Subcutaneous and visceral fat volumes measured by MRI and its relationship with nutrients intake among adults

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Running title: Fat volumes measured by MRI and nutrients intake

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ABSTRACT

Background and Objectives: Types and amounts of nutrients may influence the volume of subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT). This study targeted to investigate the relationship between SAT and VAT volumes and macro- and micronutrients intake among adults. **Methods and Study Design:** Data were collected via a private face-to-face interview, in which diet history was obtained using validated quantitative food frequency questionnaire. The different fat volumes were assessed using magnetic resonance imaging (MRI) scanning. **Results:** Participants with the lowest SAT volume had the highest intake of monounsaturated fatty acids (p<0.044) and omega-3 fatty acids (p<0.048). VAT volume was significantly associated with the highest level of total energy and energy from carbohydrate consumption among participants (p<0.037) while significantly associated with the lowest energy intake from fat among participants (p<0.013). There was a significant relationship with the highest consumption of total carbohydrate, soluble fiber, and insoluble fiber and VAT volume (p<0.05). Participants in the highest VAT volume had significantly the highest intake of vitamin A, β-carotene, and copper. **Conclusions:** This study underscores the importance of quantifying depot-specific body fat and highlights the unique responsiveness of various fat depots to dietary intake.

Key Words: macronutrients, micronutrients, subcutaneous fat, visceral fat, magnetic resonance imaging

INTRODUCTION

Obesity is an accelerating health problem worldwide. It is defined as an abnormal or excessive fat accumulation that may impair health. In 2014, more than 1.9 billion adults, 18 years and older, were overweight and over 600 million were obese.[1] In Jordan, prevalence of overweight and obesity among adults was estimated to be 30.5 and 35.9 %, respectively.[2] Obesity is associated with an increased risk of a number of co-morbidities such as hypertension, dyslipidemia, cardiovascular disease,[3] type 2 diabetes[4] and cancer.[5]

Body mass index and waist circumference are the two main measurements commonly used to assess body fat, but these anthropometric measures cannot provide information regarding the anatomical location and distribution of stored excess fat.[6,7] However, the use of imaging techniques such as computed tomography and magnetic resonance imaging (MRI) has been characterized as an incredible advance in our ability to accurately and reliably quantify individual differences in body fat distribution and to selectively distinguish specific

abdominal fat depots.[8,9] Two types of fat depots have been recognized in the android region, subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT). SAT is the adipocyte that lies just beneath the skin, while adipocyte that accumulates around the vital organs in the abdominal cavity is known as VAT. Not all adipocytes in android region induce the same degree of pathogenesis; the association between the adverse metabolic risk profile and VAT was found to be about 2-folds stronger than that for SAT.[10] Thus, quantifying total and regional fat mass as well as differentiating between the types of fat depots provide a clear picture regarding the pathogenesis of obesity and therefore, better insight to disease risk.

SAT and VAT appear to be differentially influenced by dietary factors.[9,11-13] It has been proposed that diet can explain more of the variation in VAT than in SAT,[13] and lifestyle modification has the ability to reduce VAT more than SAT.[14] For instance, several studies have revealed associations between dietary fatty acids composition and visceral fat accumulation.[15,16] A 5% increase in energy intake from animal protein substituted for carbohydrate, monounsaturated fatty acids, and polyunsaturated fatty acids was reported to be associated with higher visceral adiposity index changes after three years of follow-up among Iranian adults.[9] The consumption of high dietary protein, adjusted for total caloric intake was associated with VAT among a group of young adult Saudi females independent of body weight.[17] Chaput et al (2014) revealed that low calcium and micronutrient intake could be considered as predictor of excess body weight and gains in weight and adiposity over time.[18]

Jordan is considered one of the Middle East countries that witnesses a dramatic transition from its Mediterranean dietary and lifestyle pattern towards western pattern. This transition is accompanied by an increase in the prevalence of obesity and many other related diseases. Motivated by the current evidence that supports the associations between diet and body fat deposition and distribution, this study aimed at investigating the relationship between subcutaneous and visceral fat volumes (measured using MRI) and the intake of nutrients among Jordanian adults.

MATERIALS AND METHODS

Study Design and Participants

In this cross-sectional study, a total of 167 apparently healthy persons agreed to participate in the study. Participants were recruited from Royal Medical Services (RMS) personnel (including the security manpower, hospital cleaners, and employees in administrative positions). Eighty three males and 84 females (age range 20–51) were enrolled in the study during the period of October 2014 to July 2015. Eligibility criteria to participate in the study

were: being Jordanian and above 18 years old and free from any chronic disease (self-reported). Pregnant and lactating women and persons suffering from eating disorders or having any disease were excluded. Response rate for dietary assessment and anthropometric measurements were about 90.0%. All participants were asked to sign a formal consent according to the medical center ethics approval. The study was approved by the Institutional Review Board Ethics Committee of RMS; the ethical approval number of the study was 10205/2014. All participants completed the MRI safety questionnaire before participating in this study.

Magnetic Resonance Imaging (MRI) measurement

Abdominal MRI was performed on a 3T Siemens Trio MR system (Siemens Medical Systems, Erlangen, Germany), equipped with 4-channel phase-array body coil. The acquired axial slices covered the region between the level above of the diaphragm and the head of the femur in the supine position and full expiration. The imaging protocol included in (IP) and out (OP) of phase, non-enhanced T1 weighted sequence with the following imaging parameters; repetition time (TR=5 ms), echo time (TEOP=1.225 ms) and (TEIP=2.45 ms), flip angle (FA=10o), slice thickness (ST=5 ms), 80 slices, 256 x 192 matrix size, 380 x 285 mm^2 field of view, number of signal averages (NSA=1), acceleration factor of 2, and scan time =18 seconds. The MRI scanner calculated the "fat-only" and "water-only" images from this sequence as follows:

“Fat” image = [*IP* – *OP*]/2; “Water” image = [*IP* + *OP*]/2

Image post processing and analysis

“Fat” MR images were imported into image analysis software (SliceOmatic, Tomovision Inc., Montreal, Canada) in their standard formats (Digital Imaging and Communications in Medicine; DICOM) to segment the subcutaneous and visceral fat tissues. Slices covering the region between the top of the diaphragm and the top of the first sacral vertebra (S1) were used to segment and analyze the subcutaneous and visceral fat tissues by an expert in image analysis. Each segmented tissue was saved as a separate “tag” and the total volumes of SAT and VAT were calculated from all analyzed slices and saved into a separate file for further analysis. The model and method employed to segment the various tissues is fully described and illustrated elsewhere.[19] “Fat” images were also used to measure the waist circumference at a level just below the lower costal margin.

Dietary assessment

A validated Arabic quantitative Food Frequency Questionnaire (FFQ) was used for dietary assessment.[20] The FFQ questions tracked the information on the dietary history of study participants. The FFQ and 24-hour recalls produced similar agreement percentages ranging between 25.5% and 43.6%. Mean energy-adjusted reliability coefficients ranged from .695 to .943. A Cronbach's α for the total FFQ items of .857 was recorded. Many studies have used FFQ along with MRI to assess body fat volumes and their relationship with the type of nutrient consumed.[21,22] Trained dietitian asked participants, during face-to-face interviews, how frequently, on average, during the past year they had consumed one standard serving of specific food items in nine categories (<1/month, 2–3/month, 1–2/week, 3–4/week, 5–6/week, 1/day, 2–3/day, 4–5/day, or 6/day). Food lists in the modified FFQ questions were classified based on types of foods: 21 items of fruits and juices; 21 items of vegetables; eight items of cereals; nine items of milk and dairy products; four items of beans; 16 items of meat such as red meat (lamb and beef), chicken, fish, cold meat, and others; four items of soups and sauces; five items of drinks; nine items of snacks and sweets; and 14 items of herbs and spices.[20] For better portion size estimation food models and standard measuring tools were used. To estimate macro- and micronutrients intake, the intakes of foods collected by FFQ were analyzed using dietary analysis software (ESHA Food Processor SQL version 10.1.1; ESHA, Salem, OR, USA) with additional data on foods consumed in Jordan.[23]

Physical activity level

Physical activity level was determined using 7-day Physical Activity Recall (PAR).[24] The 7-Day PAR is a structured interview that depends on participant's recall of time spent engaging in physical activity over a seven day period of their usual days.[24] The number of hours spent in different activity levels were collected and transformed to metabolic equivalents (METs). The total physical activity MET minutes per week was calculated by summing the METs.[24]

Anthropometric measurements

All anthropometric measurements were carried out by trained dietitian at the morning between 8-10am while they were fasting. Waist circumference (WC) was measured by tape (WC_tape) at the narrowest level between the lowest rib and the iliac crest at the end of normal expiration in standing position.[25] In addition, waist circumference was measured from the MR images (WC_MRI) at a level just below the lower costal margin using an image analysis software (SliceOmatic, Tomovision Inc., Montreal, Canada) in a semi-automated

approach. Body weight was measured to the nearest 0.1 kg, with minimal clothing and without shoes, using a calibrated scale (Tanita, Model SC-331S, Japan).[25] Height was measured to the nearest 1 cm with participants in standing position without shoes using a calibrated portable measuring rod.[25] Body mass index (BMI) was calculated as the ratio of weight in kilograms to the square of height in meters.[25]

Statistical analysis

The data were analyzed using SPSS statistical package version 20. Energy, macronutrients and micronutrients were presented as mean ± standard error of mean (SEM). SAT, VAT and total fat were grouped into tertiles. Data presented in 3 tertiles to have a representative number of subjects in each tertile. Analysis of Covariance (ANCOVA) was used to assess the impact of energy, macronutrients and micronutrients intake on SAT, VAT, and total fat. Data was adjusted for energy, age, gender, physical activity and smoking. The Benjamini-Hochberg correction with critical value for a false discovery rate of 0.25 for multiple comparisons was used. Value of $p \leq 0.05$ was considered statistically significant.

RESULTS

Participants' anthropometric measurements, SAT, VAT and total abdominal fat were published previously in another publication.[26] The authors reported significant statistical differences ($p<0.05$) between males and females in height, weight, and waist circumference, with males showing higher values than females (Table 1). Regarding the physical activity level, the results of this study did not show any significant difference between male and female participants. While the SAT tissue volume in males was found to be significantly (p-value=0.001) lower (3779.4 cm^3) than in females (5222.7 cm^3), VAT tissue volume was significantly (p-value=0.001) higher (2967.1 cm3) in males than in females (1881.7 cm^3). However, the total of participants showed insignificant differences in SAT, VAT and total abdominal fat between males and females due to the equal participation of both genders.[26]

Table 2 shows the relationship between participants' daily total energy intake and macronutrients' contribution to energy intake in relation to their body fat fractions. Participants in the highest tertiles of visceral fat and total fat had the highest consumption of energy from carbohydrate ($p<0.05$). In contrast, visceral fat was significantly associated with the highest energy intake from fat among participants in the lowest tertile ($p<0.013$). There was no significant relationship between protein consumption across different tertiles of visceral fat and total fat. We also found no statistically significant relationship with total

energy and macronutrient distribution consumptions across all tertiles of subcutaneous fat. After using Benjamini-Hochberg with a false discovery rate of 25%, the participants in the lowest tertile of total fat was also found to have a significantly higher percent of energy from fats compared to the highest tertile.

Table 3 presents the participants' daily intake of the different macronutrients through different tertiles of body fat fractions. The consumption of total carbohydrate, soluble fiber, and insoluble fiber were significantly higher among participants in the highest tertiles of visceral fat and total fat ($p<0.05$). Conversely, visceral fat was significantly associated with the highest intake of total fats, saturated fats, monounsaturated fats, omega-6 fatty acids and omega-3 fatty acids among participants in the lowest tertile.

Participants in the highest tertile of visceral fat had significantly the highest intake of copper. There was no statistically significant relationship with the studied fat soluble and water soluble vitamins as well as calcium, iron and zinc intake through all tertiles of body fat fractions (Table 4).

DISCUSSION

The present study was conducted to find the relationship between the main nutrients intake, and SAT and VAT in a convenient sample of Jordanian adults. Body fat tends to accumulate in two main regions, abdominal and peripheral, the former is considered a stronger predictor of disease risks. An increase in cardiovascular risk factors from no to at least 3 factors corresponding to an increase of less than 1 kg of abdominal fat.[27] The two fat depots in the abdominal region differ in their anatomical and physiological properties, consequently, induce different degrees of pathogenicity.[28,29] Although, VAT constitutes only a small proportion of total body fat, its bad reputation persuades by its clinical impact.[28,29] VAT is more cellular, vascular, innervated, and contains a larger number of inflammatory and immune cells as compared to SAT.[28] There are more glucocorticoid and androgen receptors in VAT than SAT, also VAT is more sensitive to lipolysis and lipogenesis and more insulin-resistant compared to other fat depots.[30] Therefore, VAT can be identified as metabolically active and pathogenic fat depot that drains several adipokines directly into the portal vein.[28] The current evidence supports a strong and independent association of VAT depot, rather than SAT, with chronic diseases and metabolic abnormalities. In addition, VAT has been influenced by genetics, gender, ethnicity, age, and modifiable factors including physical activity and diet.[31,32]

This study successfully highlighted a few significant associations between food consumption and visceral fat accumulation. The results show a significantly greater

consumption of carbohydrate among subjects with the highest visceral fat deposition, which might be explained by two possible scenarios. First, is the activation of de novo lipogenesis; the process of lipid synthesis from dietary lipid or non-lipid precursors that takes place primarily in the liver. Despite the fact that de novo lipogenesis lightly contributes to fatty acid pool,[34] this process has been implicated in modulating disease risks and obesity development particularly in a situation of overfeeding.[35,36] De novo lipogenesis has been reported to be upgraded to a higher extent following a high carbohydrate diet, compared to a high-fat diet.[37] Similar pattern of consumption was found among participants in the highest tertile of VAT, where they were found to consume higher amounts of carbohydrate and energy as compared to participants in the lowest tertile. Also, adipocyte has been reported to contribute to de novo lipogenesis process,[38] therefore, even in a case of insulin sensitivity, visceral adipose tissue as being more insulin resistant than SAT, would be less likely to respond to the insulin-induced deactivation of de novo lipogenesis. Additionally, the accumulation of visceral fat has been implicated in insulin resistance,[39] which, in turn, would further stimulate the levels of hepatic lipogenesis, thereby, fat deposition in various fat depots.[40] The second suggested mechanism for the increased total and visceral fat masses associated with high carbohydrate consumption is that the higher carbohydrate consumption, mainly if based on high glycemic index food, would augment postprandial excursions of glucose and insulin. Greater levels of glucose would modulate fuel selection, thereby, increasing carbohydrate, rather than fat, oxidation and shutting down the lipolysis.[41,42] In such case, the spared fat will be further accumulated in adipocyte.

Higher consumption of insoluble fiber was found to be associated with higher visceral and total body fat. The fermentation of insoluble fiber produces short chain fatty acids (SCFA), which contribute to total energy and have been proposed to affect energy homeostasis, and therefore, body weight.[43] Such situation is most likely to happen when high fiber consumption is accompanied with high energy intake. Short chain fatty acids have been also suggested to inhibit lipolysis which might also contribute to obesity.[44] The significant relationship between high soluble fiber consumption and body fat deposition was unexpected and it is hard to give a good explanation, however, the amount or duration of consumption might have not been sufficient to induce the favorable effect of soluble fiber on weight management. it has been previously reported that short-term fiber supplementation of an ad-libitum, self-selected diet has not induced weight loss or changed the response of individuals to satiety and hunger perceptions.[45,46] A mechanism on how soluble fiber could enhance weight gain has been proposed through the gut microbiota.47 Gut microbiota could contribute to obesity through

improved energy extraction from diet by the conversion of dietary fiber to SCFA. The human intestinal microbiome is enriched in genes involved in the degradation of indigestible polysaccharides. Some of the formed SCFAs, mainly acetate and propionate, play an important role in lipid and glucose metabolism.[47] Acetate acts as a substrate for de novo lipogenesis in liver, whereas propionate can be utilized for gluconeogenesis. The conversion of fermentable dietary fiber to SCFA provides additional energy to the host which could promote obesity particularly visceral fats. Yet, some epidemiologic studies show that diets high in fiber rather prevent than promote obesity development. This may be due to the fact that SCFA are also ligands of free fatty acid receptors (FFAR).[47] Activation of FFAR leads to an increased expression and secretion of enteroendocrine hormones such as glucagon-like-peptide 1 or peptide YY which cause satiety. Indeed, further studies could be warranted to confirm the effect of fiber on body fat volumes.

The quality and quantity of dietary fat can affect obesity and chronic disease risks,[48] however, the quality of dietary fat has been suggested to have a stronger effect on body fat accumulation.[49,50] While an accumulating evidence shows a negative impact of saturated fatty acid intake on fat deposition,[51-53] monounsaturated- and polyunsaturated- fatty acids consumption was proven to have desirable effects on body weight and fat mass.[51,53,54] The overeating of saturated fatty acids stimulated hepatic and visceral fat storage, where excess energy from polyunsaturated fatty acids may promote lean tissue in healthy humans.[17] Likewise, including monounsaturated fat to the diet prevented visceral fat gain within an isocaloric design including other types of dietary fats.[55] The consumption of a monounsaturated fatty acid-rich high-fat diet (40% Kcal total fat and ~29% Kcal monounsaturated fatty acid) for 3 weeks resulted in a reduced android adiposity as compared to a high palmitic high-fat diet (40% Kcal total fat and ~16% Kcal palmitic acid).[50] The findings of this study support the importance of the quality of dietary fat. Subjects in the lowest tertile of visceral fat were found to consume a significantly higher amount of dietary fat, compared to subjects in the highest tertile of visceral fat, nevertheless, the unsaturated fat has contributed to the majority of the consumed fat. The favorable effects of unsaturated fat could be induced by directing the metabolism toward a pathway of oxidation rather than storage.[32,51] Similarly, the inverse relationship (significant or trend toward significance) between monounsaturated-, omega-3-, and omega-6- fatty acids consumption and subcutaneous and total body fat would further support the effectiveness of unsaturated fat consumption on modifying energy homeostasis and body fatness. Compared to saturated fatty acids, greater oxidation rates of polyunsaturated- and monounsaturated fatty acids augment

energy expenditure levels, and therefore, reduce the propensity of fat deposition and might promote weight management.[51] Monounsaturated fatty acid (MUFA) has been also suggested to regulate food intake by one of its metabolite, oleoylethanolamine, which influences metabolic and reward system and controls appetite sensation, and therefore, reduces energy intake.[51] Even though we didn't measure the level of oleoylethanolamine in the participants' food intake, reduced energy consumption accompanied by a high MUFA intake among participants in the lowest tertile of visceral fat could propose the influence of the oleoylethanolamine intake from MUFA on food intake. The present study found (data not displayed) significant inverse correlation (r= -.498, p<.0001) between energy intake and MUFA consumption which is in agreement with the previously mentioned studies.

The detectable significant higher consumption of copper among participants in the highest tertile of VAT might reflect the higher consumption of carbohydrate and fiber among this group. In addition to being rich in carbohydrate and fiber, many fruits and vegetables are considered as good sources of copper e.g. dark leafy vegetables, dried fruits, and sweet potato. Up to our best knowledge, no single study has been found that investigating in-depth the relationship between SAT and VAT volumes and micronutrients intake among adults, which imparts our study to be the pioneer in this topic.

One of the main limitations in this study is the one year dietary recall made by FFQ, which may be affected by behavioral and dietary changes, memory and bias. However, we believe that because food selection and taste are mostly based on availability and habits that influence deliberate choices, including endemic cultural biases, we accept that the recall period of one year is very likely reflective of the previous years. Another limitation of the FFQ is that it doesn't consider the bioavailability of the consumed nutrients as in other dietary assessment methods. In addition, our sample size is small due to the limited time available to use the MRI scanner at the medical center. Therefore, we recommend conducting additional studies on a large-scale to generalize these findings and to evaluate the differences between male and female. We also should indicate that this study had no major selection bias in the participants' weight status. The prevalence of overweight and obesity among Jordanians has been found to be above 70% of both males and females adults aged above 25 years.[56]

In conclusion, the study found that consumption of carbohydrate (soluble and insoluble fibers) and fat (monounsaturated, omega-3 andomega-6 fatty acids) from the macronutrient and copper from the micronutrients can influence the amount of fat volumes. This study underscores the importance of quantifying depot-specific body fat and highlights the unique

responsiveness of various fat depots to dietary intake. A large scale study might be needed to generalize the obtained results.

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AUTHOR DISCLOSURE

The authors declare that they have no conflict of interests.

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Table 1. Anthropometric and socioeconomic characteristics of the study sample based on gender.[26]

Parameter	Male			Female			*p*-value	Total		
	N	Mean	SEM	N	Mean	SEM		N	Mean	SEM
Height (cm)	77	172.4	0.81	81	158.6	0.66	0.001	157	165.3	0.75
Weight (kg)	77	79.1	1.7	81	68.7	1.4	0.001	157	73.8	1.5
BMI (kg/m^2)	77	26.7	0.59	81	27.5	0.63	0.255	157	27.1	0.43
Waist circumference (cm) by										
Tape	65	93.8	1.4	75	86.8	1.3	0.017	140	90.0	0.99
MRI	83	90.5	1.3	84	83.8	1.4	0.001	167	87.1	0.97
Subcutaneous fat (cm^3)	83	3779.4	220.4	84	5222.7	273.6	0.001	167	4505.4	184.1
Visceral fat (cm3)	83	2967.1	171.3	84	1881.7	100.2	0.001	167	2421.1	107.3
Total abdominal fat (cm^3)	83	6746.5	356.4	84	7104.4	358.4	0.591	167	6926.5	252.4
Physical activity (MET)	52	23080.9	2449.2	63	20463.8	1476.7	0.344	115	21647.2	1370.4
BMI Categories N (%)										
Normal	35 (45.5)			35(43.8)				70 (44.6)		
Overweight	23 (29.9)			20 (25.0)			0.458	43 (27.4)		
Obese	19 (24.6)			25 (31.2)				44 (28.0)		
Marital status N (%)										
Married	31(40.8)			24 (29.6)				55 (35.0)		
Single	45 (59.2)			53 (65.4)			0.053	98 (62.4)		
Divorced	-			4 (4.9)				4 (2.5)		
Education N (%)										
Illiterate	1 (1.5)			-			0.761	1 (0.6)		
Primary and secondary education	14 (20.6)			22 (28.2)				36 (21.4)		
Diploma	12 (17.6)			13 (16.7)				25 (14.9)		
Bachelor	39 (57.4)			42 (53.8)				101 (61.3)		
Master and Ph.D.	2 (2.9)			1 (1.3)				3 (1.8)		
Occupation N (%)										
Yes	43 (64.2)			45 (57.7)			0.063	88 (60.7)		
No	24 (35.8)			33 (42.3)				57 (39.3)		
Current smoking N (%)										
Yes	34 (50.9)			10 (13)			0.004	44 (30.6)		
No	33 (49.1)			67 (87)				100 (69.4)		

SEM: standard error of the mean; BMI: body mass index.

Table 2. Total energy intake and the percentages of energy from macronutrients intakes across different tertiles of fat fractions of the body

Nutrients		Subcutaneous fat			Visceral fat			Total fat		
		Mean±SEM	*p*-value*	Benjamini-Hochberg *p*-value	Mean±SEM	*p*-value*	Benjamini-Hochberg *p*-value	Mean±SEM	*p*-value*	Benjamini-Hochberg *p*-value
Total energy (kcal)	T1	2694.6±133.3	0.206	0.409‡	2682.5±152.9	0.115	0.085†	2744.1±139.4	0.541	0.375‡
	T2	3046.1±21.0			2947.4±173.3			3035.6±198.9		
	T3	3188.1±167.1			3298.8±177.1			3149.1±167.5		
Percent energy from proteins	T1	12.2 ±0.32	0.390	0.409‡	11.9±0.33	0.873	0.968‡	12.3±0.34	0.407	0.386‡
	T2	12.5±0.41			12.1±0.41			12.2±0.4		
	T3	11.4±0.32			12.0±0.33			11.6±0.4		
Percent energy from carbohydrates	T1	61.8±1.14	0.358	0.418‡	61.8±1.2	0.013	0.046†	60.7±1.1	0.108	0.137†
	T2	62.2 ±12.6			61.2±1.0			62.9±1.3		
	T3	65.1±1.5			66.1±1.6			65.4±1.5		
Percent energy from fats	T1	29.5±0.99	0.679	0.537‡	29.4±1.0	0.013	0.043†	30.2±0.98	0.270	0.205†
	T2	28.6±1.0			29.9±0.9			28.3±0.99		
	T3	27.3±1.2			25.9±1.2			26.9±1.2		

†Significant based on Benjamini-Hochberg *p*-value at false discovery rate=0.25
‡Not significant based on Benjamini-Hochberg *p*-value at false discovery rate=0.25
*Statistical significant difference ($p< 0.05$). All data was adjusted for energy, age, gender, physical activity and smoking.

Table 3. Energy-adjusted macronutrients intake across different tertiles of fat fractions of the body

Nutrients		Subcutaneous fat			Visceral Fat			Total Fat		
		Mean±SEM	*p*-value*	Benjamini-Hochberg *p*-value	Mean±SEM	*p*-value*	Benjamini-Hochberg *p*-value	Mean±SEM	*p*-value*	Benjamini-Hochberg *p*-value
Proteins (g)	T1	98.8±2.7	0.376	0.418‡	95.9±2.3	0.985	0.848‡	99.1±2.7	0.317	0.386‡
	T2	100.1±2.8			97.7±2.9			99.1±2.7		
	T3	93.4 ±2.5			98.8±2.8			94.2±2.6		
Total carbohydrates (g)	T1	505.9±8.7	0.196	0.366‡	510.8±11.8	0.008	0.041†	499.4±8.9	0.071	0.137†
	T2	520.6±13.4			507.0±8.9			526.1±13.1		
	T3	550.3±16.7			558.9±17.4			551.3±16.7		
Fiber (g)	T1	57.8±1.8	0.529	0.556‡	56.4±1.9	0.053	0.431‡	56.2±1.9	0.261	0.362‡
	T2	56.5±2.5			57.3±2.3			57.4±2.3		
	T3	60.6±1.9			61.2±2.1			61.3±2.0		
Soluble fiber (g)	T1	6.7±0.60	0.135	0.409‡	6.4±0.60	0.001	0.025†	6.3±0.60	0.024	0.137†
	T2	7.0 ±0.64			6.8±0.60			7.4±0.63		
	T3	8.5±0.62			9.0±0.62			8.6±0.61		
Insoluble fiber (g)	T1	11.1±0.84	0.095	0.366‡	10.7±0.91	0.001	0.024†	10.6±0.87	0.006	0.137†
	T2	12.7±1.1			12.2±0.93			12.9±1.0		
	T3	14.6±0.90			15.4±1.0			14.9±1.0		
Total Fats (g)	T1	118.4±3.71	0.352	0.418‡	117.6±4.6	0.006	0.024†	120.4±3.8	0.139	0.150†
	T2	112.3±4.9			118.6±3.3			111.1±4.8		
	T3	104.5±5.6			98.9±5.8			103.7±5.6		
Saturated fats (g)	T1	33.9±1.4	0.192	0.556‡	33.9±1.5	0.012	0.459‡	34.7±1.5	0.104	0.375‡
	T2	34.1±2.2			34.4±1.4			33.7±2.2		
	T3	30.7±2.1			30.4±2.7			30.3±2.0		
Monounsaturated fat (g)	T1	29.4±1.4	0.338	0.366‡	29.1±1.4	0.024	0.024†	29.4±1.5	0.174	0.137†
	T2	26.3±1.7			28.3±1.5			26.4±1.7		
	T3	23.6±1.7			21.9±1.8			23.5±1.7		
Trans fats (g)	T1	3.2±0.45	0.885	0.857‡	3.7±0.46	0.845	0.579‡	3.3±0.46	0.876	0.542‡
	T2	3.5±0.37			2.9±0.34			3.6±0.36		
	T3	3.2±0.35			3.2±0.37			2.9±0.35		

†Significant based on Benjamini-Hochberg *p*-value at false discovery rate=0.25.
‡Not significant based on Benjamini-Hochberg *p*-value at false discovery rate=0.25.
*Statistical significant difference ($p<0.05$). All data was adjusted for energy, age, gender, physical activity and smoking.

Table 3. Energy-adjusted macronutrients intake across different tertiles of fat fractions of the body (cont.)

Nutrients		Subcutaneous fat			Visceral fat			Total fat		
		Mean±SEM	*p*-value*	Benjamini-Hochberg *p*-value	Mean±SEM	*p*-value*	Benjamini-Hochberg *p*-value	Mean±SEM	*p*-value*	Benjamini-Hochberg *p*-value
Cholesterol (mg)	T1	229.9±23.8	0.280	0.537‡	218.9±15.7	0.404	0.671‡	239.2 ±24.4	0.085	0.240†
	T2	217.1±19.2			224.7±28.6			215.6±19.2		
	T3	188.1±17.5			191.5±13.4			180.4±15.9		
Omega 6-fatty acids (g)	T1	21.9±1.5	0.275	0.366‡	21.7±1.7	0.040	0.024†	21.6±1.6	0.304	0.205†
	T2	17.5±1.9			19.8±1.6b			17.4±1.9		
	T3	15.9±1.9			13.7±1.9b			16.1±1.9		
Omega 3-fatty acids (g)	T1	1.7±0.12	0.135	0.366‡	1.7±0.13b	0.044	0.024†	1.6±0.12	0.254	0.205†
	T2	1.3±0.17			1.4±0.14b			1.3±0.16		
	T3	1.3±0.17			1.0±0.16			1.2±0.15		

†Significant based on Benjamini-Hochberg *p*-value at false discovery rate=0.25.
‡Not significant based on Benjamini-Hochberg *p*-value at false discovery rate=0.25.
*Statistical significant difference ($p < 0.05$). All data was adjusted for energy, age, gender, physical activity and smoking.

Table 4. Energy-adjusted micronutrients intake across different tertiles of fat fractions in the body

Nutrients		Subcutaneous fat			Visceral fat			Total fat		
		Mean±SEM	*p*-value	Benjamini-Hochberg *p*-value	Mean±SEM	*p*-value	Benjamini-Hochberg *p*-value	Mean±SEM	*p*-value	Benjamini-Hochberg *p*-value
Vitamin A. IU	T1	18238.57±1814.8	0.869	0.654‡	17566.76±1699.72[a]	0.103	0.053†	15837.28±0.02[a]	0.122	0.137†
	T2	21284.61±2415.36			17604.58±2085.72			24486.55±2637.70[b]		
	T3	20660.06±2338.70			25011.91±2582.83[b]			19859.41±2255.67[b]		
β-carotene. µg	T1	7512.77±954.20	0.803	0.569‡	7009.44±833.69	0.066	0.041†	6218.60±706.68	0.115	0.137†
	T2	9521.37±1334.06			7731.74±1172.87			11002.56±1426.88[b]		
	T3	9453.99±1326.92			11746.94±1467.52[b]			9266.97±1310.39[b]		
Vitamin D. µg	T1	0.95±0.23	0.257	0.584‡	0.87±0.28	0.408	0.968‡	0.98±0.23	0.273	0.587‡
	T2	0.95±0.30			0.86±0.22			0.90±0.29		
	T3	0.63±0.11			0.80±0.16			0.65±0.12		
Vitamin E. mg	T1	17.45±2.92	0.288	0.528‡	17.62±4.02	0.284	0.848‡	17.35±2.93	0.307	0.386‡
	T2	17.78±4.17			15.25±2.55			17.85±4.15		
	T3	11.75±1.53			14.10±2.45			11.77±1.58		
Vitamin K. µg	T1	156.80±20.65	0.197	0.494‡	136.14±13.98	0.453	0.334‡	136.78±17.23	0.102	0.137†
	T2	268.86±70.40			241.28±68.28			300.27±70.28		
	T3	200.02±34.46			248.26±40.99			188.63±34.20		
Calcium. mg	T1	1486.50±214.14	0.247	0.523‡	1535.13±320.69	0.406	0.914‡	1490.70±214.69	0.319	0.375‡
	T2	1668.71±347.54			1342.73±193.46			1675.39±350.15		
	T3	1120.61±122.23			1397.97±210.40			1109.73±112.47		
Copper. mg	T1	1.30±0.8	0.094	0.418‡	1.23±0.08	0.018	0.046†	1.29±0.08	0.105	0.272‡
	T2	1.46±0.11			1.49±0.10b			1.50±0.12		
	T3	1.56±0.09			1.60±0.10b			1.53±0.09		
Iron. mg	T1	33.32±3.69	0.502	0.569‡	33.48±5.58	0.456	0.848‡	33.10±3.75	0.465	0.507‡
	T2	33.22±5.78			28.91±3.22			33.32±5.70		
	T3	26.8±1.7			30.9±2.9			26.9±1.9		
Zinc. mg	T1	21.2±3.2	0.313	0.494‡	21.6±4.4	0.246	0.737‡	21.1±3.2	0.314	0.375‡
	T2	20.6±4.6			17.5±2.8			20.4±4.6		
	T3	14.0±1.5			16.7±2.5			14.2±1.5		

†Significant based on Benjamini-Hochberg *p*-value at false discovery rate=0.25.
‡Not significant based on Benjamini-Hochberg *p*-value at false discovery rate=0.25.
*Statistical significant difference ($p<0.05$). All data was adjusted for energy, age, gender, physical activity and smoking.