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Body Fats Accumulate Metabolic Products: Physical and Chemical Analysis in Vitro

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Abstract Objective: Aim of our study is to investigate physical and chemical structure and properties of body lipids at various anatomical sites. Trial design: A pilot physical and chemical descriptive experimental trial in vitro. Methods and Participants: Adipose tissue in the amount of 252 samples from 36 individuals (17 dead females) at autopsy. The subjects had died from various injuries and were between 36-60 years old. Interventions: Chemical groups and compounds were studied on infrared spectrometry with software, and atomic adsorptive analysis on spectrometer. Elemental chemical analysis of lipids different localization carried out. Research Subject: Chemical elements and compounds. Results: The highest levels of saturated fatty acids and almost all chemical groups and compounds analysed are found in dense atherosclerotic plaque (AP). In those samples, relatively more compounds containing metabolic products were identified ($P < 0.05$, $n = 252$). Dense AP contains relatively more saturated and branched hydrocarbon chains, and they have the largest quantities of organic and inorganic elements and compounds in their structure. Conclusions: Human body lipids, especially dense AP, serve as a depot for various organic substances. Once having been formed, the AP has its own pathophysiological role in adsorption of redundant waste products.

Keywords: *body lipids, atherosclerotic plaque, physical and chemical structure, composition*

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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]. According to morphological studies, AP is an inhomogeneous structural formation [2,3]. It is known from the literature that body lipids are heterogeneous and diverse both according to location and function [4]. For example, AP has a layered or multifocal form, often with significant predominance of one or another component, usually of atheromatous masses, fibrous structures, and calcifications [5].

There is not enough data in literature on the chemical structure and composition of lipids at various body locations [6]. What does the body fat else? The aim of our study was to investigate physical and chemical structures and properties of body lipids at various anatomical sites.

2. Study Design

A pilot physical and chemical descriptive experimental trial in vitro.

2.1. 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 injuries and were between 36-60 years old. The autopsy material (lipids) was taken for research purposes after forensic medical examinations had been carried out. The criteria used for inclusion of material in this research were:

1. sampling was performed within 2 hours after death (interval of time between death and collection);
2. tissue donors had no chronic somatic diseases (such as cardiovascular, endocrine, cancer, etc) and cause of death was road accident.

Removal of autopsy material was performed at the Centre for Forensic Medical Examination of the city of Almaty. Tissue was collected from 7 various locations: visceral fat (VF), from the omentum and paranephric regions; subcutaneous fat (SF) from the buttocks, the abdomen (umbilical region), and shoulder; AP from the descending aorta; homogeneous AP, at the stage of

smooth/dense plaque (hereafter referred to as dense), and heterogeneous AP at the stage of destruction (loose plaque).

2.2. Research Methods

All the fat tissues were previously dryed by method of J. Decock and Hubert Vanhaecke (1999) [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). For the study of organic compounds by IR, wave lengths of 2–50 micrometers were used, corresponding to ν = 5000 – 200 cm–1. For positive equipment controls, potassium bromide (KBr) and sodium nitrate ($NaNO_3$), with enthalpy of melting peaks at 753.3°C (per 73.3 min) and 311.1°C (per 58.4 min), respectively, were used.

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].

Chemical groups and compounds were studied by IR: methyl groups ($-CH_3$), hydrocarbon chains ($R-(CH)_2-R$), 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-NO2), sulphide oxide, sulphides, sulfphonamides ($R2-SO_2$), phosphates ($-PO_4$), 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 lipids was carried out by passing oxygen in a fast stream (burning) using a Derivatography Simultaneous Termal 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 ($-CO_2$), calcium (Ca), and sodium (Na) contents were determined.

For statistical analysis, Student's 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 ± standard error of the mean (M ± SEM), and P values of < 0.05 were considered significant. Statistical analysis was performed using SPSS for Windows version 17.0 (SPSS: An IBM Company, Armunk, NY) and Microsoft Excel-2010.

Table 1. Content of chemical functional groups and compounds determined by IR spectrometry in various lipids sample from different anatomical sites (expressed as percent values) (n = 252)

Chemical Functional Groups and Compounds	IR length (cm-1)	AP (dense)	AP (loose)	VF (omentum)	VF (pararenal fat)	SF (buttocks)	SF (umbilical region)	SF (shoulder)
Methyl, $-CH_3$	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, $R-(CH_2)-R$	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, $R-NO_2$	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, $R_2(SO_2)$	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, $-PO_4$	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

3. Results

Qualitative and quantitative chemical composition of various lipids according to IR spectrometry are presented in Table 1 and correlated with the anatomical site of origin of the samples. As shown in Table 1, the content of

chemical radicals and compounds in lipids differed significantly according to anatomical origin. These data are also presented in a visual form in Figure 1 shows that the highest levels of saturated fatty acids and almost all chemical groups and compounds analysed are found in dense AP. In those samples, relatively more compounds - CH_3, -PO_4, -OH, saturated -C-C group, ketone, phenol, N-, S-, and Cl-containing metabolic products ($P < 0.05$) were identified. This may indicate the presence in dense AP of long-chain saturated fatty acids and relatively high concentrations of methyl groups.

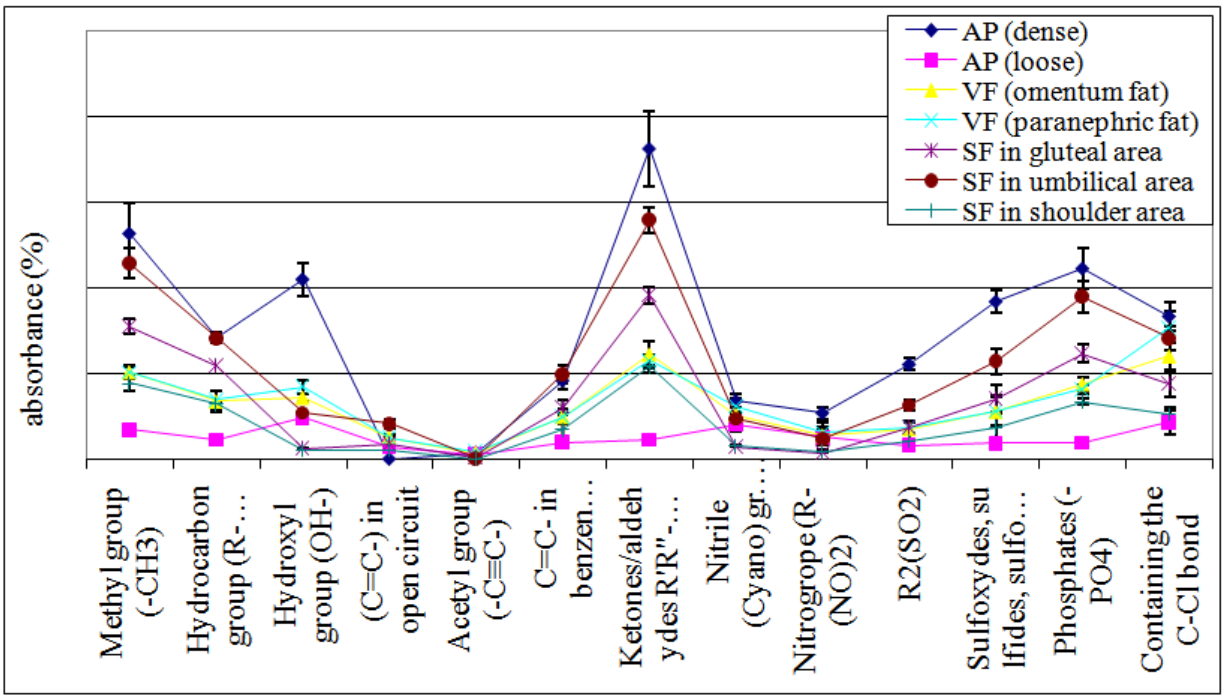

Figure 1. Graphical representation of content of chemical functional groups and compounds summarized in Table 1 ($P < 0.05$, $n = 252$)

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

The nitrogen content of the lipids in the form of urea, uric acid, creatinine, indole, skatole, cadaverine, and others may indicate the presence of proteinaceous material and (or) metabolic product [9]. The sulfur content may also indicate the presence of protein metabolic product. The content of open circuit hydrocarbon (-C = C-) indicates the presence of unsaturated fatty acids [10].

Figure 1 shows that dense AP fundamentally is able to deposit different substances of protein metabolism, fat and protein particles or lipoproteins ($P < 0.05$). This may indicate that dense AP is able to deposit different substances of protein metabolism, fats, fat and protein particles, or lipoproteins. Evidently, the type and ratio of fat and protein particles in AP are present in a dynamic state. In comparison, the content of analysed chemical groups in AP (loose) is extremely low. It is possible that this latter stage is associated with destruction of AP and calcification [11].

Chemical analysis of VF, taken from omentum and paranephric adipose tissue, shows significant differences in the content of chemical groups and compounds analysed ($P > 0.05$). IR spectroscopy data indicate the presence of high levels of ketone bodies in SF from the umbilical region of abdomen ($P > 0.05$). If we assume that accumulation of fat is high in the abdomen (leading to abdominal obesity), then the data in Table 1 and Figure 1 reveal the following: -C-C- groups, -CH_3, -OH, -S, -PO_4, and ketone/aldehydes are found at higher levels in the subcutaneous region of abdomen than in the buttocks and shoulder. It is possible that the central part of the human body, which is relatively immobile, is a favoured position for accumulation of metabolic toxins in SF.

Table 2. Elemental analysis of various lipids in tissue samples (n = 252)

	CO_2 (gr/ml)	OH- (gr/ml)	Carbon (%)	Hydrogen (%)	Oxygen (%)	Ca (mcg/ml)	Na (mcg/ml)
Atheroma (dense)	298.0 ± 15.2	124.0 ± 8.3	52.0 ± 2.3	15.1 ± 0.3	28.9 ± 1.0	16.0 ± 2.3	41.3 ± 5.9
Atheroma (loose)	52.69 ± 4.1	89.0 ± 5.3	13.6 ± 0.8	7.99 ± 0.4	25.4 ± 0.9	47.3 ± 6.8	22.3 ± 3.2
VF (omentum fat)	255.9 ± 13.2	41.6 ± 4.5	69.8 ± 2.4	10.4 ± 0.9	15.8 ± 1.0	1.9 ± 0.3	18.4 ± 3.1
VF (pararenal fat)	241.9 ± 12.9	45.9 ± 5.8	66.0 ± 1.9	10.8 ± 0.8	19.2 ± 0.9	1.4 ± 0.2	13.4 ± 2.2
SF in buttock area	243.6 ± 11.8	47.6 ± 5.4	66.5 ± 2.5	11.6 ± 0.8	17.9 ± 0.9	1.5 ± 0.3	12.7 ± 2.1
SF in umbilical area	243.3 ± 16.8	113.1 ± 7.6	67.2 ± 2.4	11.1 ± 0.9	17.8 ± 1.0	2.3 ± 0.4	13.5 ± 2.2
SF in shoulder area	176.3 ± 14.5	58.1 ± 4.9	49.2 ± 2.6	10.0 ± 0.8	16.8 ± 0.9	2.1 ± 0.4	11.3 ± 1.9

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

It is also possible that body lipid-containing structures, especially dense AP, may serve as a depot for metabolic products of proteins and fats, fats themselves, protein particles, or lipoproteins.

It is possible that alimentary postprandial hyper lipidaemia actually leads to excessive deposition of lipids in the lumen of blood vessels [12]. The AP is an “accessible” site for deposition of waste products and end

products of metabolism. The deposition of lipids in AP in blood vessel lumen may be due to an excessive amount of blood lipoproteins of low and very low density [13].

Development of the AS process can occur not only in overweight individuals, but also in those of normal body weight [14].

It is known that a minimum number of biological active substances bound to proteins are present in blood for emergency needs of the human body [15]. In this regard, lipoprotein in the blood may play a similar emergency role for supplying cells and tissues with lipids.

During evolution, it makes sense for deposition of fat in appropriate organs and tissues in order to safeguard against times of food restriction and starvation. In this regard, AP may be considered as emergency fat depots in the blood formed after caloric intake.

Elemental data analysis of studied fats in various anatomical sites sampled is presented in Table 2.

These result show that lipids significantly differ in content of chemical radicals and compounds according to site of origin ($P < 0.05$). It is noted that dense AP contain the highest diversity of chemical elements and compounds. These data are also presented graphically in Figure 2, Figure 3, Figure 4, analysed according to chemical compounds (CO_2 and OH), non-metal chemical elements (C, H, O), and metal elements (Ca, Na).

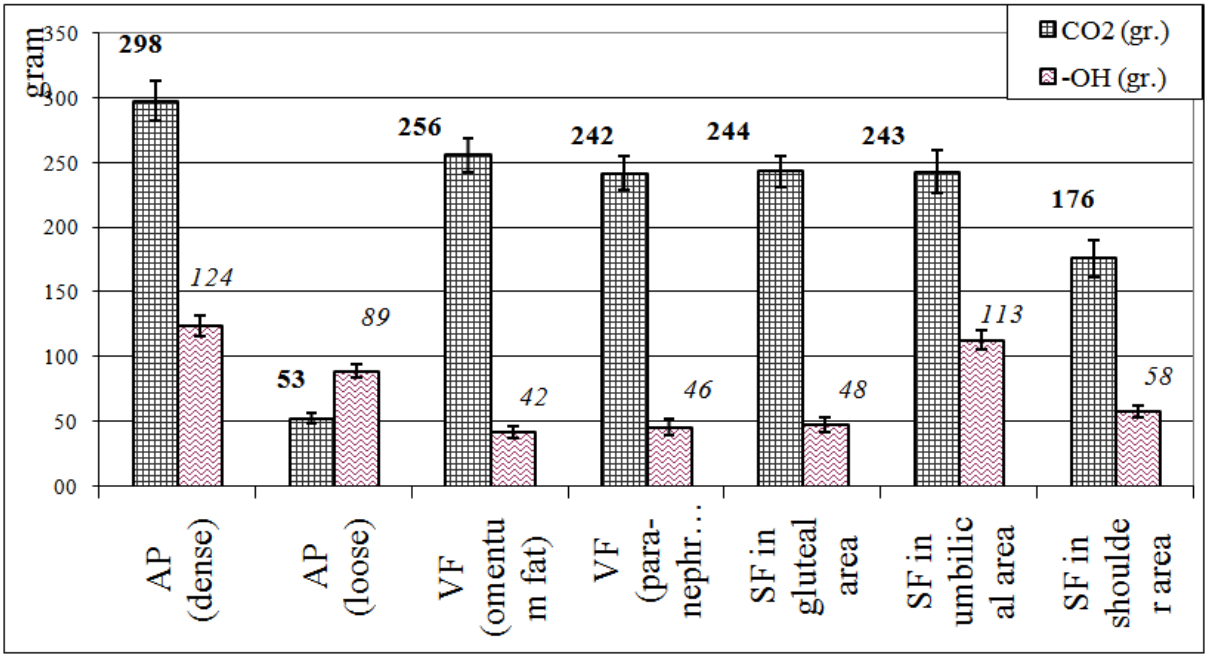

Figure 2. Content of carboxyl and hydroxyl groups in fats from different anatomic sites according to elemental analysis ($P < 0.05$; $n = 252$)

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

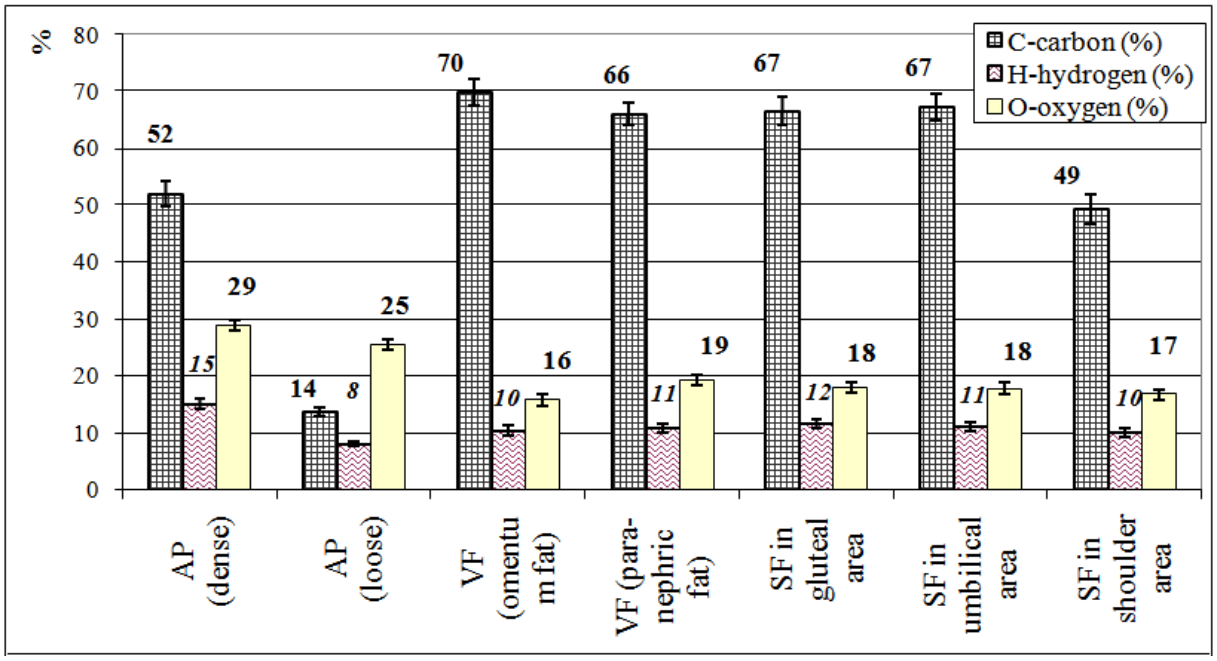

Figure 3. Percentage content of carbon (C), oxygen (O) and hydrogen (H) in fats from different anatomical sites, according to elemental analysis ($P < 0.05$; $n = 252$)

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

Figure 2 shows that the content of CO_2 and –OH groups is significantly higher in dense AP than in other lipid sources sampled ($P < 0.05$). This parallels data presented in Figure 1, in which dense AP was also found to contain relatively highest amounts of long-chain saturated fatty acids and oxidised lipids.

The process of methylation and (or) hydroxylation of lipids in the human body is related to detoxification [16].

Figure 3 shows that analysis of the percentage of H and O is highest in dense AP. It is known that calorific capacity of hydrocarbons is proportional to H content [17].

The relatively high O content in loose AP may indicate the presence of large amounts of oxidised metabolic products. It is considered that the oxidation process is aimed to decrease toxic properties of metabolic products, and this process of oxidation frequently accompanies inflammation [18]. For example, increase of saturated fatty acids in blood serum reduce anti-inflammatory activity of blood. Inflammation is enhanced during the destabilization of AP [19]. In recent studies, surface thermometry of AP has showed direct correlation between unstable plaques and level of inflammation markers [20].

In SF, the content of -OH groups is significantly higher when tissue sampling from the abdominal area (Figure 2). High level of ketones are also found in SF from this area.

As shown in Figure 4, the content of Ca is relatively higher in loose than dense AP, and the reverse is found for Na.

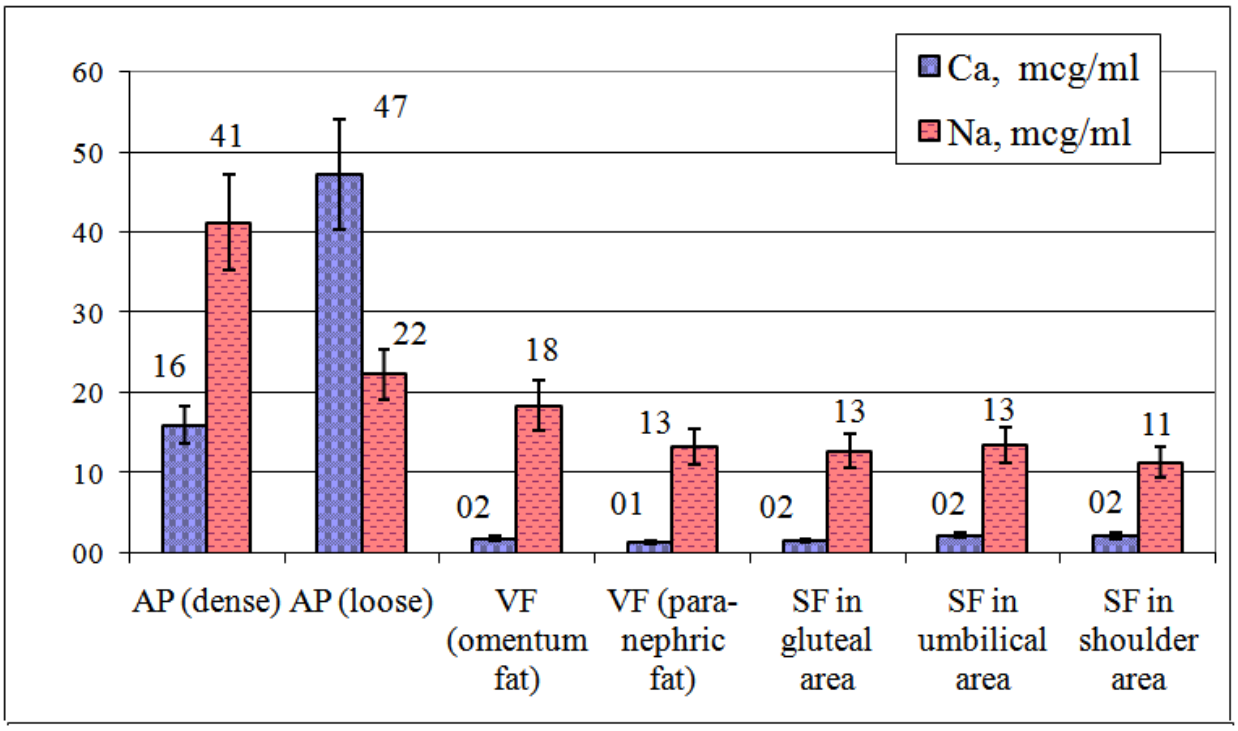

Figure 4. Calcium (Ca) and sodium (Na) composition of various body fats ($P < 0.05$; $n = 252$)

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

The Ca content in AP is comparatively higher, especially in loose AP. Probably, AP can deposit salt [21]. Na-ions play role in detoxification and transport in the body [22].

Elemental analysis of various lipids showed, that lipids from different locations in the human body differ by chemical composition. Dense AP contain the largest number of C, H, and O, metals, such as Na and Ca, and chemical compounds containing hydroxide and carboxide groups. This could be due to the deposition property of lipids at chronic metabolic intoxication [23].

4. Discussion

The process of AP formation may stretch over years due to the capacity of the body to store lipid, with transformation of body fat (24). If AP has higher energy intensity relatively to free lipids, then process of fat deposition at presence of permanent hyperlipidaemia can reflect a new method of energy resources saving.

Glycogen resources are necessary for emergency needs of the human organism. Lipogenesis occurs during periods of further increase of glucose flux. Both are resources for the long-term needs of an organism [25]. The study showed that the body lipids are heterogeneous in content, dependant on their anatomical location. Lipids in dense AP have a relatively higher content of saturated hydrocarbon chains.

AP may be a sui generis source available lipids in the blood stream. With increasing low density lipoproteins levels in blood, increased migration of leukocytes to vascular walls occurs mediated by the chemokin MCP-1 (chemotactic protein produced by monocytes) [26].

Increase of atherogenecity of lipoprotein is associated with mechanism of size increase and induration of chemical bonds of lipids [27].

Our study showed fat has ability to accumulate organic waste material, depending on anatomic site. Recent studies confirm persistent organic waste remaining in adipocytes exacerbates obesity [28,29].

The results of the investigation would be useful for improving a curative methods of atherosclerosis.

5. Conclusion

Fats in the body are heterogeneous in content and differ by properties. Dense AP contains relatively more saturated and branched hydrocarbon chains, and they have the largest quantities of organic and inorganic elements and compounds in their structure. Human body lipids, especially dense AP, serve as a depot for various organic substances. Possible, lipids including AP can have important pathophysiological meaning in adsorption of metabolic product sat chronic metabolic intoxication.

Abbreviations

atherosclerotic plaque (AP)
atherosclerosis (AS)
visceral fat (VF)
subcutaneous fat (SF)
Infrared (IR)
carbon (C)
oxygen (O)
hydrogen (H)
hydroxyl groups (-OH)
carboxyl groups (-CO_2)
calcium (Ca)
sodium (Na)
odds ratios (OR)
confidence interval (CI)
mean ± standard error of the mean (M ± SEM)

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Competing Interests

Conflicts of interest were not declared by any author.

Endnotes

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. Fourth, the heterogeneous content of organic substances in the human fats was not analyzed 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 of other countries.

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Statement of Author Contributions and Acknowledgements

Kuat P. Oshakbayev: design and performance, scientific executor, collecting of the practical material, bibliography review, scientific analysis, statistical advancing, writing of the paper.

Kenneth Alibek: design and performance, scientific executor, paper review, writing of the manuscript.

Igor O. Ponomarev: design and performance, scientific executor, collecting of the practical material, scientific analysis, writing of the paper.

NurlybekUderbayev: design and performance, scientific executor, collecting of the autopsy material.

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Pernekul Oshakbayev: design, bibliography search and review, scientific analysis and writing the paper.

Sholpan Kaliyeva: review the paper, collect of the clinical material, patient’s diagnosis, scientific analysis.

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