70	2.26 ± 0.31
Hepatic lipid contents			
Hepatic TC (mg/g liver)	3.9 ± 0.5	6.3 ± 1.1	7.1 ± 0.9
Hepatic TG (mg/g liver)	31.8 ± 7.0^{v}	58.6 ± 12.3^{vw}	74.3 ± 8.6^{w}
Histopathological findings (score)			
Steatosis (0/0.5/1/1.5/2)	3/1/2/0/0	1/4/1/0/0	1/3/2/1/0
Lobular inflammation (0/1/2)	4/2/0	3/3/0	2/4/1
Hepatocyte ballooning (0/0.5/1)	6/0/0	3/2/1	2/4/1
NAS (0-2/2.5-4.5/5-8)	6/0/0	4/2/0	5/2/0
Fibrosis (0/1-4)	6/0	6/0	7/0

Values are means ± SE. Different superscript letters within the same parameter indicate significant differences at $p<0.05$.
CT, computed tomography; BW, body weight; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; FFA, free fatty acid; NAS, NAFLD activity score.

Hepatic lipid concentrations

Hepatic TC concentrations were highest in the H group and lowest in the C group, but there were no significant differences. Hepatic TG concentrations were significantly higher in the H group than those in the C group (p=0.017, Table 2).

Histopathological observation

There were no significant differences in score/grade of hepatic steatosis, lobular inflammation, hepatocyte ballooning, or NAS among the three groups. NAS of 5 points or greater, or hepatic fibrosis was not observed in any rats (Table 2).

Correlations between adipose and liver tissue variables

Correlations between adipose and liver tissue variables by anthropometric, CT, biochemical, or histopathological evaluation are summarized in Table 3. Abdominal SAT weight/body weight ratio was correlated positively and strongly with abdominal VAT weight/body weight ratio ($r=0.801$, $p<0.001$). Abdominal SAT weight/body weight ratio and abdominal VAT weight/SAT weight ratios revealed a strong negative correlation ($r=-0.765$, $p<0.001$).

Hepatic fat accumulation indices, i.e., fat percentage of liver on CT evaluation, hepatic TG content, and hepatic steatosis score by histopathological evaluation showed positive correlations with one another (Figure 2). Of these hepatic fat accumulation indices, fat percentage of the liver by CT evaluation was positively correlated with both abdominal VAT weight/body weight ratio and SAT weight/body weight ratio. Hepatic TG content was also positively correlated with both abdominal VAT weight/body weight ratio and SAT weight/body weight ratio. Moreover, hepatic TG content was negatively correlated with abdominal VAT weight/SAT weight ratios. On the other hand, hepatic steatosis score by histopathological evaluation was not correlated with any of abdominal VAT or SAT weight/body weight and VAT weight/SAT weight ratios (Figure 3).

Table 3. Correlations between adipose and liver tissue variables by anthropometric, CT, biochemical, or histopathological evaluation.

Parameter	LW/BW	VAT/BW	SAT/BW	VAT/SAT	L-%fat	H-TC	H-TG	HS	LI	HB	NAS
LW/BW	1.000										
VAT/BW	0.002	1.000									
	(0.994)										
SAT/BW	0.101	0.801	1.000								
	(0.682)	(<0.001)									
VAT/SAT	-0.366	-0.342	-0.765	1.000							
	(0.123)	(0.152)	(<0.001)								
L-%fat	-0.010	0.663	0.623	-0.420	1.000						
	(0.969)	(0.002)	(0.004)	(0.074)							
H-TC	0.174	0.400	0.475	-0.454	0.665	1.000					
	(0.477)	(0.090)	(0.040)	(0.051)	(0.002)						
H-TG	0.181	0.478	0.605	-0.587	0.749	0.900	1.000				
	(0.459)	(0.038)	(0.006)	(0.008)	(<0.001)	(<0.001)					
HS	0.267	0.233	0.216	-0.336	0.546	0.743	0.771	1.000			
	(0.270)	(0.336)	(0.375)	(0.159)	(0.016)	(<0.001)	(<0.001)				
LI	0.222	0.351	0.321	-0.284	0.470	0.614	0.665	0.580	1.000		
	(0.360)	(0.141)	(0.180)	(0.238)	(0.042)	(0.005)	(0.002)	(0.009)			
HB	0.259	0.533	0.390	-0.239	0.709	0.548	0.547	0.495	0.434	1.000	
	(0.284)	(0.019)	(0.099)	(0.325)	(0.001)	(0.015)	(0.015)	(0.031)	(0.063)		
NAS	0.353	0.395	0.360	-0.369	0.661	0.769	0.802	0.848	0.833	0.757	1.000
	(0.138)	(0.095)	(0.130)	(0.120)	(0.002)	(<0.001)	(<0.001)	(<0.001)	(<0.001)	(<0.001)	

Data are shown in terms of r values (p values).
LW/BW, Liver weight/body weight (BW) ratio; VAT/BW, Abdominal visceral adipose tissue (VAT) weight/BW ratio by computed tomography (CT) evaluation; SAT/BW, Abdominal subcutaneous adipose tissue (SAT) weight/BW ratio by CT evaluation; VAT/SAT; Abdominal VAT weight/SAT weight ratio by CT evaluation; L-%fat, Fat percentage of liver by CT evaluation; H-TC, Hepatic total cholesterol; H-TG, Hepatic triglyceride; HS, Hepatic steatosis by histopathological evaluation; LI; Lobular inflammation by histopathological evaluation; HB, Hepatocyte ballooning by histopathological evaluation; NAS, NAFLD activity score

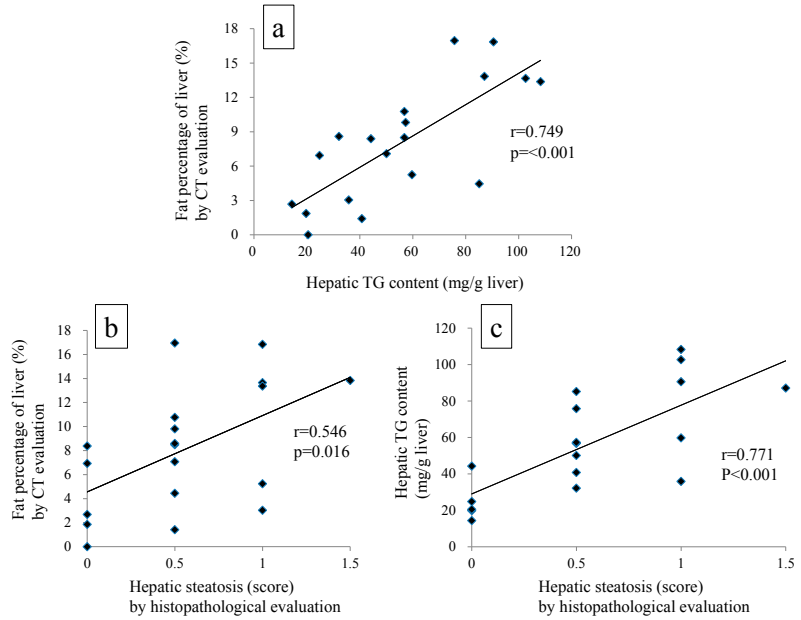

Figure 2. Correlations between hepatic fatty accumulation indices. Fat percentage of the liver by CT evaluation and hepatic TG content (a), fat percentage of the liver by CT evaluation and hepatic steatosis score by histopathological evaluation (b), and hepatic TG content and hepatic steatosis score by histopathological evaluation (c) showed positive correlations. CT, computed tomography; TG, triglyceride.

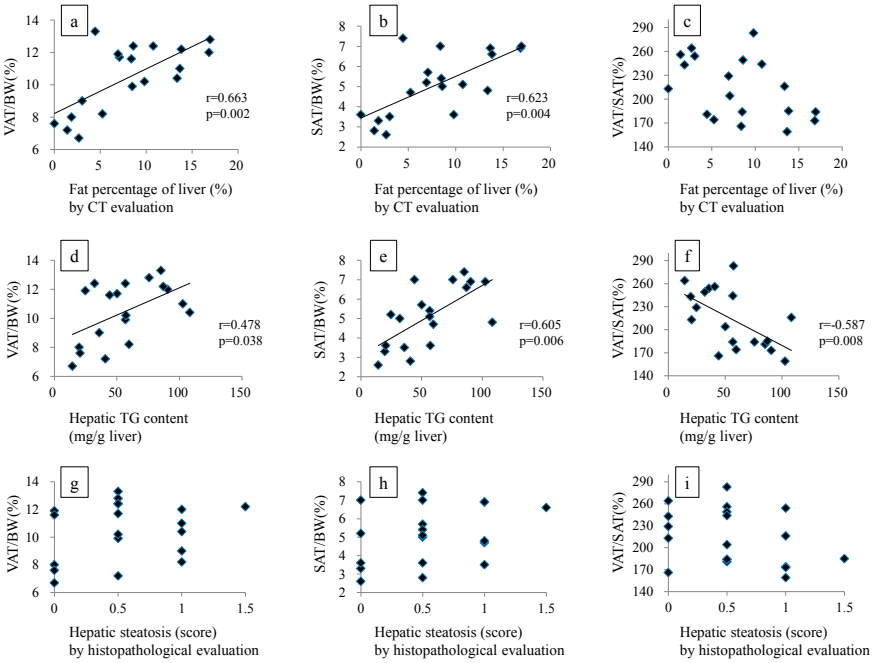

Figure 3. Correlations between VAT/BW, SAT/BW, or VAT/SAT and fat percentage of the liver by CT evaluation (a-c), hepatic TG content (d-f), or hepatic steatosis score by histopathological evaluation (g-i). VAT/BW, abdominal visceral adipose tissue (VAT) weight/body weight (BW) ratio by computed tomography (CT) evaluation; SAT/BW, abdominal subcutaneous adipose tissue (SAT) weight/BW ratio by CT evaluation; VAT/SAT, abdominal VAT weight/SAT weight ratio by CT evaluation.

Discussion

In order to elucidate the correlation between abdominal VAT or SAT volume and severity of hepatic fat accumulation, SD rats were fed a normal diet or high-fat diet (containing 45% or 60% fat) for 16 weeks in the present study. In SD rats fed a high-fat diet for a relatively short period (3 to 12 weeks), hepatic fat accumulation can be observed before a significant increase in peripheral fat deposition.[13] In the present study, abdominal VAT and SAT weight/body weight ratios were significantly larger at 22 weeks of age in groups fed a high-fat diet (M and H group) than those fed the normal diet (C group). Hepatic fat accumulation as evaluated by CT measurement of fat percentage of the liver and hepatic TG concentration were also significantly larger in high-fat diet groups (M or H group) than those in the normal diet group (C group). Because final body weight and weight gain during the 16-week study period were not correlated with abdominal VAT weight/SAT weight ratios, it is unlikely that SAT deposition occurred prior to VAT deposition. As abdominal VAT weight/body weight ratio revealed a strong positive correlation with abdominal SAT weight/body weight ratio, there were no correlations in abdominal VAT weight/SAT weight ratios among the three groups.

Visceral and subcutaneous adipose tissues have different structures and functions. Visceral adipose tissue is more cellular, vascular, innervated, and contains a large number of inflammatory and immune cells, lesser preadipocyte differentiating capacity and a greater percentage of large adipocytes than SAT. Moreover, VAT is also metabolically more active in producing adipocytokines such as leptin, tumor necrosis factor-alpha (TNF-alpha), interleukin 6, angiotensinogen, plasminogen activator inhibitors-1 (PAI-1), and adiponectin. These adipocytokines flow directly into the liver via the portal vein.[2,3,7,14] Therefore, visceral fat accumulation may be a more important risk factor for the development of metabolic syndrome or hepatic fat accumulation than subcutaneous fat.[2-5] In the present study, hepatic fat accumulation indices such as fat percentage of the liver by CT evaluation and hepatic TG content were positively correlated with abdominal VAT weight/body weight ratio, as reported previously.[6]

In the present study, abdominal SAT weight/body weight ratio also revealed positive correlations with hepatic fat accumulation indices. It was particularly noteworthy that hepatic TG content was negatively correlated with abdominal VAT weight/SAT weight ratios. This suggests that hepatic TG content was correlated more strongly with abdominal SAT weight than VAT weight. Subcutaneous fat accumulation represents the normal physiological buffer for excess energy intake with limited energy expenditure. In humans, about 80% of all body fat is in the subcutaneous area and visceral fat accounts for up to 10-20% of total fat in men and 5-8% in women.[14] Therefore, it is possible that SAT volume actually contributes to more absolute risk for metabolic syndrome or hepatic fat accumulation than VAT volume because SAT volume is greater than VAT volume.[3]

Hepatic fat accumulation is reported to be associated with hepatic insulin resistance,[15] although hepatic fat accumulation was not correlated with insulin resistance in the present study (Table 2). Adipocytes from VAT were reported to be more insulin-resistant than those from SAT.[14] However, Garg reported that SAT was more strongly associated with insulin resistance than VAT in human studies.[16] Maruyama et al. also reported that changes in fasting insulin concentration were correlated more strongly with changes in SAT volume than those in VAT volume in 744 adults.[17] In this context, Ishikawa et al. reported that SAT regulates systemic insulin sensitivity by altering fat storage and the expression of TNF-alpha by adipocytes in VAT. Therefore, the balance between SAT and VAT accumulation may be important in systemic insulin resistance in metabolic syndrome.[18] Amati et al. also reported that VAT and thigh SAT had markedly opposite associations with insulin sensitivity; thigh SAT exerted a protective influence against peripheral insulin resistance.[19] However, this was not evident in the present study, as "adipo-IR index" was not statistically different among the C, M and H groups (Table 2), and fasting serum insulin and glucose were not significantly correlated with abdominal SAT weight/body weight ratio or abdominal VAT weight/body weight ratio (data not shown).

In the present study, hepatic fatty accumulation indices such as fat percentage of the liver by CT evaluation, hepatic TG content, and hepatic steatosis score by histopathological evaluation showed positive correlations with one another (Figure 2). However, correlations between such hepatic fat accumulation indices and abdominal VAT or SAT deposits did not show identical results. Measurement of fat percentage of the liver by CT is a simple, non-invasive method for evaluating all parts of the liver, but this method requires expensive equipment and exposure to radiation. Chemical measurement of hepatic lipid concentration has the advantages of being precise and able to measure TG, as well as TC, but this method evaluates only a small part of the liver tissue, and therefore runs the risk of "sampling error". Histopathological evaluation of the liver can evaluate both steatosis and other features, such as lobular inflammation, hepatocyte ballooning, and fibrosis. However, this method

also evaluates a small part of the liver tissue, and therefore runs the risk of "sampling error". In clinical settings, percutaneous liver biopsy has a risk of complications such as bleeding, pain, and hypotension.[20] In the present study, it should therefore be noted that hepatic fat accumulation as evaluated by fat percentage of the liver by CT evaluation, hepatic TG content, and hepatic steatosis score by histopathological evaluation were negatively correlated with abdominal VAT weight/SAT weight ratios (r=-0.420, -0.587, and -0.336, respectively) (Table 3 and Figure 3), although only hepatic TG content showed a significant difference. This finding indicates that hepatic fat accumulation is more closely correlated with abdominal SAT weight than with VAT weight.

It is known that increased serum TG and decreased serum HDL-C levels are associated with severity of NAFLD.[21] However, serum TC levels were significantly lower in the H group than those in the M group, and serum TG and FFA levels were significantly lower in the H group than those in the C group in the present study (Table 2). This may be due to the "12-hour fasting" and the subsequent imbalance in TG acquisition and removal in the liver of rats fed a high-fat diet. Cahova et al. reported that serum TG and FFA in hereditary hypertriglyceridemic (HHTg) rats fed a high-fat diet and fasted for 24 hours were lower than those in HHTg rats fed a high-sucrose diet.[22] The authors concluded that high-fat diet associated steatosis was characterized by downregulated FFA synthesis *de novo*, increased FFA oxidation, and significantly impaired very low density lipoprotein (VLDL) output.[22]

In conclusion, our results showed that abdominal SAT accumulation, rather than abdominal VAT accumulation, is positively correlated with hepatic steatosis in SD rats. Therefore, physicians, nurses, dieticians, and investigators should pay closer attention to SAT status, in addition to VAT. Further investigations will be needed in order to clarify the precise mechanisms responsible for the effects of SAT and VAT on the development of NAFLD.

Conflict of interest

The authors have no conflicts of interest to declare.

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