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Assessment of visceral and subcutaneous fat tissue size using the stereological methods with the combination of computed tomography images: A simple accurate approach

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Abstract

Objective: Several methods have been used to evaluate the accurate volume of visceral adipose tissue and obesity. In this study we describe a new accurate approach for the assessment of visceral and subcutaneous fat tissue size using the stereological methods with the combination of computed tomography images.

Methods*:* Abdominal CT scans of 52 subjects (Male 26, Female 26) were examined retrospectively. The mean age of the males and females (±SD) were 49.95±15.36 and 45.31±14.57, respectively. The CT scanning of patients was performed using a multislice CT machine. The volume of abdominal cavity (AC) and abdominal body region (ABR) were estimated using the planimetry approach of Cavalieri principle. Subcutaneous fat tissue (SFT) in ABR and visceral fat tissue (VFT) within the AC were estimated using point-counting approach of Cavalieri principle. The volume fraction of the SFT within the ABR and VFT within the abdominal cavity were also estimated.

Results: The volume of SFT was higher in females (males 5481.40cm^3 and females 8032.85cm^3), ($p < 0.05$). The results were similar for the volume of VFT ($p < 0.05$). The volumes of VFT in male and female were 4462.51cm^3 and 3154.95cm^3, respectively. The bodyweight of the males was 11.25% heavier than the females. The volume fraction of the VFT to the AC of the males and females were 36.35 and 29.37%, respectively, while the volume fraction of the SFT to the (ABR) was significantly higher in the females than the males (males 18.78% and females 28.91%), ($p < 0.05$). There was a close correlation between the BMI and the volume of VFT and SFT ($r=0.708$ and 0.714, respectively), ($p < 0.05$).

Conclusion: The results revealed that method described in this study is simple, reliable, accurate and fast, and it can be used to measure visceral and subcutaneous fat tissue precisely

Keywords: Tissue; Fat; Stereology; Images

1 Introduction

Obesity is caused by an imbalance between energy intake and expenditure and is characterized by the accumulation of adipose tissue in both visceral and subcutaneous depots (Farkas et al., 2019). Obesity can also alter the endocrine and metabolic functions of adipose tissue and is a risk factor for many metabolic diseases (White and Tchoukalova, 2014). The regional distribution of adipose tissue has been shown to be a stronger predictor of health risk than overall excessive adiposity (Farkas and Gater, 2018). Excess of visceral adipose tissue facilitates high doses of adipokines in the portal vein to the liver and other body tissues, resulting in serious effects such as diabetes, non-alcoholic fatty liver diseases, kidney disease, cancer and other health problems (Dhawan and Sharma, 2020).

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Adipose tissue is an anatomically distributed throughout the human body, and the pattern of adipose tissue distribution is influenced by many factors, including sex, age, genotype, diet, physical activity level, socioeconomic factors, hormones, and drugs (Camilleri et al, 2021; Maria and Philipp, 2006; Sakamoto et al, 2025; Ren. Z, 2025). The human organs do, however, vary widely in size.

Over the years, several methods have been used to evaluate the accurate volume of visceral adipose tissue and obesity. Magnetic resonance (MR) imaging, computed tomography (CT) ultrasonography, bioelectrical impedance, dual energy X-ray absorptiometry, and anthropometric measurement are the common tool to obtain information on the volumetric analysis of the visceral fat tissue (Armao et al, 2006; Sarma et al., 2020; Meriño-Ibarra et al., 2005; Miwa et al., 2005, Kong M et al., 2023). To date, there is no simple and reliable method to measure visceral adipose tissue. However, the volume fraction of a component within a reference volume is a simple and very widely used parameter in biomedical science (Mattfeldt et al., 2003; Mattfeldt et al., 2004) which can be used to express the proportion of a component within whole structure. Hence, it is possible to use this proportion to compare the organ size and any part of organ between the groups and also to estimate the volume of structures by the combination of the Cavalieri principle, a method of stereology, and sectional imaging techniques (Altunkayank et al., 2009).

However, we have not found any study on the estimation of visceral fat volume using stereological methods with the combination of CT imaging. In this study, we first time used a new approach to evaluate the volume fraction of an individual visceral fat volume using the Cavalieri principle on abdominal CT scans.

2 Materials and Methods

The ethics board of the university approved the present retrospective study. The abdominal CT scans of 52 subjects (Male 26, Female 26) who applied for the CT scanning because of kidney stone complaints were examined retrospectively. The mean age of the males and females (±SD) were 49.95±15.36 and 45.31±14.57, respectively. There was not age difference between the males and females ($p > 0.05$).

The volume of abdominal cavity (AC) and abdominal body region (ABR) were estimated using the Cavalieri principle of the stereological methods. The above and below limits of the AC is accepted between the sections showing the highest point of the dome of the diaphragm and pelvic floor, respectively. The above and below limits of the ABR is accepted between the sections showing xiphoid process and circular view of the femoral head above and below, respectively. As a second step, subcutaneous fat tissue (SFT) in the described ABR and visceral fat tissue (VFT) within the AC were estimated (Fig. 1). The volume fraction of the SFT within the ABR and VFT within the abdominal cavity were also estimated using the obtained volume data.

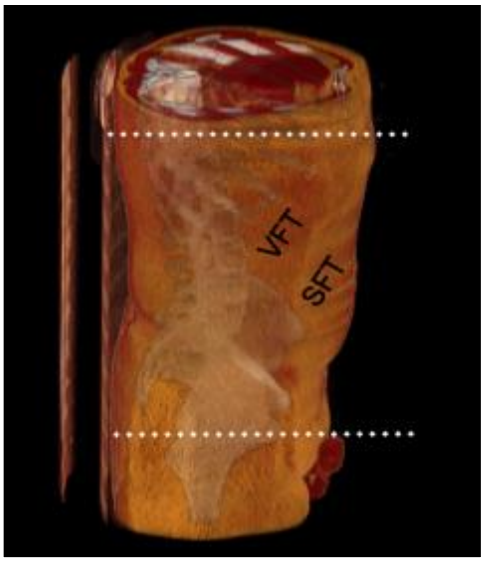

Figure 1 The upper and lower limits (dotted lines) of the ABR and distribution of the fat tissue within the body

The CT scanning of patients was performed using a multislice CT machine (Toshiba TSX-101A, Aquilion 16 Slice, Tochigi, Japan). The scanning was performed with the following parameters: kVP: 120, mAs: 150, msec: 500, section thickness: 5 mm. The imaging data were stored as Dicom files.

The consecutive CT scans were converted into PNG formatted images (Fig. 2A) using a software namely DicomWorks (Version 1.3.5). The images were transferred into Image (Version 1.42q) which is distributed freely by the National Institutes of Health, USA. First the images were converted into stacks then the program was matched using the reduction scale of the images. Since the consecutive images were more than required, systematic random sampling procedure was used and every 1 out of 6 sections was used for the volume estimations. Sectional cut surface areas of the AC and ABR were estimated using the planimetry method. For the AC, the innermost borders of the cavity were delineated using polygonal selection tool of the program (Fig. 2B). The threshold function of the software was used to determine the outermost borders of the abdomen and it was automatically delineated using wand tool of the software (Fig. 2C). The software automatically calculated the number of pixels enclosed by the traced structure contours on each section and provided the cross-sectional area of the interested organ on a slice-by-slice basis. The sum of the areas multiplied by the section thickness provided the volume of structure as shown below formula (Sahin et al., 2007):

$$V = t \times \sum A \quad \text{............} \quad (1)$$

where t is the section thickness of consecutive sections (3 cm) and *Σ*A is the total sectional area of the consecutive sections.

Since the fat tissue dispersed within the images planimetry method could not be applied for the assessment of volume of SFT and VFT. Therefore, we applied the point-counting approach of the Cavalieri method for the volume estimation of both tissues. After threshold process, point counting grid was superimposed over the sections using the grid plugin of the ImageJ (Fig. 2D). The representative is per point was 2 cm in the point counting grid. The numbers of points hitting the visceral and SFT in the sections were counted and the obtained point numbers were used for the estimation of fat volume using the following formula (Sahin et al., 2003):

$$V = t \times a / p \times \sum P \quad \text{.............} \ (2)$$

Where t is the section thickness of consecutive sections (3cm), *a/b* is the representative area per point in the grid (2cm) and *ΣP* is the total sectional area of the consecutive sections.

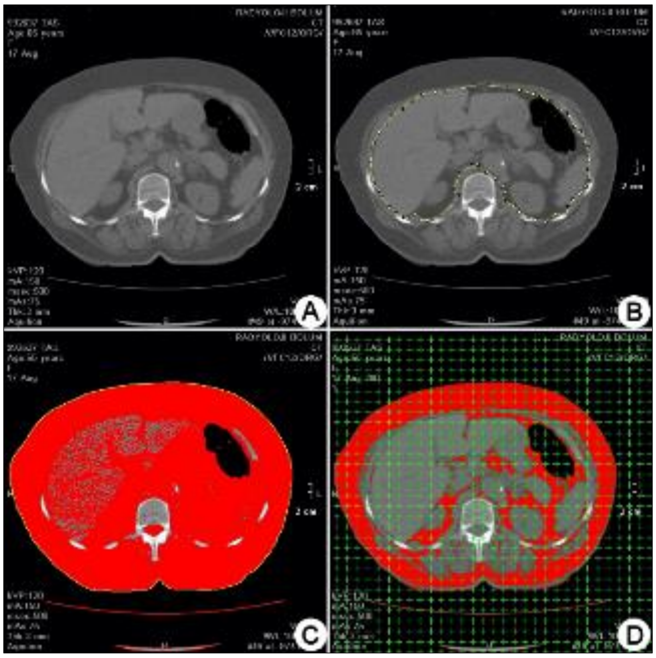

Figure 2 Usage of the ImageJ software for the estimation of volumes. A; an axial section of the abdominal CT, B; delineation of the innermost borders of the abdominal cavity, C; thresholding and semiautomatic delineation of the abdominal body region, D; superimposition of the point counting grid for the estimation of volume of visceral fat tissue

Organ size of the subjects is a function of the body size. Therefore, larger bodies have larger organ size. Naturally, the males' body size is bigger than females. As the result of this fact, the visceral tissue size of the males is larger than females which make the size comparisons between genders impossible. For this reason, the fat tissue size of the females should be corrected regarding the males body size.

The volume fractions of the fat tissue to the abdominal cavity and abdominal body region were estimated using the following formula (Basoglu et al., 2007):

$$VV\ (VFT, AC) = (\text{Volume of VFT} / \text{volume of AC}) \times 100 \quad \ldots\ldots \quad (3)$$

$$VV\ (SFT, ABR) = (\text{Volume of SFT} / \text{volume of ABR}) \times 100 \quad \ldots\ldots \quad (4)$$

The results of section cut surface areas were transferred into the worksheet which is produced in Microsoft Excel and the volume and the volume fraction data were obtained automatically. Obtained data were compared between males and females using Independent Samples Test. A p value equal or less than 0.05 was accepted as statistically significant.

3 Results

The body weights of the males and females were 78.69 and 69.96 kg, respectively. The males were heavier than males ($p < 0.05$). The height of males and females were 1.71 and 1.61 m, respectively. The difference of heights between the genders was significantly different ($p < 0.05$). The body mass index (BMI) of the males and females were 26.87 and 27.29, respectively. There was no difference in terms of body mass index between the groups ($p > 0.05$). The detail of the age, weight, height and BMI is given in the Table 1.

The volume of SFT was higher in females (males 5481.40cm^3 and females 8032.85cm^3), ($p < 0.05$). The results were similar for the volume of VFT ($p < 0.05$). The volumes of VFT were 4462.51cm^3 and 3154.95cm^3, respectively (Table 2).

The body weight of the males was 11.25% heavier than the females. For the normalization correction we multiplied the fat tissue volume with the weight difference rate, i.e. 11.25%. After the normalization, the mean SFT volume of the females was 9035.27±4607.41cm^3 which is still statistically significant ($p < 0.05$) as it is the case before. However, the normalized mean VFT volume of females was 3548.67±2639.63cm^3. After normalization, there was not a statistical difference between the males and females ($p > 0.05$), Fig. 3).

The volumes of AC and ABR of males were larger than the females ($p < 0.05$). After the normalization on the basis of males' body size, both sizes did not show statistical differences between the genders ($p > 0.05$). The details are given in the Table 2 and Fig. 3.

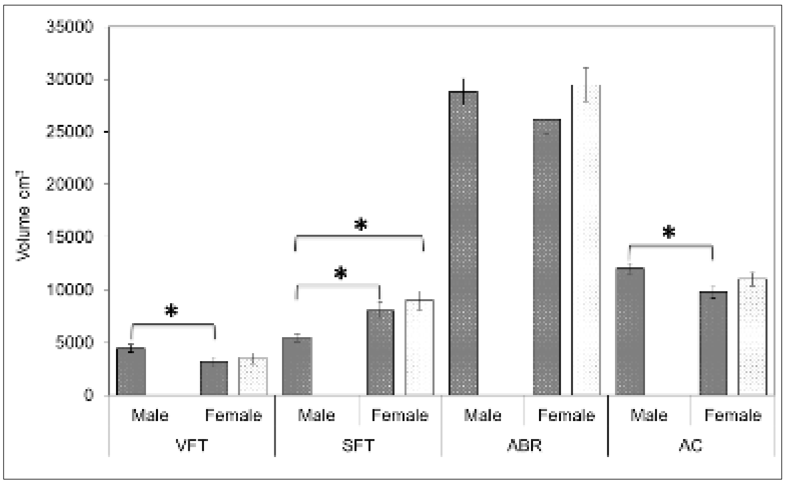

Figure 3 Volumetric data of the subjects. Light bars are the normalized data. *; the comparison is statistically significant

The volume fraction of the VFT to the AC of the males and females were 36.35 and 29.37%, respectively. However, the difference between the genders did not rise to a statistically significant level ($p > 0.05$). The volume fraction of the VFT to the ABR was significantly higher in the males than the females (males 15.28% and females 10.91%), ($p < 0.05$). The volume fraction of the subcutaneous fat tissue to the abdominal body region was significantly higher in the females than the males (males 18.78% and females 28.91%), ($p < 0.05$), (Fig. 3). The details of the volume fraction data are given in Table 3 and Fig. 4.

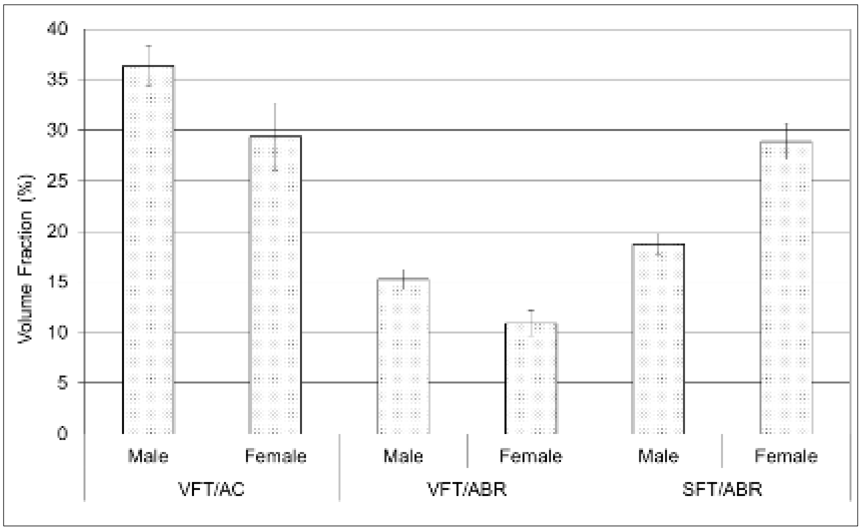

Figure 4 Volume fraction data of the subjects

There was a close correlation between the BMI and the volume of VFT and SFT (r=0.708 and 0.714, respectively), ($p < 0.05$) and Fig 5. The details of correlation analysis of the certain features are given in Table 4. The following regression formulas were calculated for the prediction of volume fraction of visceral fat volume to the abdominal cavity. Anyone who knows the BMI may predict possible percentage of visceral fat tissue using the given formulas:

Regression for Males

$$\text{VF of VFT/AC} = -2.87 + (1.46 * \text{BMI}) \quad \ldots\ldots\ldots\ldots \quad (5)$$

Regression for Females

$$\text{VF of VFT/AC} = -12.192 + (1.523 * \text{BMI}) \quad \ldots\ldots\ldots \quad (6)$$

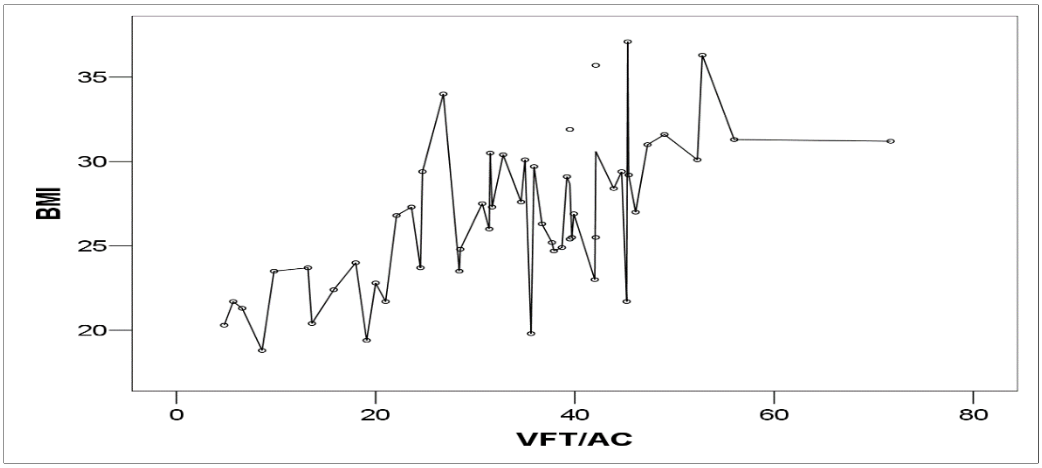

Figure 5 The graphic showing the relation between BMI and AC

Table 1 Demographic features of the subjects

		Mean	Minimum	Maximum	Std. Deviation
Age (year)	Male	49.65	19.00	77.00	15.36
	Female	45.31	20.00	71.00	14.57
Weight (kg)	Male	78.69	56.00	105.00	10.88
	Female	69.96	48.00	100.00	14.47
Height (m)	Male	1.71	1.65	1.85	0.05
	Female	1.61	1.46	1.70	0.06
BMI	Male	26.87	19.40	36.30	3.57
	Female	26.47	18.80	37.10	5.20

Table 2 The details of the volumetric data (cm^3) of the subjects

		Mean	Minimum	Maximum	Std. Deviation
VFT	Male	4462.51	1026.00	8791.20	1832.51
	Female	3154.95	331.20	8384.40	2346.78
SFT	Male	5481.40	1396.80	8960.40	1943.11
	Female	8032.85	1566.00	16380.00	4096.23
ABR	Male	28840.63	19872.00	52199.10	6455.73
	Female	26206.44	14514.20	43929.60	7238.36
AC	Male	12028.83	8727.90	17569.00	2495.64
	Female	9812.43	5215.50	18501.50	2918.46

Table 3 Volume fraction (%) of the fat tissue within AC and ABR

		Mean	Minimum	Maximum	Std. Deviation
VFT/AC	Male	36.35	9.80	52.80	10.27
	Female	29.37	4.80	71.70	16.79
VFT/ABR	Male	15.28	4.70	26.40	4.97
	Female	10.91	2.10	25.60	6.33
SFT/ABR	Male	18.78	6.40	31.00	5.13
	Female	28.91	9.80	45.20	9.01

Table 4 Correlation analysis of the certain features of males (lower left values) and Females (upper right values)

	BMI	VFT	SFT	VFT/AC	VFT/ABR	SFT/ABR
BMI		0.669 (**)	0.822 (**)	0.613 (**)	0.564 (**)	0.672 (**)
VFT	0.703 (**)		0.761 (**)	0.930 (**)	0.950 (**)	0.598 (**)
SFT	0.663 (**)	0.617 (**)		0.710 (**)	0.632 (**)	0.910 (**)
VFT/AC	0.508 (**)	0.851 (**)	0.572 (**)		0.973 (**)	0.673 (**)
VFT/ABR	0.424 (**)	0.861 (**)	0.343	0.918 (**)		0.561 (**)
SFT/ABR	0.355	0.352	0.830 (**)	0.550 (**)	0.332	

** Correlation is significant at the 0.05 level (2-tailed)

4 Discussion

Overweight and obesity are associated with a range of chronic illnesses including cardiovascular disease, diabetes mellitus and cancer (Crudele et al, 2021); they are also strong predictors of increased mortality (Ladeiras et al 2016). In recent decades, body fat distribution has become a major focus of research, as there is evidence that it is more important than total body fat mass (BF) in predicting obesity-related diseases (Tchernof and Despres, 2013). The proportion of abdominal fat tissue comprising visceral (VAT) and subcutaneous adipose tissue (SAT) is a critical correlate for all health complications related to overweight and obesity (Despres et al 2008; Kuk et al, 2006).

4.1 Measuring the fat quantity and distribution in human is generally difficult and imprecise

Numerous techniques have been developed to assess visceral fat. Comparison of the different methods shows that imaging technique, such as CT and MR imaging, are the optimal techniques available for accurate assessment of visceral fat (Yuta et al 2024; Kong et al, 2022). Methods other than imaging techniques have limited potential in the measurement of changes in visceral fat deposition (Shuster et al 2012). Gender related patterns of body fat deposition become established during puberty and as with total body fat, show significant familial associations (Karastergiou et al 2012). Power and Schulkin (2008) reported that men have significantly more fat within the abdominal cavity and woman have similar total fat, but store a greater proportion of it in their subcutaneous tissues.

Although CT and MR imaging provide volumetric information about fat distribution in males and females, we have not found a stereological study about VFT and SFT. Therefore, we propose a new approach to evaluate the volume fraction of an individual visceral fat volume using the Cavalieri principle on abdominal CT scans. Bonaventura Cavalieri who is an Italian mathematician described the Cavalieri principle over three centuries ago. He proposed that the volume of an irregular shaped object could be estimated from a set of two-dimensional slices through the object, provided that they are parallel separated by a known distance, and begins randomly within the object. All these criteria are met by standard CT procedure (Clatterbuck and Sipos, 1997).

In the present study, we evaluated the volume of VFT, SFT, AC, and ABR in males and females. The volume fraction of the SFT within the ABR and VFT within the abdominal cavity were also estimated using the obtained volume data. The volume of the VFT was found greater in males from females.

Organ size of the subjects is a function of the body size. Therefore, larger bodies have larger organ size. Naturally, the males' body size is bigger than females. As the result of this fact, the visceral tissue size of the males is larger than females which make the size comparisons between genders impossible. For this reason, the fat tissue size of the females should be corrected regarding the male's body size. As a result, the corrected volume of VFT of females increased. There was no difference between the corrected the volume of VFT of females and the volume of VFT of male.

According to our results, we should not compare subjects using volume of VFT itself. It is true to use the fraction of VFT/ABR. Obtained fraction of VFT/ABR was found to be greater in males than females. In contrast, the fraction of SFT/ABR was greater in females than males.

5 Conclusion

In conclusion, the results demonstrate that the proposed method is simple, reliable, accurate and fast, and it can be used to estimate the volume of VFT, SFT, AC, ABR, global fat tissue, and the fraction between visceral and subcutaneous fat tissue in a supervised and effective manner.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Statement of ethical approval

The ethics board of the Gezira University approved the present retrospective study.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study

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