



# 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.

- The samples' donors had no chronic diseases (such as cardiovascular, endocrine, cancer, etc) prior to death, i.e. they were healthy;
- A cause of the death was road accident;
- 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>-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

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