The Influence of Anatomical Boundaries, Age, and Sex on the Assessment of Abdominal Visceral Fat

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Abstract:

Single-slice abdominal computed tomography (CT) scanning has been used extensively for the measurement of abdominal visceral fat (AYF). Optimal anatomical scan location and pixel density ranges have been proposed and are specifically reported to allow for the replication and standardization of AVF measurements. Standardization of the anatomical boundaries for CT measurement of AVF and the influence of age and gender on results obtained with different boundary locations have received much less attention. To determine the influence of three boundary analysis methods (AVF-1, AVF-2, and AVF-3) on the measurement of AVF by CT, 54 older (60 years to 79 years) and 37 younger (20 years to 29 years) healthy men and women were examined. The measurement boundary for AVF-1 was the internal most aspect of the abdominal and oblique muscle walls, and the posterior aspect of the vertebral body. AVF-2 used fat measurements enclosed in a boundary formed by the midpoint of the abdominal and oblique muscle walls, and the most posterior aspect of the spinous process. AVF-3 used fat measurements enclosed in a boundary formed by the external border of the abdominal and oblique muscle walls, and the external border of the erector spinae. Greater AVF measures were obtained with AVF-2 and AVF-3 compared with AVF-1 ($p<0.0001$). These differences were greater in older compared with younger subjects ($p<0.0001$) and greater in women compared with men ($p<0.02$). The significantly greater AVF measurements obtained with AVF-2 and AVF-3 resulted from the inclusion of larger amounts of fat that are not drained by the portal circulation. This included retroperitoneal, intermuscular, and intramuscular lipid droplets, which increase with aging. On the basis of these results, we recommend the AVF-1 anatomical boundaries for the measurement of AVF in clinical investigations, particularly with older subjects. These data demonstrate the importance of precise and reproducible anatomical boundaries for the measurement of AVF, particularly in longitudinal studies.

Keywords: computed tomography | body composition | regional distribution of fat

Article:

Introduction

There is a growing body of evidence that suggests that the regional distribution of body fat is more strongly related to the risk of developing disease than is percent body fat (4,8,12). Specifically, it has been shown that individuals with elevated levels of abdominal visceral fat (A VF) have an increased incidence or risk of metabolic disorders such as hyperinsulinemia, hypertension, and hyperlipidemia (5,9, 11, 14, 17,24,29,30). The development of precise measurements of the regional distribution of body fat by computed tomography (CT) has facilitated investigations relating the amount of AVF to the aforementioned metabolic disorders.

The technical aspects of A VF measurement by CT have been reviewed (33). Pixel densities between -190 and -30 Hounsfield units (HU) are widely accepted as the attenuation range for fat determination by CT (15,20,26). Additionally, AVF areas from a single CT slice scan at the intra vertebral space between L4 and LS have been shown to be highly correlated with total AVF volume calculated by multiple CT scans, thus defining the optimal anatomical scan location (19,25). However, the anatomical boundaries used to distinguish AVF from total abdominal fat are more subjective and may vary between investigators. The difficulty of using the peritoneum, vessels, and/or organs that may be visible on CT scans to distinguish between various anatomical locations of abdominal adipose tissue has been reported (27,33). Because the amount of AVF is affected by both age (older individuals have increased levels) (6) and sex (men have increased levels) (21), it can be hypothesized that the accumulation of intermuscular fat and intramuscular lipid droplets may also be affected by age and sex. If this is the case, then the anatomical boundaries chosen for AVF determinations could markedly affect age and sex comparisons. This study examined the effects of anatomical boundaries on the assessment of AVF in young and old adults.

Methods and Procedures

Subjects

Subjects included 20 young men (ages, 20 years to 29 years), 17 young women (ages, 23 years to 29 years), 31 older men (ages, 58 years to 76 years), and 23 older women (ages, 60 years to 79 years). None of the subjects were taking medications that are known to alter or suspected of altering lipid metabolism or visceral fat accumulation. Subject characteristics are shown in Table 1. All subjects underwent a detailed medical history and physical examination and provided written informed consent in accordance with guidelines established by the Human Investigation Committee of the University of Virginia.

Table 1. Physical characteristics of the subjects (n = 91)

Variable	Older Mean (± SEM)		Younger Mean (± SEM)	
	Men (n=31)	Women (n=23)	Men (n=20)	Women (n=17)
Age (yrs)*	66.0 (1.0)	67.0 (1.0)	24.0 (1.0)	25.0 (0.1)
Height (cm)†	176.8 (1.2)	162.5 (1.2)	175.4 (1.8)	167.7 (1.6)
Weight (kg)*†	90.3 (3.6)	69.7 (2.7)	77.4 (1.5)	65.4 (2.4)
BMI (kg/m^2)*†	27.8 (0.5)	26.3 (0.9)	24.8 (0.5)	23.3 (0.7)

*Significant differences ($p<0.05$) between older and younger subjects. BMI, body mass index.
†Significant differences ($p<0.05$) between men and women.

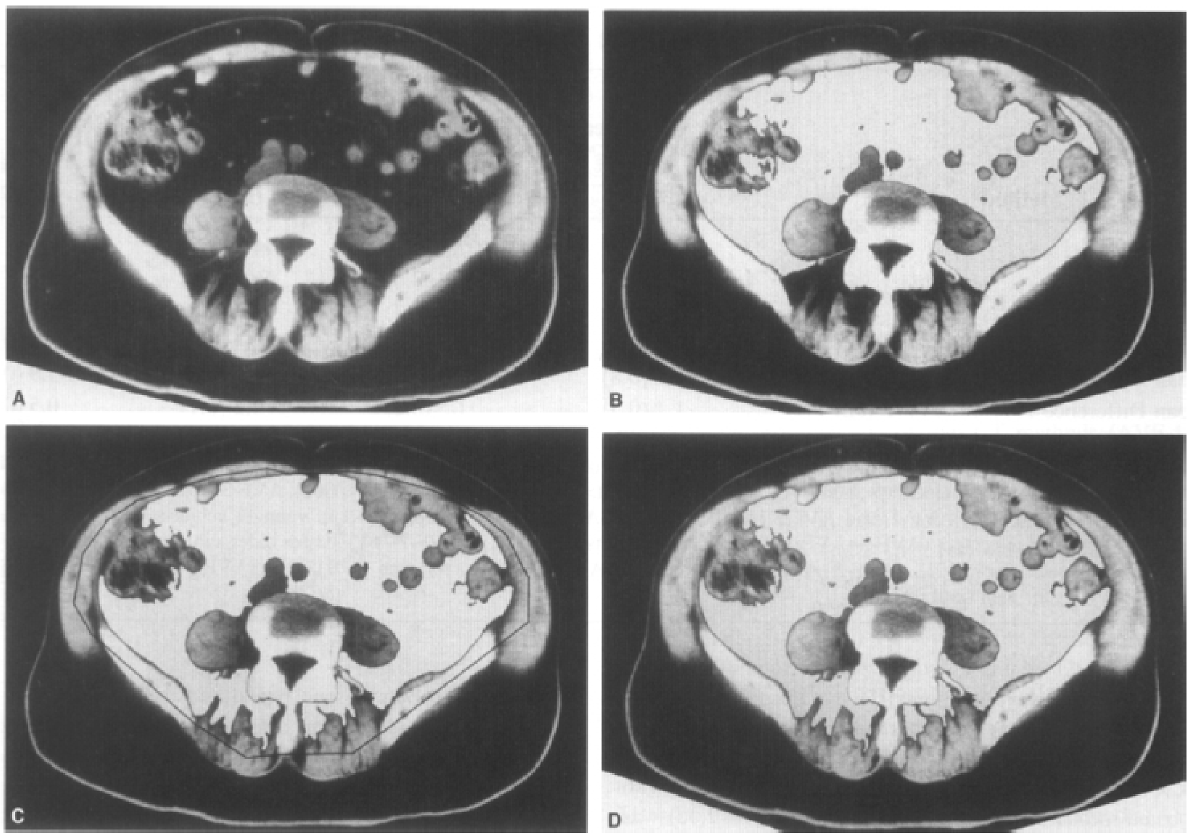

Figure 1: (A) Cross-sectional image of the abdomen obtained by CT at the L4 to L5 level in a 68-year-old man. (B) AVF area (206.2 cm^2) using AVF-1 methodology highlighted in the image for a 68-year-old man. The internal most aspect of the abdominal and oblique muscle walls, and posterior aspect of the vertebral body were used as the anatomical boundaries for AVF-1. (C) AVF area (213.2 cm^2) using AVF-2 methodology highlighted in the image for a 68-year-old man. The midpoint through the abdominal and oblique muscle walls, and the posterior aspect of the spinous process were used as the anatomical boundaries for AVF-2. (D) AVF area (220.2 cm^2) using AVF-3 methodology highlighted in the image for a 68-year-old man. The external border of the abdominal and oblique muscle walls, and the external border of the erector spinae were used as the anatomical boundaries for AVF-3.

Measurement of AVF by CT

CT measures of AVF were performed with a Picker PQ 5000 and analyzed with a newly developed tissue quantification analysis package with a Picker Voxel Q 3D imaging station (Picker International). The scanning was performed with 140 kV and a slice thickness of 0.5 cm. Briefly, the subjects were clothed only in a loose gown and examined in a supine position with their arms stretched above their heads. An abdominal scan at the level of the L4 to LS intravertebral space was performed with no angulation with a lateral pilot for location (Figure 1A). A VF cross-sectional areas (cm2) were calculated by delineating, with a mouse computer interface, the designated areas and then computing the adipose tissue using an attenuation range from -190 to -30 HU.

Three procedures were used to determine AVF cross-sectional area. The measurement boundary for procedure 1 (AVF-1) was the internal most aspect of the abdominal and oblique muscle walls, and the posterior aspect of the vertebral body (Figure 1B). The boundaries were developed by our group to provide the most conservative estimate of AVF. Procedure 2 (A VF-2) used fat measurements enclosed in a boundary formed by the midpoint of the abdominal and oblique muscle walls, and the most posterior aspect of the spinous process (Figure 1C). This is the procedure recommended by Despres et al. (12). Procedure 3 (AVF-3) used fat measurements enclosed in a boundary formed by the external border of the abdominal and oblique muscle walls, and the external border of the erector spinae (Figure 1D). This. anatomical boundary was chosen to provide the most liberal estimate of AVF. It should be noted that the peritoneum is not visible on the CT scans; therefore, the defined boundaries of AVF-1, AVF-2, and AVF-3 include both intraperitoneal and retroperitoneal fat. The technical difficulty in excluding the retroperitoneal fat from the CT scan analysis has been recognized, and attempts have been made to develop measurement boundaries using additional anatomical landmarks to distinguish the two fat compartments (2,22). Similar to previous attempts discussed by van der Kooy and Seidell (33), proposed boundary-determining techniques were not possible because the additional anatomical landmarks including blood vessels and aspects of the colon and/or kidneys were poorly visible on our scans.

Duplicate measurements using each AVF analysis method were performed by two independent investigators on a subset of 33 scans. The intraobserver and interobserver reliability for each method showed that all methods were highly reproducible ($r \geq 0.99$; coefficient of variation$\leq$0.4%).

Statistical Analyses

A 3x2x2 analysis of variance (method x age x sex) with post-hoc mean comparisons was used to quantify the effects of different anatomical boundaries and the influence of age and sex on the assessment of A VF. Linear regression was used to determine the correlation among A VF areas determined by the three measurement methods. An *a* level of 0.05 was used to determine statistical significance. Additionally, using A VF-1 as the criterion method, the standard error (total error) from the line of identity (TE) were determined for the four groups of subjects examined.

Results

The assessments of AVF tissue using the AVF-1, AVF-2, and AVF-3 methods are shown in Table 2. As expected, AVF measurements were significantly greater in the older compared with the younger subjects ($p<0.0001$); however, when sex differences were examined, AVF measurements were not significantly different between the men and women tested ($p = 0.12$). A significant main effect was found for method (p<0.0001), and all mean comparisons were significantly different (AVF-1 vs. AVF-2, p<0.0001; AVF-1 vs. AVF-3, p<0.0001; AVF-2 vs. AVF-3, *p*<0.03).

Table 2. CT assessment of AVF using three anatomical boundaries

Method*	Older Mean (± SEM) Men (n=31)	Older Mean (± SEM) Women (n=23)	Younger Mean (± SEM) Men (n=20)	Younger Mean (± SEM) Women (n=17)
AVF-1 (cm^2)	127.0 (8.7)	107.6 (9.1)	44.1 (4.8)	33.6 (2.8)
AVF-2 (cm^2)	137.4 (9.1)	122.7 (9.6)	46.5 (4.8)	36.9 (3.0)
AVF-3 (cm^2)	138.5 (9.0)	124.2 (9.7)	46.7 (4.8)	37.5 (3.2)
Mean Difference AVF-2–AVF-1 (cm^2)	10.4 (0.8)	15.0 (1.3)	2.4 (0.4)	3.3 (0.5)
Mean Difference AVF-3–AVF-1 (cm^2)	11.6 (0.8)	16.5 (1.4)	2.6 (0.4)	3.9 (0.6)
Mean Difference AVF-3–AVF-2 (cm^2)	1.1 (0.3)	1.5 (0.1)	0.2 (0.1)	0.5 (0.2)

*See text for definition of AVF-1, AVF-2, and AVF-3. Analyses of variance and post-hoc procedures revealed the following: AVF-3 measurement>AVF-1 measurement (*p*<0.0001). AVF-2 measurement>AVF-1 measurement (*p*<0.0001). AVF-3 measurement>AVF-2 measurement (*p*<0.05). AVF-1, AVF-2, and AVF-3 in older subjects>AVF-1, AVF-2, and AVF-3 in younger subjects (*p*<0.0001). (AVF-3–AVF-1) for older subjects > (AVF-3–AVF-1) for younger subjects (*p*<0.0001). (AVF-2–AVF-1) for older subjects > (AVF-2–AVF-1) for younger subjects (*p*<0.0001). (AVF-3–AVF-1) for women > (AVF-3–AVF-1) for men (*p*<0.0001). (AVF-2–AVF-1) for women > (AVF-2–AVF-1) for men (*p*<0.0001).

Significant two-way interactions were observed between age and method and between sex and method (p<0.0001). A significant three-way interaction was also observed (age x sex x method, p<0.02). Two-way analysis of variance with repeated measures (age x method, and sex x method) was also performed. Results revealed the following. A comparison of AVF-1 vs. AVF-2 and AVF-1 vs. AVF-3 revealed that AVF-2 and AVF-3 resulted in greater AVF area than AVF-1 for both age groups, independent of sex (p<0.0001). Method by sex comparisons revealed that the measurement differences (AVF-2 – AVF-1 and AVF-3 – AVF-1) were greater in women compared with men (p<0.0001); method by age comparisons revealed that measurement differences (AVF-2 – AVF-1 and AVF-3 – AVF-1) were greater in older than younger subjects (p<0.0001).

Figure 2 shows the relationships among the three methods of AVF measurement. The line of identity is shown for each method comparison, and the TE was calculated for each group (young men, young women, older men, and older women). The correlations among AVF measurement methods ranged from $r = 0.983$ to $r = 1.000$ for all groups. The total errors were larger in older compared with younger subjects and in women compared with men.

Discussion

Single-slice CT scans are commonly used for the measurement of AVF, and the accuracy of these scans is thought to be surpassed only by the accuracy of multislice CT imaging or cadaver analysis (26,33). The accuracy of the measurement is highly dependent on the proper selection of scan location, the range of Hounsfield units used to distinguish adipose from other tissue types, and the identification of anatomical boundaries for the separation of specific fat depots. The scan location and pixel identification range commonly used are consistent and widely accepted.

However, much less standardization in practice has been given to careful delineation of the boundaries for cross-sectional area analysis.

This study demonstrates that small changes in anatomical boundary line location result in significantly different AVF cross-sectional area measurements. The major finding of this study was that the AVF-2 and AVF-3 methods yielded significantly greater AVF estimates than did the AVF-1 method. The absolute and relative differences between the abdominal fat measurements using the anatomical boundaries of AVF-1 compared with AVF-2 and AVF-3 were significantly greater in older than younger subjects (mean differences in older subjects ranged from 10.4 cm^2 to 16.5 cm^2 compared with 2.4 cm^2 to 3.9 cm^2 in younger subjects; Table 2) and greater in women than in men. Furthermore, the methodology differences were influenced by age to a greater extent than sex.

Although AVF-1, AVF-2, and AVF-3 measurements were highly reliable, AVF-1 appears to provide a more accurate measure of the AVF cross-sectional area than do the AVF-2 and AVF-3 methods. As noted previously, separation of the intraperitoneal and retroperitoneal fat was not possible using our scans because of poor visibility of blood vessels and organs. Therefore, even the most conservative estimates of AVF (such as with AVF-1) contain both intraperitoneal and retroperitoneal fat, which previously has been defined as visceral fat (33). The increased fat areas obtained with the A VF-2 and A VF-3 methods were likely the result of the inclusion of intermuscular fat and/or intramuscular lipid droplets from the abdominal, oblique, and back muscles. In spite of the inclusion of retroperitoneal fat by the AVF-1, AVF-2, and AVF-3 methods, this study demonstrates that CT analysis boundaries, which include subcutaneous fat, intermuscular, and/or intramuscular lipid droplets (as in A VF-2 and A VF-3), may further compromise the accuracy of the measurement of visceral adipose tissue. Furthermore, in longitudinal or interventional studies requiring precise visceral adipose tissue measurements, variability in the analysis method will likely affect the interpretation of the effectiveness of the intervention of appreciation of the change over time.

The intraperitoneal fat, which includes both omental and mesenteric fat, possesses unique metabolic properties resulting in a high free fatty acid flux (4). Increases in the amount of visceral fat have been associated with increased free fatty acid concentrations, which may lead to local and systemic adverse metabolic consequences. Adverse changes at the liver include a decreased extraction of insulin and increases in gluconeogenesis, very low-density lipoprotein (VLDL) secretion, and hepatic triglyceride lipase activity. Local alterations in skeletal muscle tissue may include an increase in free fatty acid oxidation and a decrease in both glucose oxidation and insulin sensitivity. Systemic alterations may include a decrease in blood high-density lipoprotein cholesterol levels and an increase in insulin, free fatty acid, glucose, and VLDL cholesterol concentrations (4,10,22,23,28,30). These adverse metabolic alterations are known to be cardiovascular disease risk factors (10). Although one recent study suggests that subcutaneous abdominal fat is a major determinant of peripheral and hepatic insulin sensitivity (1), the vast majority of studies suggest that adverse metabolic consequences are specifically related to the intraperitoneal fat (5,9,11,14,17,24,29,30). Therefore, it is important that precise anatomical boundaries for CT analysis that minimize the inclusion of extraperitoneal fat be chosen for the estimation of AVF. Several anatomical boundaries for the measurement of AVF have been described. Although the brevity of the descriptions provided in many reports often makes it difficult to discern exact boundary locations, most authors report the inclusion of abdominal intermuscular fat in their measurement of AVF (3,7,16,18,31,32,34). We propose that this is not the best course of action and recommend that as little as possible of the fat contained in the muscle wall surrounding the abdominal cavity be included within the boundaries for the measurement of AVF. The AVF-1 procedure will accomplish this goal.

In conclusion, the results of this study indicate that changes in anatomical boundary selection affect the measurement of AVF. The observed effects were considerably greater in older subjects and in women. On the basis of known anatomical compartments and corresponding circulatory drainage, we suggest that AVF-1 is the superior method of measuring visceral fat and recommend that analyses of CT scans for this purpose use this boundary line placement consistently over time.

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