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DSC ANALYSIS OF HUMAN FAT TISSUE IN IDIOPATHIC AVASCULAR NECROSIS OF THE FEMORAL HEAD
A preliminary study

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Avascular necrosis of human femoral head (ANFH) causes incongruity in the joint that leads to disability in patients requires total hip arthroplasty (THA). Several etiological factors of ANFH have been proposed in the literature but there are cases of idiopathic origin. We observed macroscopic variation in quality of the subcutaneous fat tissue in patients with ANFH compared to patients with osteoarthritis or hip fracture during THA procedures.

The samples were analysed by histology, gas chromatography (GC) and differential scanning calorimetry (DSC).

Conclusion: the alteration in the fatty acid profile did not cause histological changes, however we could detect biochemical changes using DSC and GC.

Keywords: *DSC, fatty acids, gas chromatography, osteonecrosis*

Introduction

The origin of the avascular necrosis at first was unknown therefore was considered idiopathic [1]. The ANFH is thought to have ischaemic origin but other pathways may also play a role in the pathogenesis of the disease [2–4].

During the surgical procedure of advanced stage of ANFH the authors observed alterations in colour and consistency of subcutaneous fatty tissue compared to patients suffering from trauma or osteoarthritis requiring hip surgery. The authors observed visible differences depending on the etiology of the disease, e.g. alcoholic, steroid treated and idiopathic etiological cases. We proposed, that the changes due to the altered fat metabolism that contributed to the ANFH can be observed in the subcutaneous fat tissue histologically or at molecular-biochemical level. The goal of the present study was to investigate the subcutaneous fat tissue structure by histology, to analyse its fatty acid composition by GC, and demonstrate its thermal consequences by DSC.

In our daily routine through history and physical examination, getting details of present and past illnesses, and laboratorial investigation we can exclude most of the known etiological factors, but one can not reveal the entire pathophysiological pathway (Table 1).

Table 1 Suspected etiological factors playing role in cuasing ANFH

Possible ethiologic factors associated with osteonecrosis	
Metabolic diseases	**Traumatic**
Hyperlipidemia	Femoral neck fracture
Hyperuricemia/gout	Dislocation or fracture
Hepatopathy	Minor trauma
Diabetes mellitus	**Drug induced or toxic Corticosteroid th.**
Pancreatitis	Alcohol use
Cushing syndrome	Cigarette smoking
Gaucher's disease	**Inflammatory diseases**
Hemorheologic	Systemic lupus erythematosus
Sickle cell anaemia	Scleroderma
Intravascular coagulation	Rheumatoid arthritis
Thrombophlebitis	Psoriatic arthritis
Coagulopathies-Thrombophilia	**Other**
Caisson (dysbarism) disease	Pregnancy
Chronic renal failure	Radiation
Hemodialysis	Organ transplantation
Angiopathic	HIV infection
Raynaud syndrome	Idiopathic
Diabetic angiopathy	

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The remaining cases are the so-called ANFH cases are considered of idiopathic origin [1, 3–10].

Experimental

Materials and methods

In this study we compared the results of two groups, one of patients suffering from advanced stage ANFH with undiscovered etiology and the other of otherwise healthy patients with hip region fracture, without X-ray sign of any femoral head pathology. The groups were formed by 5–5 patients; all of them were male, with normal hematologic values. The mean age of the ANFH group was 55 years, (from 49 to 62), mean age of control group was 53 years (from 48 to 56).

ANFH patients were operated by THA, anterolateral surgical exposure was performed, the control group underwent different kinds of osteosynthesis.

Tissue samples

Fat tissue samples were taken from the subcutaneous fat by anterolateral incision of the surgical exploration site during total hip arthroplasties (ANFH group) and by other surgical exposures during different kinds of osteosynthesis at the same region (control group).

The patients gave informed consent before the procedure and it was approved by the local ethical commitee.

(Our activities were done under the proper law paragraphs and valid permission.)

Histology

For the histological examination the specimens were fixed in 10% neutralized formaline for a week at room temperature, embedded in paraffin, and cut to a thickness of 5 μm. All sections were stained with oil red and hematoxylin-eosin. The histological morphometry was performed with Nikon Eclipse E400 light microscope (usual magnification 400×) to examine the suspected changes of the fat tissue structure.

The remaining tissue samples were frozen and kept in a freezer (–20°C) until further analysis, but no longer than 24 h.

Fatty acid analysis

Boron-trifluoride methanol extraction was used for the fatty acid-profile definition in 10 samples, then the fatty acids were transformed to fatty acid-methyl-esters (FAME) using Morrison and Smith method, then were stored in N-hexane medium until the FAME profile was analysed by a Chrompack CP 9000 type gas chromatograph.

FAMEs were separated on a 100 m×0.25 mm quartz capillary column (CS-Sil 88). Both the injector (splitter) and detector (FID) were maintained at temperature 270°C. Helium gas was used as the carrier gas using pressure 235 kPa, with flow rate at the detector 1 mL min^{-1}. The temperature-programme of column: maintain the temperature of 140°C for 10 min; then rise to 10°C min^{-1} until 235°C, and maintain isotherm until 26 min.

Differential scanning calorimetry (DSC)

The thermal unfolding of the fat tissue samples were monitored by Setaram Micro DSC-II calorimeter. The experiments were conducted between 0 and 100 as well as –20 and 100°C. The heating rate was 0.3 K min^{-1} in all cases. Conventional Hastelloy batch vessels were used during the denaturation experiments. Typical sample wet masses for calorimetric experiments were between 200–250 mg. Pure alcohol was used as a reference sample. The sample and reference vessels were equilibrated with a precision of ±0.1 mg. There was no need to do any correction from the point of view of heat capacity between sample and reference vessels. Calorimetric enthalpy was calculated from the area under the heat absorption curve by using two-point setting Setaram peak integration.

Results and discussion

Histological findings

Normally the adipose tissue mass contains 80% of lipids, stored mainly as triglycerides in the adipocytes. Other cells, as preadipocytes, fibroblasts and macrophages also can be found, constituting the other part of the cellular content in fatty tissues.

We could not identify any histological changes in the shape or size of adipocytes and other cells between the two groups (Fig. 1).

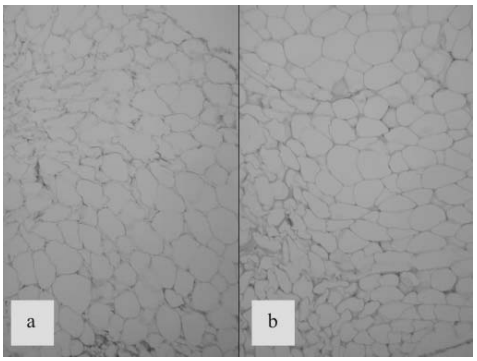

Fig 1 Characteristic histological picture of subcutaneous fatty tissue (a – control group, b – ANFH group)

Table 2 Distribution of FAMEs by GC (data in average±sd)

Type of fatty acid	Idiopathic	control
	Fatty acid methyl esther/%	
lauric acid 12 :0	0.23±0.02	0.63±0.05
myristic acid 14:0	1.89±0.17	1.98±0.11
myristoleic acid 14:1	0.33±0.03	0.23±0.02
pentadecanoic acid 15:0	0.19±0.02	0.16±0.01
palmitic acid 16:0	20.20±2.01	20.19±2.06
palmitoleic acid 16:1	7.01±0.68	4.95±0.47
margaric acid 17:0	0.22±0.02	0.22±0.02
stearic acid 18:0	2.62±0.02	3.63±0.33
oleic acid 18:1	50.72±5.01	45.93±4.31
linoleic acid 18:2n6	14.00±1.07	18.75±1.78
eicosenic acid 20:1	0.73±0.06	0.88±0.08
alpha-linolenic acid 18:3n3	0.36±0.03	0.47±0.05
heneicosanoic acid 21:0	0.23±0.02	0.20±0.02
eicosadienoic acid 20:2	0.32±0.03	0.41±0.04
eicosatrienoic acid 20:3n6	0.22±0.02	0.36±0.03
arachidic acid 20:4n6	0.53±0.05	0.33±0.02
docosapentanoic acid 22:5n3	0.20±0.02	0.66±0.06

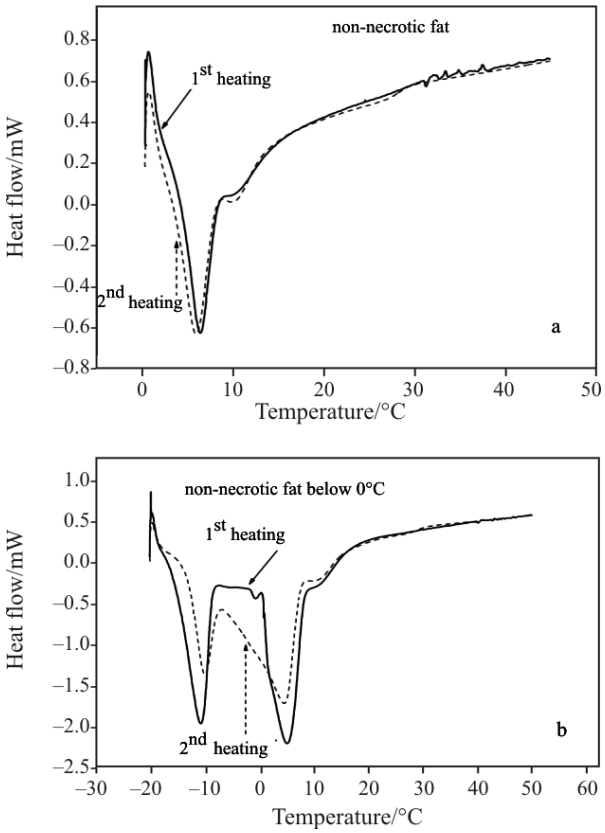

Fig. 2 a – Thermal denaturation of control samples in 0–100°C range; b – –20+100°C range

GC findings

The amounts of saturated fatty acid (SFA), mono-unsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) fractions of fatty acid methyl esthers (FAME) of the idiopathic and control samples were compared quantitatively (Table 2). There were no obvious differences in SFA content of the two groups (idiopathic: 25.58%, control: 27.01%).

The MUFA fraction of the idiopathic group was remarkably higher than the control (59.69% *vs*. 51.99%). The PUFA fraction of the control group yielded higher values (idiopathic 15.31% *vs*. control 20.57%). Normally oleic acid (46%), palmitic acid (20%) and linoleic acid (19%) can be found in the adipocytes forming the main part of fat tissue. Patients with idiopathic ANFH have the same proportion of palmitic acid (20%), an increased proportion of oleic acid (51%) and decreased percentage of linoleic acid. There were no appreciable difference between the mean values of the two groups in myristic acid, pentadecanoic acid, margaric acid, heneicosanoic acid.

GC analysis of the FAME samples revealed slightly decreased amounts of long-chained MUFAs, except arachidic acid in the idiopathic ANFH group. During our analysis we have found relatively wide range in the measured values, that might have been due to biological variaton, since the samples were from different donors and from different age group. The compared values represent the mean value of the ANHF and the control group.

DSC findings

DSC revealed similar changes in the thermodynamic parameters as it was found by GC experiments: the non-necrotic samples during heating between 0–100°C exhibited: two separable transitions with T_m=5.7 and 9.9°C with total calorimetric enthalpy change ΔH= –20.8 J g^{-1} (Fig. 2a); as well as heating between –20–100°C resulted a transition with data: T_m= –10.9 and 4.95°C, total ΔH= –75.8 J g^{-1} (Fig. 2b). In case of idiopathic avascular necrosis the DSC scans exhibited significant alterations compared to the control samples: during heating between 0–100°C: T_ms=8.0 and 11.7°C, total ΔH= –14.25 J g^{-1} (Fig. 3a); heating between –20–100°C: T_ms= –0.1 and 7°C, total ΔH= –100.9 J g^{-1} (Fig. 3b). In case of second heating we could observe the biggest change in the thermal parameters between the 0–100°C range.

The ANFH occurs mainly among males, caused mostly by increased alcohol intake [1–3]. In addition several, relatively rare conditions may play a role in development of the disease. The ANFH is a hetero-genoius disorder, sometimes starts with sudden onset

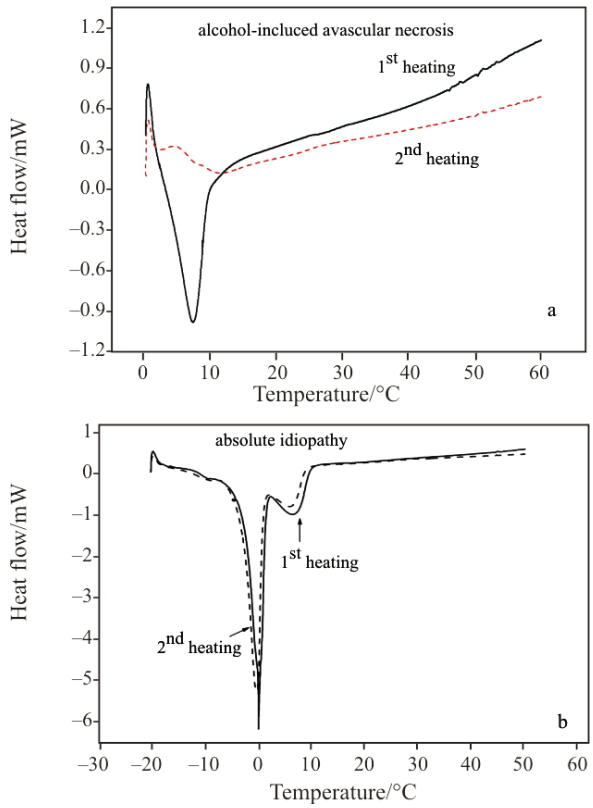

Fig. 3 a – Melting characteristic of avascular necrotic samples in 0–100°C interval; b – –20+100°C range

of sharp pain or can start gradually and the signs and symptoms of formation are masked [11]. Decreased circulation can lead to oedema in bone marrow in the femoral head [5, 11]. In this early, sometimes subclinical, painless stage only MRI can be the only diagnostic method [1, 3–5, 11, 13, 14]. Later as the pathologic process goes on, more bone cell death occurs and leads to microfractures in antero-superior, mass bearing part of the femoral head [5, 11]. The sinking bone surface causes cartilagineous incongruity and yields to secunder degenerative changes, end stage osteoarthritis after several years, in this advanced stage it is clearly visualised by X-ray [12].

The ANFH disease occurs tipically in 4^{th} or 5^{th} decade, affecting the working age group [1–4, 12]. Due to the different pathophysiological processes and the marked osseal changes the treatment of ANFH mostly surgical. The femoral head preserving operations such as core decompression drilling and rotational osteotomies give sufficient outcomes only in early stages [15–17]. In advanced stages only total hip arthroplasty provides good result, respecting the patients relatively young age we use cementless total hip prostheses [12, 18, 19].

There are well known etiological factors such as alcohol abuse, chemotherapy and steroid drug administration that often cause ANFH, and there are other rarer conditions and diseases that can lead to ANFH by different pathways [20, 21]. In our daily practice we observed changes in colour of subcutaneous fatty tissue, however we could not discover any known etiological factor in some ANFH cases. In these fat tissue samples GC clearly identified alterations in fatty acid composition compared to control group [22, 23]. DSC results supported the GC findings, thus both methods are suitable to investigate healthy and pathologic human tissues in terms of fatty acid alteration [24, 25].

The findings of the fat tisssue analysis are preliminary, and to our knowledge no database exist regarding fatty acid composition of human tissues. Based on our findings we propose that increased proportion of oleic acid and decreased percentage of linoleic acid predisposes the patients to develop ANFH. The pathogenesis is not clear and requires further investigation.

Conclusions

We have observed macroscopic variation in color and consistency of the subcutaneous fat tissue in patients with ANFH compared to osteoarthritis or hip fracture patients during THA procedure or other surgical procedure performed in the hip region. We have also found differences between ANFH developed on the bases of chronic alcohol abuse, steroid therapy and so-called idiopathic origin. In the recent study we have investigated the subcutaneous fatty tissue of patients, in which cases none of well-known etiological factor was identified by history and physical exam. In some of these cases we suspect concealed moderate alcohol intake and neuropathic joint changes. Although we have not found histological changes, the tissue fatty acid analysis by GC revealed some significant alterations; these outcomes were further supported by DSC. These alterations in fat metabolism may play a pivotal role not only in pathogenesis of ANFH, but also may predispose patient for increased cardiovascular risk due to changes in blood lipid content.

The thermal consequences of the macroscopic observed differences in fat tissue are well detectable by DSC, while the fatty acid composition changes are identifiable with GC. The two methods supplementing each other can yield better understanding of the fat metabolism changes occurring in ANFH.

Further investigations needed regarding the fatty tissue and plasma lipid level alterations, whether they could have an effect in the outcomes of the femoral head preserving techniques done in early stages of ANFH. Better understanding the pathogenesis of the disease may decrease the number of so-called idiopathic ANFH cases.

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