PhD Thesis

HISTOLOGICAL STUDY OF HUMAN FETAL ADIPOSE ORGAN DEVELOPMENT AND ITS INVOLVEMENT IN THE INFLAMMATORY REACTION IN OBESITY

ABSTRACT

Scientific Supervisor

Prof. Ștefania Crăițoiu

PhD Student

Ana Marina Fusaru

2012
Invest in people!
EUROPEAN SOCIAL FUND
Human Resources Development Operational Sectoral Program 2007 – 2013

Priority axis: 1
“Education and professional formation supporting economical growth and social development”

Major Intervention Domain: 1.5
“Doctoral and Post-Doctoral Programs supporting research”

Project title:
“Supporting young PhD students through doctoral grants”

Project code:
POSDRU/88/1.5/S/52826

Beneficiary:
University of Medicine and Pharmacy of Craiova.
# TABLE OF CONTENTS

**ABBREVIATIONS**  
8

**INTRODUCTION**  
11

**CURRENT DATA**  
1. HISTOLOGY AND HISTOPHYSIOLOGY OF THE ADIPOSE ORGAN  
15
2. ADIPOSE TISSUE DEVELOPMENT  
34
3. THE ADIPOSE ORGAN IN ADULTS AND ITS INVOLVEMENT IN THE PATHOGENESIS OF INFLAMMATION IN OBESITY  
45

**PERSONAL STUDY**  
MOTIVATION AND WORK HYPOTHESIS  
60
AIMS OF THE THESIS AND STUDY DESIGN  
62
4. MATERIAL AND METHODS  
65
5. HISTOLOGICAL STUDY OF FETAL ADIPOGENESIS  
82
6. IMMUNOHISTOCHEMICAL STUDY OF FETAL ADIPOGENESIS  
132
7. IMMUNOHISTOCHEMICAL STUDY OF ADIPOSE TISSUE INVOLVEMENT IN THE PATHOGENESIS OF INFLAMMATION IN OBESITY  
153

GENERAL CONCLUSIONS  
173

REFERENCES  
175

---

**Key words**: adipose tissue, fetal adipogenesis, obesity, metabolic syndrome, immunohistochemistry, TNFα, TLR, VEGF, UCP-1, electron microscopy.
INTRODUCTION

Obesity is one of the biggest health problems currently facing both developed and developing countries.

The adipose tissue development and the correlation of its functions within the adipose organ - an integrative concept, recently accepted by more and more morphology experts - have become a priority in the current studies undertaken by researchers belonging to a wide range of medical specialties.

There are still many uncertainties and scientific controversy about the embryonic origin and the differentiation of adipose tissue in humans, current knowledge being based on extrapolation of the results obtained on animals or cell cultures.

The main objective of this study was to find some new data on the origin, differentiation and development of the adipose organ in human fetus through a systematic approach of the histological aspects that define each adipose depot during its fetal development.

Excess body fat entails a series of clinical complications, such as the metabolic syndrome, some biliary conditions, Alzheimer's disease and even some types of cancer. After identifying that the adipose tissue secretes many molecules with proinflammatory role - adipokines - central obesity is considered "metabolically active".

The second major objective of our study was to assess the involvement of some adipokines from the adipose tissue in the development of metabolic syndrome in adults.

The study of the fetal adipose tissue development was performed in the Laboratory of Human Morphology from the Department of Clinical and Experimental Medicine, University of Ancona (Politecnica delle Marche), Italy. The study of the involvement of the adipose tissue in the pathogenesis of inflammation in obesity was conducted in the Laboratory of Biochemistry, Faculty of Pharmacy and the Laboratory of Histology, Faculty of Medicine, University of Craiova.

The samples were obtained from the Department of Pathology, Municipal Hospital "Filantropia" and the Surgical Clinic No.1, County Emergency Hospital, Craiova.

CURRENT DATA

Chapter I – Histology and histophysiology of the adipose organ - describes the main histological and physiological features of the two types of adipose tissues: WAT (White Adipose Tissue) and BAT (Brown Adipose Tissue).

Chapter II – Adipose tissue development - describes the two steps of adipogenesis: (i) the generation of preadipocytes from mesenchimal cells (the determinant phase), followed by (ii) the differentiation of the preadipocytes into mature, functional adipocytes (terminal differentiation). This chapter also describes the main theories regarding the origin of adipoblasts, as well as the most important transcriptional factors involved in the adipose tissue development. The last part of this chapter presents the most important knowledge about human fetal adipogenesis.
Chapter III – The adipose organ in adults and its involvement in the pathogenesis of inflammation in obesity - outlines the main characteristics of the two types of adipose tissues - BAT and WAT- in adults. In the first part of this chapter we focus on the main scientific evidence on the existence of metabolically active BAT in adults. The expansion of adipose tissue in adults, which finally leads to obesity, is due to both adipocyte hypertrophy and hyperplasia, but the development of the metabolic syndrome is more likely linked to the expansion of the visceral adipose tissue.

PERSONAL CONTRIBUTION

Aims of the thesis

- Defining the histological stages of the human fetal adipose organ development and the morphological characterization of fetal adipogenesis
- Identifying the relationship between the heterogeneity of adult adipose depots and the synthesis of proinflammatory cytokines and the relationship between these cytokines and immunity, related to the metabolic status of individuals.

Chapter IV - Material and methods

For the study of human fetal adipogenesis we used fragments of adipose tissue from several subcutaneous (cervical, axillary, interscapular, thoracic and abdominal) and visceral (periaortie/pericardic, intraabdominal – mesenteric and omental, and perirenal) depots from human fetuses aged between 10 to 39 weeks of gestation. Human fetuses were classified in four groups of age: (i) very early, aged 10-12 weeks; (ii) early, aged 16-20 weeks; (iii) intermediate, aged 20-26 weeks; (iii) late, aged 36-39 weeks. For the histological study we used the usual staining techniques (hematoxylin-eosin and Masson trichrome staining) followed by transmission electron microscopy technique. We also used the immunohistochemical technique to highlight the presence of UCP1 and CD31 in the same adipose depots.

For the study of the adipose organ in adults and its involvement in the pathogenesis of inflammation in obesity we used adipose tissue samples from subcutaneous (periumbilical) and visceral (peritoneal) depots from patients between 45-73 years distributed into three groups: (i) control, lean patients, (ii) obese patients with BMI>30 and (iii) obese and diabetic patients. Samples were processed for paraffin embedding and sections were routinely stained with haematoxylin-eosin and Masson trichrome. We also did an immunohistochemical study using the following antibodies

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Dilution</th>
<th>Source</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD68</td>
<td>1:200</td>
<td>Abcam - KP1ab955</td>
<td>ABC</td>
</tr>
<tr>
<td>TNFα</td>
<td>1:100</td>
<td>Abcam - ab6671</td>
<td>ABC</td>
</tr>
<tr>
<td>TLR2</td>
<td>1:100</td>
<td>Abcam - ab24192</td>
<td>ABC</td>
</tr>
<tr>
<td>TLR 4</td>
<td>1:100</td>
<td>Abcam - ab13556</td>
<td>ABC</td>
</tr>
<tr>
<td>VEGF</td>
<td>1:50</td>
<td>Dako (VG1) - ab1316</td>
<td>LSAB</td>
</tr>
<tr>
<td>CD31</td>
<td>1:40</td>
<td>Dako - clona JC70A cod M0823</td>
<td>EnVision</td>
</tr>
</tbody>
</table>
Chapter V - Histological study of fetal adipogenesis

Our approach involved a detailed and systematic study of the appearance and evolution of the adipose lobules - lobulogenesis – in some subcutaneous and visceral depots and an assessment of the histological features of the adipocytes differentiation - adipogenesis.

We show here that the appearance and the development of the adipose tissue is a two step process, involving the following stages:

**Stage I** - mesenchymal tissue - characteristic for all the adipose depots in subjects aged 10-12 weeks.

**Stage II** – appearance and development of the adipose lobules, which can be divided into 3 shapes:

- **Mesenchimal lobules** – well vascularisated and innervated mesenchimal islets that are formed by the condensation of the mesenchimal tissue around the blood vessels.

- **Intermediary lobules** – characterised by the appearance of the first adipose cells. This category is the most heterogenous as these kind of lobules may contain unilocular and/or multilocular preadipocytes, as well as fully differentiated adipocytes. The intermediary lobules were present from age of 16-17 weeks until 36 weeks of gestation.

- **Mature lobules** – characterised by the lack of mesenchimal cells.

We concluded that:

1. The adipose depots differentiation include lobulogenesis – the formation of the adipose lobules - and adipogenesis – the differentiation of precursor cells into mature adipocytes.

2. Lobulogenesis does not start simultaneous in all the adipose depots, but it takes place continuously during the second and the third trimester of pregnancy.

3. The subcutaneous adipose depots develop before the visceral ones, starting with those located in the cervical area.

4. The full-term human fetus is characterised by the presence of multilocular or mixed adipose lobules in the same areas where PET-CT revealed the presence of fuctional BAT in adults: cervical, interscapular and pericardic.

5. BAT and WAT adipogenesis occurs in similar ways, starting from undifferentiated precursors located in pericytic position that primarily formes the preadipocites and finally the mature unilocular or multilocyular adipocytes

6. The adipoblasts are microscopic identical, but the unilocular and multilocular preadipocytes are morphologically distinct. Preadipocitelor differentiate multilocular and those uniloculare a common endothelial precursot is an argument adipocitară transdifferentiation phenomenon with deep meanings in noninvasive treatment of obesity.

7. The existence of a common precursor for the two types of preadipocytes – unilocular and multilocular- may be an evidence for the transdifferentiation properties of the adipose tissue.
Chapter VI - Immunohistochemical study of the fetal adipogenesis

We performed a systematic immunohistochemical study to highlight the presence of UCP1 and CD31 in the same adipose depots used for the research of fetal adipogenesis. Our study revealed that:

1. Fetal Adipose tissue displays an UCP1 immunoreactivity starting with age of 19-20 weeks which progressively intensifies until birth.
2. UCP1 is present in fetal adipose tissue where PET-CT revealed the presence of functional BAT in adults: cervical, interscapular and pericardic.
3. We noted that not only the multilocular adipocytes express UCP1, but also some immature unilocular adipocytes from WAT depots.
4. The immunoreactivity for CD31 of the endothelial cells showed differences depending on the degree of maturation of the adipose lobules.

Chapter VII - Immunohistochemical study of adipose tissue involvement in the pathogenesis of inflammation in obesity

White adipose tissue from different locations is characterized by significant differences in the structure of adipocyte secretoma. We compared the expression of TNFα, TLR2 and TLR 4 in peripheral subcutaneous and central peritoneal adipose depot in three different conditions: lean, obese and obese diabetic. We sustain the correlation between the incidence of TNFα and TLR4 positive stromal vascular cells and adipocytes in abdominal obesity and obesity associated with type 2 diabetes mellitus. The adipocytes close the vicious circle of inflammation in obesity, representing both the source and the effectors of proinflammatory factors synthesis.

Results of the immunohistochemical reactions for TNF-α, TLR2 and TLR4 in various adipose depots

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Cells</th>
<th>LEAN</th>
<th>OBESE</th>
<th>OBESE DIABETIC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>sc</td>
<td>per</td>
<td>sc</td>
</tr>
<tr>
<td>TNF-α</td>
<td>icc</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>ves</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>adc</td>
<td>-</td>
<td>-</td>
<td>+/-</td>
</tr>
<tr>
<td>TLR2</td>
<td>icc</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td></td>
<td>ves</td>
<td>-</td>
<td>-</td>
<td>+/-</td>
</tr>
<tr>
<td></td>
<td>adc</td>
<td>-</td>
<td>-</td>
<td>+/-</td>
</tr>
<tr>
<td>TLR4</td>
<td>icc</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td></td>
<td>ves</td>
<td>+/-</td>
<td>-</td>
<td>+/-</td>
</tr>
<tr>
<td></td>
<td>adc</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Legend: sc-subcutaneous depot, per-peritoneal depot, icc-immunocompetent cell, ves-vessels, adc-adipose cells
Fat pads displayed a similar incidence of CD31 immunoreactivity in both subcutaneous and peritoneal location. The positivity for VEGF in obese diabetic adipose samples was present in numerous structures, both of the stromal vascular fraction, blood vessels and stromal cells, as well as in the cytoplasm of adipocytes. We concluded that VEGF synthesis in visceral adipose tissue is inefficient, being not followed by angiogenesis to counterbalance tissue hypoxia.

References