Difference in the indices of adipose tissue and peripheral blood cells of individuals with overweight

Gogiashvili L.1 Tsagareli Z.1 Nikobadze E.1 Melikadze E.1 Dgebuadze M.1 Kvachadze T.1

Abstract

Background: Overweight is one of the predisposing risk factors for the development of different diseases, pathogenesis of which is based on the various types of disturbances of the adipose tissue structure, impeding both local and systemic metabolism. Recent experimental and clinical studies have revealed a new phenomenon: overweight leads to inflammation of adipose tissue and dynamically acts on the peripheral blood monocytes. In this correlation, the utmost importance is given to macrophages of the white adipose tissue (WAT) and monocytes, which account for not only the necrosis of adipocytes and utilization of the cellular debris, but also to the formation and persistence of the chronic inflammation sites.

Aim of the study: To identify the dynamic morphologic changes in adipose tissue in individuals with overweight and with different degrees of obesity, taking into account the ability of leptin to activate PMNs; find out the correlation between the altered level of inflammation, plasma levels of leptin and changes of cells in peripheral blood, monocytes/tissue macrophages and PMNs; perform the quantitative assessment of the data.

Methods: Puncture biopsy samples from the gluteal area white adipose tissue and 2 ml of venous blood samples were collected from male (n=14) and female patients (n=11) with age ranging in 41.5±12.3 years (mean ± SD), receiving treatment for overweight and increased body mass. The body mass index (BMI) was calculated by Quetelet index: BMI = body weight (kg) / height² (m). The patients were classified depending on BMI according to the degree of obesity (WHO, 1997). The leptin was measured in the blood serum by immunoenzyme method using the reagents kit (“Leptin (Sandwich) ELISA”, DRG Instruments GmbH, Germany). Histological and Electron microscopic methods were used.

Results and conclusion: Based on the given data, statistically significant differences were obtained in all studied parameters between group I (normal weight + overweight) and group II (I, II and III degree obesity) (t-criterion of Student and U-criterion of Mann-Whitney) (Table 2a, 2b, 3). The state of monocytes/ macrophages and peripheral blood cells reflects not so significantly obvious changes on the body weight, increased production and utilization of leptin activates the recruitment of macrophages from peripheral blood, simultaneously promoting their phagocytic function, which eventually leads to lipophagocytosis, necrosis and build-up of cellular debris with repeated activation of macrophages. Consequently, the interrelation between increased production of leptin, breakdown of adipocytes from WAT and characteristic reaction of monocytes and macrophages on increased body weight represents the cause for existing sites of necrosis in adipose tissue and formation of chronic inflammatory response, supporting to remodeling of adipocytes and progression of derangements in fat metabolism from increased body weight to different degrees of obesity, having at the same time the important diagnostic and prognostic potential.

Keywords: Obesity, inflammation, leptin, macrophages, monocytes, adipocytes, electron microscopy

Introduction

Overweight is one of the predisposing risk factors for the development of different diseases, pathogenesis of which is grounded on the various types of disturbances of the adipose tissue structure, impeding both local and systemic metabolism1,2. Recent experimental and clinical studies have revealed a new phenomenon: overweight leads to inflammation of adipose tissue1. In this correlation, the utmost importance is given to macrophages of the white adipose tissue (WAT), which account for not only the necrosis of adipocytes and utilization of the cellular debris, but also to the formation and persistence of the chronic inflammation sites4. Morphologic studies have shown that macrophages in fat tissue are localized predominantly around hypertrophic and/or necrotic adipocytes, forming the characteristic i.e. ring-like structure; at the same time most of the macrophages have pro-inflammatory effects stimulating the apoptosis of altered adipose cells. During the process of apoptosis in adipocytes, half of the macrophages surrounding the adipose cell will die. The exact mechanism of the death pathway for macrophages is still unclear5.
The obesity involves the pathologic positive feedback circle: hypertrophic adipocytes producing chemokines and their receptors, which initiate the recruitment of monocytes/macrophages; the latter contribute to the hypertrophy of adipocytes with greater extent and in addition, produce the adhesion proteins, which finally leads to the further development of inflammatory reaction. The given hypothesis about close interactions between macrophages and adipocytes is also supported by our studies, which alters the functional state of both types of cells\textsuperscript{6,7,8}.

Significant role in the regulation of fat tissue metabolism is played by the fat hormone synthesized in the adipocytes – leptin – one of the adipokines produced by fat tissue: signaling protein the serum concentration of which reflects the summary energetic reserve of fat tissue. High levels of leptin lead to the decreased secretion of Insulin, thereby representing the central factor in the pathogenesis of type II Diabetes Mellitus. In addition to the regulation of energetic balance, leptin is able to activate the inflammatory cells as macrophages, polymorphonuclear leukocytes (PMN), neutrophils, T-lymphocytes and infiltration by these cells even comes in advance to the infiltration by macrophages at the initial stages of the process\textsuperscript{9,10}; in addition, leptin stimulates the secretion of cytokines in these cells\textsuperscript{11}. It has been demonstrated that one of the regulatory mechanisms of leptin is related to the initiation of apoptotic death in adipose cells\textsuperscript{5}. Acting as modulator on T-cells, leptin plays the key role in the pathogenesis of the number of inflammatory diseases. Under obesity, physiologic effects of leptin are not revealed, which is related to the development of leptinoresistance that despite of the high level of leptin, accounts for the further increase in the body weight. Consequently, increased secretion of leptin in obesity supports to the development and perseverance of the reaction.

The presence of inflammatory sites in the adipose tissue presently is considered as one of the most significant and early signs of progression of obesity, especially in the patients with type II Diabetes Mellitus\textsuperscript{12}, as the recruitment of macrophages and the build-up of tumor necrosis factor \(\alpha\) (TNF-\(\alpha\)) stimulate the secretory activity of adipocytes, including the secretion of leptin, which, on its account, diminishes the expression of adiponectin, which decreases the secretion of TNF-\(\alpha\), including the chemokines and diminishes the transcription of NF-KappaB\textsuperscript{13,14} – the key player of inflammatory reaction. Diminished secretion of adiponectin under obesity leads to decreased suppressing effect on NF-KB and thus supports to inflammation. These alterations in complex accounts for the anti-inflammatory action of adiponectin, although the cause-effect relationship between the inflammation and hypoadiponecetinemia under obesity remains obscure. Presumably these conditions worsen each other by direct positive feedback principle and form the pathologic blind circuit.

Stimulating effect of macrophages of adipose tissue on angiogenesis has been established as well\textsuperscript{15}. This action is accomplished via increased expression of angiogenesis factor by macrophages – platelet-derived growth factor (PDGF), which regulates the formation of endothelial cells.

In our previous studies, we defined the role and importance of tissue macrophages of WAT in supporting to chronic inflammation and the correlation of their pro-inflammatory activities with increased size of adipocytes and body mass index (BMI)\textsuperscript{2}; the correlation between morphologic changes of adipose tissue in individuals with overweight and different levels of obesity was also established. We studied the link between obesity and the altered degree of inflammation in adipose tissue, with qualitative assessment of changes in leptin and morphologic specificities of adipose tissue in individuals with different degrees of obesity\textsuperscript{16}.

Taking into account the close functional link of macrophages and adipocytes with leptin synthesis, we formed the hypothesis of our study: being the resident cells of WAT, macrophages form the syncitial structures non-characteristic for given location, providing the recruitment of new cells from their predecessors – monocytes from circulating blood and thus supporting to both the persistence of population in the sites of chronic infiltrates in WAT as well as hypertrophy of the adipocytes.

**Aim of the study**

To identify the dynamic morphologic changes in adipose tissue in individuals with overweight and with different degrees of obesity, taking into account the ability of leptin to activate PMNs; find out the correlation between the altered level of inflammation, plasma levels of leptin and changes of cells in peripheral blood, monocytes/tissue macrophages and PMNs; perform the quantitative assessment of the data.

**Material and methods**

Puncture biopsy samples from the gluteal area white adipose tissue and 2 ml of venous blood samples were collected from male (\(n=14\)) and female patients (\(n=11\)) with age ranging in 41.5±12.3 years (mean ± SD), receiving treatment for overweight and increased body mass in the clinic of National Endocrinology Institute between 2011-2013 (informed consents were obtained from all participants before inclusion in the study) (1.6). The body mass index (BMI) was calculated by Quetelet index: BMI = body weight (kg) / height\(^2\) (m). The patients were classified depending on BMI according to the degree of obesity (WHO, 1997).

The leptin was measured in the blood serum by immunoenzyme method using the reagents kit (“Leptin (Sandwich) ELISA”, DRG Instruments GmbH, Germany).

The body mass index BMI (mean ± SD) from male (31.3 ± 8.5) and female (35.2 ± 7.9) patients (\(p=0.34\)) did not differ statistically significantly. 7 similar samples from the individuals with normal BMI served as control. Histological studies of the samples of gluteal tissue were performed on the films stained by Hematoxylin & Eosin.
without alcohol fixation. The images were analyzed and pictured on the microscope “Micos Daffodil” MCs 100/ version 2/09 (Austria) with digital camera “Sony” MC30.

The following hematologic parameters were studied: the number of monocytes (M), overall number of leukocytes (OL), basophils and lymphocytes was measured. The studies were conducted on automatic hematologic analyzer BC 5800, Mindray (China), counting of leukocyte formula (the number of red and segmented-nuclear neutrophils) was performed on films stained by Giemsa.

The ultrastructure of peripheral blood cells (monocytes and PMNs) from the same patients were studied after fixation of centrifuged peripheral blood precipitate in 2% buffered solution of gultaraldehyde and 1% solution of OsO4 (pH = 7.34), the samples were embedded in the mixture of Epon-Araldite. Ultrathin sections after double contrasting were studied in Transmission Electronic Microscope (TEM) Tesla BS 500 at the voltage of 70 kV.

Morphometry of adipocytes was performed by following steps: after digitalization of the images, the surface area of adipose cells, number of lipid vacuoles, leptin, macrophages and their structure was evaluated, monocytes of peripheral blood were studied as well, counting their number at different degrees of obesity.

The acquired data were assessed by different statistical analysis methods: Student’s t-criterion was accounted. The difference between two comparable groups was considered statistically significant when \( p \leq 0.05 \). The single-factorial dispersed discriminative analysis with defining Mann-Whitney and Tamhein criteria was applied.

**Results and discussion**

Macrophages were identified on histological and ultrathin sections of cells, aggregated with adipocytes, with singular neutrophils and lymphocytes; their number accounted for 10.2 ± 1.4 per 100 adipocytes, which was 30 times and more higher than the same index from the control group samples; increased area of active macrophages was also observed. According to electronic microscope data (EM) we observed that macrophages surrounded the adipose cells with characteristic signs of necrosis – disruption of plasma membrane, swelling (ballooning degeneration) of endoplasmic reticulum and cellular debris in intercellular space (ECM). The appearance of small lipid droplets in the cytoplasm was also notable (Figure 1).

Morphologic studies showed that macrophages are localized predominantly around hypertrophic and/or dead adipocytes, forming the characteristic so-called “scavenger” cells – the final product of residual lipid droplets of dying adipocytes. The understanding of necrosis implied the disruption of basement membrane, debris and appearance of true lipid droplets – electronically dense particles – lipophagocytosis (Figure 2).

These adipocytes have all signs of necrosis exactly, not the apoptosis, which represents itself a particular issue of interest, in relationship with the hypothesis about the actual role of leptin-activated apoptotic process in adipocytes [17]. Death of adipocytes, associated with obesity, occurs in contact with macrophages, which, by themselves, form de novo syncitial structures, having the features of compartmentalization and phagocytosis of lipid droplets. In obesity, adipocytes produce increased amount of chemokines [18], responsible for adhesion of monocytes and their transformation into macrophages. The study of monocytes in peripheral blood showed their increased number, which indicates the development of tissue inflammation, as the monocytes represent the predecessors of tissue macrophages. In addition, increased number of monocytes is accompanied by changed characteristics of these cells – immature monocytes appear in peripheral blood (Figure 3).

As known, such monocytes do not activate T-cells and have decreased phagocytic activity. In addition, our study showed the appearance of “foam” cells with so-called fenestrated cytoplasm and signs of activation of plasmolemma.

From the abovementioned data, it is clear that the cytoplasm of PMNs from peripheral blood at II and III degree obesity shows the cytoplasm homogenization, disruption of the granule structure and release of their contents, leading to the sequestration of the cytoplasm (Figure 4).

Hypertrophy of adipocytes in white adipose tissue – WAT and association of the given process with recruitment of and affect to peripheral blood monocytes indicate on the fact that hypertrophy of adipocytes in WAT can support to the necrosis of adipose cells accompanied by formation of “scavenger” macrophages around freely distributed lipid droplets. Analyzing the results of the study, we can draw the conclusion that macrophages of bone marrow origin accumulate in white adipose tissue (WAT), where they support to formation and persistence of the sites of chronic inflammation and development of metabolic complications, related with obesity [19,20,21].

Our data indicate that under activation of proteolytic cascade, typical necrotic changes are predominantly demonstrated in cells. Our material showed the positive correlation of the death fact (necrosis) of adipocytes in individuals with obesity in 19 from 23 biopsy samples (BMI ≥ 30-39), in difference with 2 from 7 biopsies in individuals with normal BMI (≤ 30) (p=0.05) (Table 1, a, b).

Taking into account the fact that adipocytes are a main secretory proteins of adipocytes, and the degree of death of adipocytes in increased in individuals with overweight, we studied the correlation between the changed plasma levels of leptin and morphologic alterations in adipose tissue (Table 2, a, b, 3). Hypersecretion of leptin is known to promote adipocytes to produce proteins of Major Histocompatibility Complex II (MHC II), playing the key role in the development of immune response, which, by itself, activates adipose-resident T cells (ARTs) – proper immune cells of adipose tissue (CD4 T-lymphocytes), promoting them to produce signaling molecules γ-interferon. Such influence of adipocytes on ARTs lies in the basis of adaptive immunity trigger mechanism for secondary inflammatory response in adipose tissue under the high calorie diet. The further augmentation of the process solely results from the involvement of macrophages / monocytes.

The quantitative data of changes in adipocytes allow to...
formulate the conclusion that there is no difference by the amount of debris (statistical significance of criterion H3P 0.387 > 0.05), necroses and balloonization of cells (statistical significance of criteria H3P 0.699 > 0.05), and also the phenomenon of lipophagocytosis (statistical significance of Tamhein criteria 0.859 > 0.05) between the individuals with normal and increased body weight; there is no difference by content of scavenger macrophages (statistical significance of criterion H3P 0.116 > 0.05) in the individuals with overweight of I degree obesity; there is no difference by content of leptin (statistical significance of criterion of Tamhein 0.382 > 0.05) and degree of appearance of lipophagocytosis (statistical significance of Tamhein criteria 0.859 > 0.05) in individuals with I and II degree obesity; same was true for the number of necroses and balloonization (statistical significance of criterion of H3P 1.000 > 0.05), lipophagocytosis (statistical significance of Tamhein criteria 0.448 > 0.05) and scavenger macrophages (statistical significance of criteria of H3P 0.235 > 0.05) in individuals with II and III degree obesity. The number of overall leukocytes was not statistically different neither from control group nor among the study groups; there was also no significant difference demonstrated between groups by the content of segmented-nuclear and rod neutrophils (statistical significance of criterion of H3P 0.456 > 0.05); in all studied cases, the number of rod neutrophils was within the limits of 3.4 – 3.9%, while the segmented-nuclear neutrophils this range was within 55.8 – 63.1%. The content of monocytes was increased in individuals with overweight by 1.8 times compared with the number of monocytes in individuals with normal body weight, and by 1.8 – 2.1 – 2.5 times compared with the control in individuals with I, II and III degree of obesity, respectively. Considering the number of foam cells, the changes in these indices are not statistically reliable among all groups, namely in the individuals with normal and increased body weight (statistical significance of Tamhein criteria 0.999 > 0.05), in individuals with overweight and I degree obesity (statistical significance of Tamhein criteria 0.133 > 0.05), also in individuals with II and III degree obesity (statistical significance of Tamhein criteria 0.988 > 0.05).

Summarizing the acquired data about correlation of changes in blood plasma level of leptin, lipid droplets, “scavenger” macrophages and “foam cells” in gluteal tissues of the individuals with overweight or different degrees of obesity, we can conclude the following: statistically significant identifiable (by percentage ratio) changes are ongoing in cells and extracellular matrix (necrosis, balloonization, amount of debris and foam cells, lipophagocytosis) in individuals with normal and increased body weight, also in individuals with I, II and III degree obesity. However, statistically significant difference in amount of leptin, lipid droplets and scavenger macrophages in the same groups was not found (Table 1a, 1b). Based on the given data, statistically significant differences were obtained in all studied parameters between group I (normal weight + overweight) and group II (I, II and III degree obesity) (t-criterion of Student and U-criterion of Mann-Whitney) (Table 2a, 2b, 3).

**Conclusion**

The state of monocytes, blood cells and macrophages reflects not so significantly obvious changes on the body weight, increased production and utilization of leptin activates the recruitment of macrophages from peripheral blood, simultaneously promoting their phagocytic function, which eventually leads to lipophagocytosis, necrosis and build-up of cellular debris with repeated activation of macrophages. Consequently, the interrelation between increased production of leptin, breakdown of adipocytes from WAT and characteristic reaction of monocytes and macrophages on increased body weight represents the cause for persisting sites of necrosis in adipose tissue and formation of chronic inflammatory response, supporting to remodeling of adipocytes and progression of derangements in fat metabolism from increased body weight to different degrees of obesity, having at the same time the important diagnostic and prognostic potential.

**Conflicting Interests**

The author(s) declared no potential conflicts of interest with respect to the authorship and/or publication of this article.
Table 1a. Quantitative assessment of indices in adipose tissue WAT and composition of peripheral blood samples in individuals with normal and increased body mass, as well as individuals with different degrees of obesity, by method of single-factorial dispersed analysis.

| Body mass          | Leptin | Necrosis, ballooning | Debris | Lipid droplets | lipophagocytosis | Scavenger macrophages | Foam cells | Overall number of leukocytes | Number Of monocytes |
|--------------------|--------|----------------------|--------|----------------|------------------|-----------------------|------------|-----------------------------|-------------------|
| 1 Normal body mass | 24,06±1,51 | 0,70±0,48           | 0,60±0,52 | 0,20±0,42       | 0,30±0,48        | 0,60±0,52            | 4,8±0,32 | 3,4±0,32                     |                   |
| 2 Increased body mass | 21,94±1,27   | 0,80±0,79           | 0,80±0,63 | 1,60±0,52       | 0,80±0,42        | 1,10±0,57            | 0,70±0,82 | 4,7±0,44                      | 5,8±0,43          |
| 3 I degree obesity | 29,86±3,95   | 1,40±0,52           | 1,30±0,48 | 2,10±0,74       | 2,20±0,42        | 1,50±0,53            | 1,7±0,82 | 4,8±0,53                      | 5,9±0,61          |
| 4 II degree obesity | 32,84±1,57    | 2,60±0,52           | 2,70±0,48 | 2,20±0,42       | 2,50±0,53        | 2,30±0,67            | 2,80±0,42 | 5,1±0,68                      | 6,5±0,25          |
| 5 III degree obesity | 48,17±6,58   | 2,60±0,52           | 2,80±0,42 | 2,80±0,42       | 2,90±0,32        | 2,60±0,52            | 2,60±0,52 | 5,5±0,34                      | 6,9±0,48          |

Table 1b. Comparable analysis of indices in adipose tissue WAT and composition of peripheral blood samples in individuals with normal and increased body mass, as well as in individuals with different degrees of obesity by method of Tamhein criteria.

| body mass          | Leptin | Necrosis, ballooning | Debris | Lipid droplets | lipophagocytosis | Scavenger macrophages | Foam cells | Overall number of leukocytes | Number of monocytes |
|--------------------|--------|----------------------|--------|----------------|------------------|-----------------------|------------|-----------------------------|-------------------|
| Normal body weight | +      |                     |        |                |                  |                       |            |                             |                   |
| Increased body mass | +      |                     |        |                |                  |                       |            |                             |                   |
| I degree obesity   |        | +                   |        |                |                  |                       |            |                             |                   |
| II degree obesity  |        |                     |        |                |                  |                       |            |                             |                   |
| III degree obesity |        |                     |        |                |                  |                       |            |                             |                   |
### Table 2a. Mean and standard deviations of indices of WAT adipose tissue cells and composition of peripheral blood samples in individuals with normal and increased body weight, as well as individuals with I, II and III degree obesity.

| Body Mass                        | Leptin (ng/ml) | Necrosis, balloonization | Debris | Lipid droplets | Lipophagocytosis | Scavenger macrophages | Foam cells | Overall number of leukocytes | Number of monocytes |
|----------------------------------|----------------|--------------------------|--------|----------------|------------------|------------------------|------------|------------------------------|---------------------|
| Increased body mass              | 21,94±1,27     | 0,80±0,79                | 1,60±0,52 | 0,80±0,42      | 1,10±0,57        | 0,70±0,82              | 4,7±0,44   | 5,8±0,43                     |
| I, II and III degree obesity     | 36,96±1,25     | 2,20±0,76                | 2,37±0,61 | 2,53±0,51      | 2,13±0,73        | 2,37±0,76              | 5,1±0,52   | 6,4±0,27                     |

### Table 2b. Mean and standard deviations of indices of WAT adipose tissue and composition of peripheral blood samples in individuals with normal and increased body weight, as well as individuals with I, II and III degree obesity.

| Body Mass                        | Leptin (ng/ml) | Necrosis, balloonization | Debris | Lipid droplets | Lipophagocytosis | Scavenger macrophages | Foam cells | Overall number of leukocytes | Number of monocytes |
|----------------------------------|----------------|--------------------------|--------|----------------|------------------|------------------------|------------|------------------------------|---------------------|
| Normal mass + increased body weight | 23,00±0,74     | 0,75±0,64                | 0,70±0,57 | 0,90±0,85      | 0,55±0,51        | 0,70±0,66              | 0,65±0,67  | 4,75±0,22                    | 4,6±0,63            |
| I, II and III degree obesity     | 36,96±9,25     | 2,20±0,76                | 2,27±0,83 | 2,37±0,61      | 2,53±0,51        | 2,13±0,73              | 2,37±0,76  | 5,13±0,42                    | 6,43±0,27           |

### Table 3. Correlation of changes of the basic parameters in individuals with normal and increased body weight, as well as in individuals with I, II and III degree obesity (t-criterion (Student’s) and U-criterion (Mann-Whitney)).

|                  | Leptin | Necrosis, balloonization | Debris | Lipid droplets | Lipophagocytosis | Scavenger macrophages | Foam cells | Overall number of leukocytes | Number of monocytes |
|------------------|--------|--------------------------|--------|----------------|------------------|------------------------|------------|------------------------------|---------------------|
| Normal weight    | +      | ↑                        | +      | +              | +                | +                      |            |  |                             |
| Increased body weight | +     | ↑                        | +      | +              | +                |  | +                      | +                      |
| I, II and III degree obesity | + | ↑                        | +      | +              | +                | +                      | +                      | +                      |

Statistical significance p < 0.05

+ The studied parameters are statistically significantly different

↑ The studied parameters are not statistically significantly different
Fig1. Macrophage from gluteal tissue biopsy, obesity II-III. Balloonization of endoplasmic reticulum (ER), small lipid droplets in cytoplasm (EM), X 10 000.

Fig2. “Scavenger”macrophage, obesity II-III, plasmolysis, lipophagocytosis, karyopiknosis, EM, X 10 000.

Fig3. Immature monocytes in peripheral blood, obesity II-III. Large polymorph nucleus, few cytoplasmic organelles, EM, X 9 000.

Fig4. Neutrophils in peripheral blood, obesity II-III, injury of granules, sequestration of cytoplasmic area, EM, X 9 000.

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