



# Accumulated substances and calorific capacity in adipose tissue: Physical and chemical clinical trial



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## ABSTRACT

**Aim:** To study physical and chemical structures and properties including calorific value of human adipose tissue in different anatomical location in autopsy-assigned clinical trial.

**Methods:** A pilot physical and chemical descriptive randomized autopsy-assigned trial. Adipose tissue 252 sampled from 36 individuals at autopsy who between 36 and 63 years old died from road accidents. Interventions: Chemical functional groups and calorific value were studied using infrared and atomic adsorptive spectrometries, elemental chemical analysis and differential scanning calorimetry. Adipose tissue was sampled from the 7 various anatomical locations.

**Results:** The highest levels of the analysed chemical substances were found in dense atherosclerotic plaque. Dense atherosclerotic plaque contains the most of metabolic products, organic and inorganic elements. Dense atherosclerotic plaque has the most of calorific value. The lowest calorific capacity has a pararenal fat.

**Conclusions:** Human body lipids serve as a harbor for various organic substances, they may absorb different metabolic products, and they have different calorific capacity depending on their location and forms. Atherosclerotic plaque contains the most of organic and inorganic elements, and brings the highest energy potential.

## 1. Introduction

The atherosclerotic plaque (AP) is one type of naturally occurring lipid-containing structures, which is a basic pathological element found in atherosclerosis (AS) [1]. AP is a heterogeneous layered structural formation [2]. Adipose tissue is an origin of energy and diverse according to its location [3]. Adipose tissue distributes in the body throughout, and it represents in different forms, such as saturated, non-saturated, atheromatous, fibrated, etc. [4,5]. There is not enough data in literature on the chemical structure, functional groups, composition and calorific value of adipose tissue at various locations [6]. What does adipose tissue have else? Does adipose tissue content else except for being lipids? The aim of the study was to investigate physical and chemical structures and properties including calorific value of human adipose tissue of different anatomical location in autopsy-assigned trial.

## 2. Methods

### 2.1. Study design

A pilot physical and chemical descriptive randomized autopsy-assigned trial.

### 2.2. Participants

Adipose tissue in the amount of 252 samples was obtained from 36 individuals (19 males, 17 females) at autopsy. The subjects had died from various car accidents and were between 36 and 63 years old. The autopsy material (adipose tissue) was taken after forensic medical examinations. The study inclusion criteria were:

- 1– The samples were performed by autopsy within 2 h (the time interval between death and sampling) after death of the subjects;

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**Table 1**

The IR spectrometry content of chemical functional groups of adipose tissue from different anatomical locations (expressed as percent values) (n = 252).

Chemical functional groups	IR length (cm <sup>-1</sup> )	AP (dense)	AP (loose)	VF (omentum)	VF (para-renal fat)	SF (buttocks)	SF (umbilical area)	SF (shoulder)
Methyl, –CH <sub>3</sub>	2922.1	1.32 ± 0.18	0.17 ± 0.03	0.51 ± 0.03	0.51 ± 0.02	0.78 ± 0.04	1.15 ± 0.08	0.45 ± 0.04
Hydrocarbon, R-(CH <sub>2</sub> )-R	2852.0	0.81 ± 0.009	0.11 ± 0.007	0.34 ± 0.06	0.35 ± 0.05	0.55 ± 0.03	0.71 ± 0.03	0.32 ± 0.01
Hydroxyl, –OH	3296.0	1.05 ± 0.09	0.24 ± 0.01	0.36 ± 0.02	0.43 ± 0.04	0.06 ± 0.006	0.28 ± 0.03	0.06 ± 0.002
–C=C– in open circuit	3008.1	0.01 ± 0.001	0.07 ± 0.006	0.13 ± 0.01	0.13 ± 0.006	0.09 ± 0.005	0.21 ± 0.028	0.06 ± 0.003
Acetyl, –C≡C–	2128.0	0.04 ± 0.007	0.03 ± 0.005	0.04 ± 0.006	0.05 ± 0.005	0.01 ± 0.002	0.01 ± 0.001	0.01 ± 0.001
–C=C– in benzene (aromatic) nucleus	1465.0	0.46 ± 0.05	0.09 ± 0.005	0.25 ± 0.02	0.25 ± 0.04	0.31 ± 0.04	0.52 ± 0.05	0.18 ± 0.05
Ketones/aldehydes, R'R''-C=O	1743.2	1.82 ± 0.22	0.11 ± 0.025	0.61 ± 0.08	0.78 ± 0.03	0.96 ± 0.04	1.42 ± 0.07	0.55 ± 0.04
Nitrile (cyano-), R'R''-C≡N-R	1645; 1652	0.34 ± 0.04	0.20 ± 0.04	0.26 ± 0.04	0.31 ± 0.05	0.08 ± 0.003	0.24 ± 0.04	0.08 ± 0.003
Nitro, R-NO <sub>2</sub>	1541.0	0.27 ± 0.03	0.13 ± 0.04	0.14 ± 0.05	0.16 ± 0.06	0.04 ± 0.003	0.13 ± 0.04	0.05 ± 0.005
Sulfide oxide, R <sub>2</sub> (SO <sub>2</sub> )	1416; 1398; 1378; 1240	0.56 ± 0.03	0.08 ± 0.004	0.18 ± 0.04	0.19 ± 0.03	0.19 ± 0.03	0.32 ± 0.03	0.11 ± 0.04
Sulfide oxide, sulfides, sulfonamides	1113; 1089	0.92 ± 0.07	0.09 ± 0.008	0.28 ± 0.08	0.28 ± 0.09	0.35 ± 0.08	0.57 ± 0.07	0.19 ± 0.09
Phosphates, –PO <sub>4</sub>	1161	1.12 ± 0.12	0.09 ± 0.01	0.44 ± 0.04	0.41 ± 0.02	0.62 ± 0.05	0.95 ± 0.09	0.34 ± 0.02
–C–Cl-bond	753	0.74 ± 0.09	0.19 ± 0.07	0.55 ± 0.08	0.71 ± 0.09	0.44 ± 0.07	0.65 ± 0.07	0.24 ± 0.04
	723	0.86 ± 0.09	0.22 ± 0.07	0.66 ± 0.09	0.82 ± 0.11	0.49 ± 0.07	0.78 ± 0.07	0.31 ± 0.05
	697	0.91 ± 0.09	0.24 ± 0.05	0.62 ± 0.08	0.81 ± 0.14	0.39 ± 0.05	0.73 ± 0.05	0.25 ± 0.05

Abbreviations: IR, infrared; AP, atherosclerotic plaque; VF, visceral fat; SF, subcutaneous fat.

- 2– The samples' donors had no chronic diseases (such as cardiovascular, endocrine, cancer, etc) prior to death, i.e. they were healthy;
- 3– A cause of the death was road accident;
- 4– Every Monday (after weekend) the four tissue donors were included in the study during nine weeks of a summer season of a year (a total of 36 tissue donors).

The autopsy was performed at the Centre for Forensic Medical Examination of the city of Almaty (the Republic of Kazakhstan). Adipose tissue was sampled from the 7 various anatomical locations: 1) visceral fat (VF) from the omentum; 2) VF from paranephric regions; 3) subcutaneous fat (SF) from the buttocks; 4) SF from the abdomen (umbilical region); 5) SF from shoulder are; 6) AP from the descending aorta, homogeneous AP, at the stage of smooth/dense plaque (hereafter referred to as dense); 7) heterogeneous AP, at the stage of destruction (loose plaque). Every sample from the different anatomical locations was collected for further chemical/physical analysis in size of up to 10 g, and was put in freezer at minus 15 °C.

### 2.3. Research methods

All the tissue samples were previously dried by method of Decock and Vanhaecke [7]. Infrared (IR) spectrometry was performed on a Termo Nicolet 5700 spectrometer (USA) using OMNIC software. Atomic adsorptive analysis was done on an AAS-1 spectrometer (Germany). The chemical and physical investigations were performed at the Institute of Chemical Sciences named A.B.Bekturov in Almaty (the Republic of Kazakhstan).

For the study of organic functional groups by IR, wave lengths of 2–50 μm were used, corresponding to  $\nu = 5000\text{--}200\text{ cm}^{-1}$ . For significant equipment controls, potassium bromide (KBr) and sodium nitrate (NaNO<sub>3</sub>), with enthalpy of melting peaks at 753.3 °C (per 73.3 min) and 311.1 °C (per 58.4 min), respectively, were used. There were duplicate samples used for each measurement, and average results were presented in the results. The adipose tissue samples for IR spectrometry were dried, grinded and put it in solid forms of KBr and NaNO<sub>3</sub>. Then, that it absorption bands were subtracted for further analysis.

The number and position of peaks in the IR absorption spectrum have been previously discussed with respect to the nature of the substance measured (qualitative analysis) and the intensity of the absorption edge (quantitative analysis) [8].

Functional groups were studied by IR: methyl groups (–CH<sub>3</sub>),

hydrocarbon chains (R-(CH<sub>2</sub>)<sub>2</sub>-R), hydroxyl groups (–OH), unsaturated hydrocarbon groups (–C=C–) in open circuit, acetyl groups (–C≡C–), unsaturated hydrocarbon chains (–C=C–) in benzene (aromatic) nuclei, ketones/aldehydes (R'R''-C=O), nitrile (cyano-) groups (R'R''-C≡N-R), nitro groups (R-NO<sub>2</sub>), sulfide oxide, sulfides, sulfonamides (R<sub>2</sub>-SO<sub>2</sub>), phosphates (–PO<sub>4</sub>), and –C–Cl-bonds.

Characteristic vibrations measured were those with hydrogen and deuterium atoms, as well as with groups containing double and triple bonds: –OH, –NH, –SH, CH, C=C, C=O, C=N, C=C=O, N=O, S=O, P=O, etc. Sets of frequencies of characteristic oscillations were tabulated in a correlation table.

Elemental chemical analysis of various adipose tissue was carried out by passing oxygen in a fast stream (burning) using a Derivatography Simultaneous Thermal Analysis-409 with PC Luxx computer processing (NETZSCH, Germany) with a category temperature range of 120 °C to 1650 °C. The temperature in the muffle furnace gradually rose to 120 °C, and at 600 °C only ash remained in the crucible. For determination of sodium and calcium ions, atomic adsorptive spectrometry was used. Carbon (C), oxygen (O), hydrogen (H), hydroxyl groups (–OH), carboxyl groups (–CO<sub>2</sub>), calcium (Ca), and sodium (Na) contents were determined.

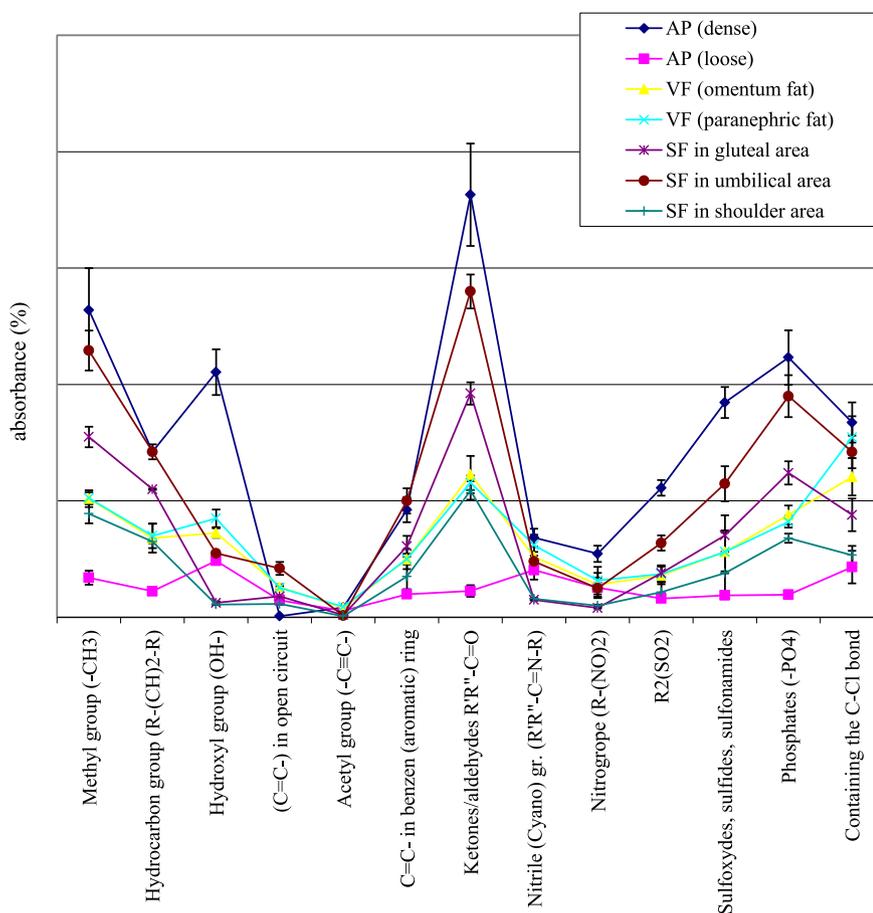
Differential scanning calorimeter (“Mettler Toledo”, USA) was used with an increments temperature of 10.37 °C (t °C, temperature measured in Celsius) per minute. In an experimental set up specimens were heated up from 26.0 °C to 700.0 °C for 70.0 min. The calorific value of adipose tissue was determined according to the heat capacities of lipids. Calorific value was determined indirectly, by measuring heat capacities of organic substances. The more a temperature difference between the sample (sample) and the standard (reference) the more a substance releases heat [9].

### 2.4. Statistical analysis

Student's two-t-test (without Bonferroni correction because n = 252) and odds ratios (OR) with confidence interval (CI) were used. The study data are presented in tables as mean with its standard error of the mean (M ± SEM). P values of < 0.05 were considered significant. Statistical analysis was performed using SPSS for Windows version 17.0 (SPSS: An IBM Company, Armonk, NY) and Microsoft Excel-2010.

## 3. Results

Qualitative and quantitative chemical composition of various



**Fig. 1.** Graphical representation of content of chemical functional groups ( $P < 0.05$ ,  $n = 252$ ). Abbreviations: AP, atherosclerotic plaque; VF, visceral fat; SF, subcutaneous fat.

adipose tissue according to IR spectrometry are presented in Table 1 and Fig. 1.

As shown in Table 1 and Fig. 1, the content of chemical radicals and functional groups in lipids differed significantly according to anatomical origin. The highest levels of saturated fatty acids and almost all analysed chemical functional groups are found in dense AP in which relatively more were identified such functional groups as  $-\text{CH}_3$ ,  $-\text{PO}_4$ ,  $-\text{OH}$ , saturated  $-\text{C}-\text{C}$  group, ketone, phenol, N-, S-, and Cl-containing metabolic products ( $P < 0.05$ ). The dense APs have of long-chain saturated fatty acids and high concentrations of methyl groups.

Chemical analysis of the VFs from omentum and pararenal adipose tissue showed differences in the content of the analysed chemical functional groups. IR spectroscopy of SF from umbilical region of abdomen indicated the presence of higher levels of ketone/aldehydes,  $-\text{C}-\text{C}-$  groups,  $-\text{CH}_3$ ,  $-\text{OH}$ ,  $-\text{S}$ ,  $-\text{PO}_4$ , then in other SFs (buttocks and shoulder regions) ( $P > 0.05$ ).

Elemental data analysis of the fat samples in the different anatomical locations is presented in Figs. 2–4.

Figs. 2–4 show that the elemental content of chemical radicals and functional groups in adipose tissue differ in anatomical locations significantly ( $P < 0.05$ ). The AP (dense) contains the highest diversity of chemical elements and functional groups such  $\text{CO}_2$ ,  $-\text{OH}$ , non-metal chemical elements (C, H, O), and metal elements (Ca, Na) and percentage of H and O ( $P < 0.05$ ). These data correlates with data from the Fig. 1. The relatively high content an oxygen in loose AP may indicate the presence of large amounts of oxidised metabolic products. Fig. 4 shows that the content of Ca is relatively higher in AP (loose) than AP (dense), and the ratio is a reverse for Na content.

In the SF (umbilical area) the content of  $-\text{OH}$  groups is significantly higher than adipose tissue from other SF areas and VFs (Fig. 2). High level of ketones also was found in the SF of umbilical area (Fig. 1). The elemental analysis of the different adipose tissue showed that they

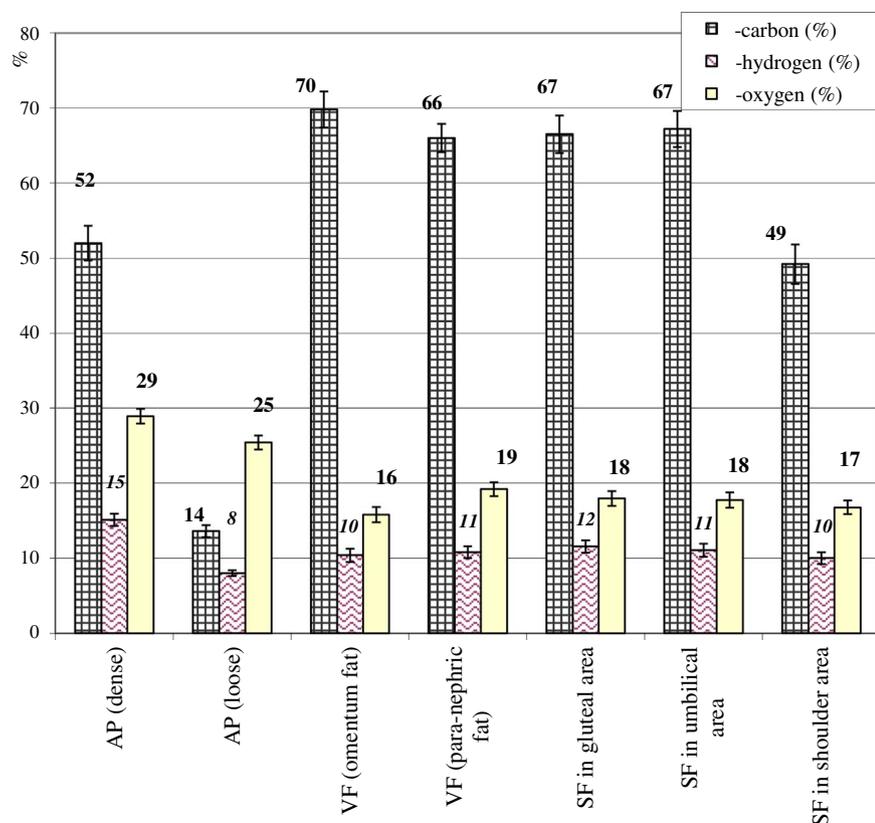
contain different chemical composition depend on their anatomical locations.

Calorific value of the adipose tissue from different anatomical locations is presented in Table 2.

Adipose tissue have different ability to store a heat depend on their anatomical location. The calorific capacity of the fats decreases in a row from AP (dense) to AP (loose), VF (omentum fat), SF (umbilical area), SF (shoulder), SF (buttocks) and VF (pararenal fat). Both APs have the highest calorific capacity among the lipids, but AP (dense) has higher a heat capacity ( $-3.97 \pm 0.16$  °C;  $P = 0.02$ ) than AP (loose) ( $-3.44 \pm 0.15$  °C). The lowest calorific capacity has VF (pararenal fat) ( $-1.25 \pm 0.21$  °C) in compare with SF (buttock area) ( $P = 0.027$ ).

Calorific value of adipose tissue in different anatomical locations is presented in correlation with temperature dynamics (Fig. 5). Calorific capacity of the adipose tissue changes during of combustion process. Both AP, dense and loose are almost below zero in the scale of t °C difference between the sample and the standard. This evidence of an intense heat absorption in the calorimeter. That indicates the APs have a relatively higher heat capacity comparison in other fats. In contrast to APs other fats have relatively similar combustion characteristics: they absorbs the heat actively at approximately 200 °C, and they actively releases the heat starting from 300 °C to 500 °C and completely burns after 600 °C.

The VF (omentum area) have an intermediate position between the APs and the other fats. This can suggest that the VF (omentum) is close to atherosclerotic fats, and they have a high calorific thermal capacity as the APs. APs (dense and loose) bring a higher energy potential in compare with the rest of the body fats.



**Fig. 2.** Content of carboxyl and hydroxyl groups in the different anatomical locations of adipose tissue according to elemental analysis ( $P < 0.05$ ;  $n = 252$ ).

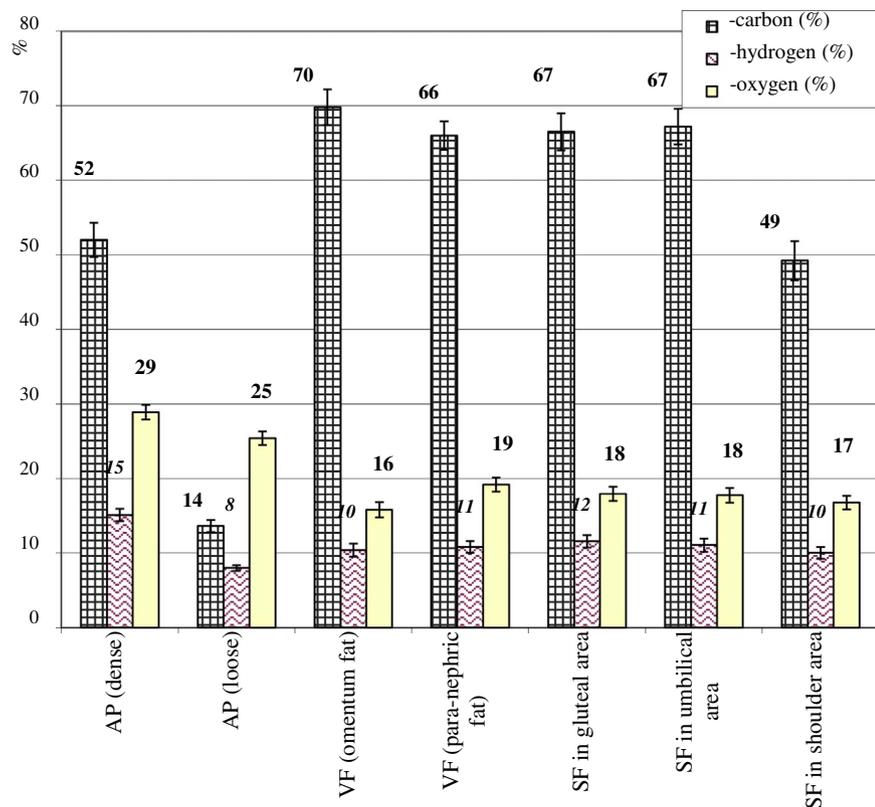
Abbreviations: AP, atherosclerotic plaque; VF, visceral fat; SF, subcutaneous fat.

#### 4. Discussion

The adipose tissue contains nitrogen components including urea, uric acid, creatinine, indole, skatole, cadaverine, and others that may indicate a presence of protein ingredient and/or metabolic products

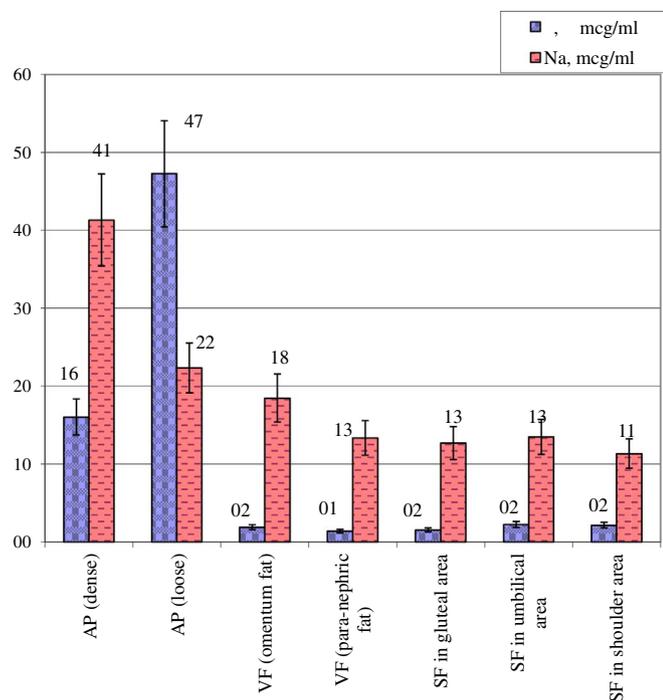
[10,11]. Open circuit hydrocarbon ( $-C=C-$ ) in the tissue can indicate the presence of unsaturated fatty acids [12].

The AP (dense) fundamentally is able to harbor different substances of protein metabolism, fat and protein particles like lipoproteins ( $P < 0.05$ ). Evidently, the type and ratio of fat and protein particles in



**Fig. 3.** Percentage content of carbon (C), oxygen (O) and hydrogen (H) in the different anatomical locations of adipose tissue according to elemental analysis ( $P < 0.05$ ;  $n = 252$ ).

Abbreviations: AP, atherosclerotic plaque; VF, visceral fat; SF, subcutaneous fat.



**Fig. 4.** Calcium (Ca) and sodium (Na) contents in the different anatomical locations of adipose tissue according to elemental analysis ( $P < 0.05$ ;  $n = 252$ ). Abbreviations: AP, atherosclerotic plaque; VF, visceral fat; SF, subcutaneous fat.

AP are present in a dynamic state. In comparison, the content of the analysed chemical groups in AP (loose) is extremely low. It is possible that this latter stage is associated with destruction of AP and calcification [13].

The study found that the SF in abdomen area contains higher level of metabolic products than in other SF in buttocks and shoulder areas. It can be an evidence that the central abdomen part of the body, which is relatively immobile, is a favoured position for accumulation of metabolic products.

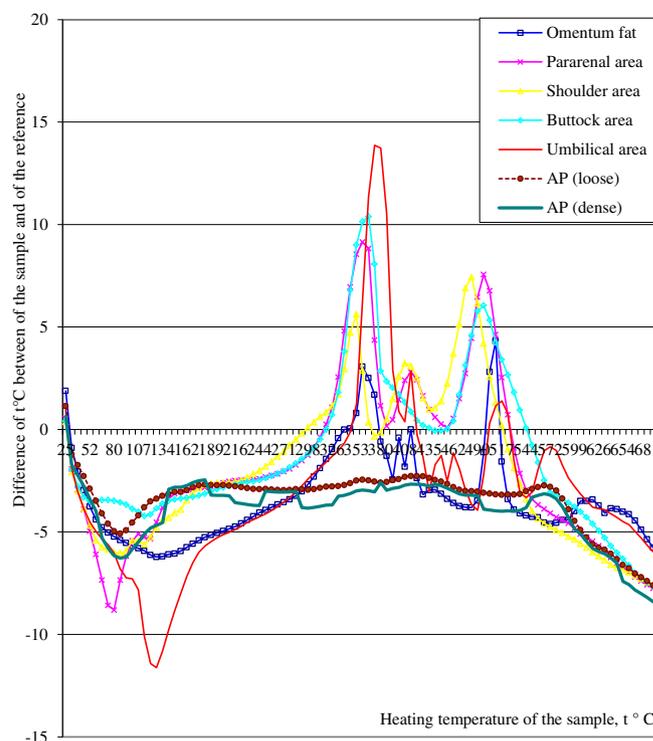
It is also possible that body lipid-containing structures, especially the AP (dense), and it can serve as the harbor for metabolic products such as protein and fat particles, or lipoproteins.

It is possible that permanent postprandial hyperlipidemia leads to excessive deposition of lipids in the lumen of blood vessels. Also, the AP is an “accessible” place for deposition of waste products and end products of metabolism [14]. Development of the AS process can occur not only in overweight people, but also in those of normal body weight [15].

Lipoproteins in the blood can play an emergency supplying role for cells and tissue by lipids. It makes sense for deposition of fats in order to survive within starvation days. In this regard, AP as compacted fats may be considered as emergency fat depots in the blood.

The methylation and/or hydroxylation of lipids in the body can be related to detoxification function of adipose tissue [16].

Hydrocarbons calorific capacity is proportional to  $H^+$  content. Oxidation process is aimed to decrease in toxic properties of metabolic



**Fig. 5.** Comparative values of calorific capacity of adipose tissue in different anatomical locations (temperature dynamics between of the sample and of the reference). Abbreviations: AP, atherosclerotic plaque.

products, and this process of oxidation accompanies an inflammation [17]. Increase in saturated fatty acids in blood serum led to reduce of anti-inflammatory activity of blood [18,19].

The study showed  $Ca^{2+}$  content is higher in AP (loose) than AP (dense), whereas  $Na^+$  is higher in AP (dense) than AP (loose). AP (dense) can deposit a salt [20].

The study showed that body lipids have an ability to accumulate organic waste material depend on its anatomic location. Recent studies confirm persistent organic metabolic waste harbor in adipocytes [21,22].

APs have the most of energy potential in compare with the rest of the studied body lipid. AP can be an emergency available energy source in the blood vessel. Calorific capacity of substances depends on its chemical composition, structure, and biological nature [23].

Fats are energy accumulators, but they are a different compared in each other. Triglycerides containing saturated fatty acids are main energy source in the body. The harder the fat the greater is the content of saturated fatty acids [24]. Calorific value of lipids according to the chemistry rules depends on the content of saturated and branched hydrocarbon chains.

APs develop in vessel wall based on a permanent hyperlipidemia [25]. Our guess that genesis of AP is the result of the transformation of body fat which was not used. AP intrinsically contains a high calorific value despite own small volume. Probably, if the body does not use own adipose tissue for a long time, then the fats transform into a more

**Table 2**

Calorific value of the adipose tissue from different anatomical locations (the temperature difference between the sample and the reference).

	AP (dense)	AP (loose)	VF (omentum)	SF (umbilical area)	SF (shoulder)	SF (buttocks)	VF (pararenal fat)
Difference between sample and reference (t °C)	$-3.97 \pm 0.16$	$-3.44 \pm 0.15$	$-3.35 \pm 0.23$	$-2.87 \pm 0.44$	$-1.97 \pm 0.23$	$-1.81 \pm 0.19$	$-1.25 \pm 0.21$
CI max	-3.65	-3.15	-2.91	-2.00	-1.23	-0.99	-0.49
CI min	-4.28	-3.73	-3.79	-3.73	-2.69	-2.63	-2.01

Abbreviations: AP, atherosclerotic plaque; VF, visceral fat; SF, subcutaneous fat; CI, confidence interval.

compact forms, but energy rich lipids. Perhaps, this process is need for saving body space without loss of energy resources.

Our findings might allow to look at the nature of the lipid transformation, and telling about “physiological” changes of body fats. Probably, atherosclerotic process is not a random event in the body, but it is a logical pathophysiological process as result of fat compaction. The “old” adipose tissue begins to absorb waste and end products of metabolism.

The results of the study would allow to develop new treatment methods of atherosclerotic diseases in the future.

## 5. Conclusions

Thus, adipose tissue in the body is heterogeneous in content and differs in property. The APs contain the largest amount of organic and inorganic functional groups, and dense AP has more saturated and branched hydrocarbon chains. Adipose tissue may a depot for various organic substances and absorb of different metabolic products. Adipose tissue has different calorific capacity depending on its locations and forms. Dense AP keeps the highest of energy potential in compare to the other body fats.

## Competing interests

Conflicts of interest were not declared by any author.

## Study limitation

Several limitations of the study deserve comment. First, the design of the present study was experimental-based, which is susceptible to selection bias. Second, the sample size was moderate, limiting its ability to detect significant results. Third, the chemical and physical investigations indicated only some of organic substances, and calorific value was estimated by specific heat capacity. Fourth, the heterogeneous content of organic substances in the human fats was not analysed in the present study. Finally, it is important to mention that our study was performed on Kazakhstan citizens, and our findings may not be relevant to people to other countries.

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## Trial national registration

State registration #0109RK000079, code O.0475 at the National Center for Scientific and Technical Information, the Republic of Kazakhstan.

## Trial international registration

ClinicalTrials.gov NCT01700075.

## Transparency document

The <http://dx.doi.org/10.1016/j.bbacli.2017.05.002> associated with this article can be found, in online version.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.bbacli.2017.05.002>.

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