Dysfunction of human subcutaneous fat arterioles in obesity alone or obesity associated with Type 2 diabetes

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ABSTRACT

The aim of the present study was to examine the effects of obesity alone and obesity associated with Type 2 diabetes on the structure, vascular reactivity and response to insulin of isolated human subcutaneous fat arterioles; these effects were correlated with the expression of insulin signalling proteins. Periumbilical subcutaneous adipose tissue was explanted during surgery, small arterioles (internal diameter 220 ± 40 μm) were dissected out and investigated by electron microscopy, myography and immunoblotting. Compared with the subcutaneous arterioles of lean subjects, obesity activated the endothelium, enhanced the accumulation of collagen within vascular wall and increased the sensitivity of adrenergic response; obesity also diminished eNOS (endothelial NO synthase) protein expression, NO production, and endothelium-dependent and insulin-induced vasodilatation, as well as the protein expression of both IRS (insulin receptor substrates)-1 and IRS-2 and of the downstream molecules in the insulin signalling pathway, such as PI3K (phosphoinositide 3-kinase), phospho-Akt and Akt. When obesity was associated with Type 2 diabetes, these changes were significantly augmented. In conclusion, obesity alone or obesity associated with Type 2 diabetes alters human periumbilical adipose tissue arterioles in terms of structure, function and biochemistry, including diminished eNOS expression and reduced levels of IRS-1, IRS-2, PI3K and Akt in the insulin signalling pathway.

INTRODUCTION

Obesity, insulin resistance and endothelial dysfunction closely coexist throughout the natural history of Type 2 diabetes. Insulin resistance contributes to endothelial dysfunction through a complex cascade of events that are not fully understood. In addition, obesity, known as a well-established risk factor, generates adverse health effects, including cardiovascular disease, diabetes and cancer [1]. On the other hand, obesity, particularly ‘central or abdominal’ obesity, usually involving increased visceral fat, leads to an imbalanced production of several metabolic products, hormones and cytokines (adipocytokines), which favour decreased insulin sensitivity in liver and skeletal muscle and impair endothelial function through direct and/or indirect mechanisms [2]. In humans, the subcutaneous adipose tissue has a greater volume and is more strongly associated with insulin resistance than visceral adipose tissue [3]. Type 2 diabetes significantly alters adipose
tissue distribution with less influence on subcutaneous adipose tissue and with more influence on visceral and intermuscular adipose tissues, both fat depots known to exacerbate insulin resistance [4]. The interest in studying the subcutaneous adipose tissue arterioles comes not only from the need to understand the interplay between the adipocytes and vasculature within this fat depot, but also from the therapeutic potential of adipose tissue-derived stroma cells in physiological and pathological wound healing [5].

Considering the rather disparate studies on subcutaneous adipose tissue arterioles, the aim of the present study was to understand the structural–functional correlations and cellular mechanisms by which obesity and obesity aggravated by Type 2 diabetes induce local effects in this particular microvascular bed, with adverse consequences on vascular wall–adipocyte cross-talk.

MATERIALS AND METHODS

Reagents
NA (noradrenaline), KCl, ACh (acetylcholine), insulin, L-NAME (Nω-nitro-L-arginine methyl ester) and sodium orthovanadate were purchased from Sigma. The primary antibodies to IRS (insulin receptor substrates)-1, IRS-2, PI3K (phosphoinositide 3-kinase) kinase, Akt, phospho-Akt, eNOS (endothelial NO synthase) and phospho-eNOS were purchased from Santa Cruz Biotechnology. A rabbit polyclonal antibody to β-actin was from Abcam, and the HRP (horseradish peroxidase)-conjugated goat anti-(rabbit IgG) secondary antibody was from Sigma. All others reagents used were of analytical grade.

Study groups and samples
In collaboration with CFR 2 University Hospital Bucharest, the study was performed on 60 middle-aged subjects (44.32 ± 4.83 years of age; 35 men and 25 women). The subjects were divided into three groups: (i) healthy individuals as control subjects, (ii) obese patients, and (iii) obese patients with Type 2 diabetes. At the hospital, evaluation of patients consisted of physical, electrocardiographic and echocardiographic examinations, establishment of BMI (body mass index), and measurement of WC (waist circumference) and of SBP (systolic blood pressure) and DBP (diastolic blood pressure). Blood was collected by venipuncture and subsequently used for the assay of glucose and cholesterol concentrations. Small pieces of subcutaneous adipose tissue were removed during surgery from the periumbilical area, at 1.5–2.0-cm deep from the skin surface. These specimens (otherwise discarded) were subsequently transferred to the laboratory, where the arterioles (internal diameter, 220 ± 40 μm) were dissected out and used in physiological and biochemical assays.

Exclusion criteria for the three experimental groups were current tobacco use, the presence of established cardiovascular disease, and the use of medication for hypertension and hyperlipidaemia. All of the subjects had SBPs/DBPs of ≤140/90 mmHg, fasting total cholesterol levels of ≤200 mg/dl and no family history of cardiovascular disease. The controls were healthy volunteers, all in good physical health and did not take any medication at the time the experiments were performed. None had a family history of diabetes or obesity among first-degree relatives. In addition, they had BMI, WC and glucose concentrations within the normal range. For obese patients, we included subjects with evidence of increased BMI and WC values, but with normal baseline blood tests and without medication. Obese patients with Type 2 diabetes were maintained in a good metabolic control with standard oral anti-diabetic agents (see Table 1). They discontinued all drug treatment for 3 days before the study. The small pieces of subcutaneous adipose tissue were removed during aesthetic surgery for the controls, and during necessary surgery for obese patients and obese patients with Type 2 diabetes.

The experiments associated with this study have been carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association and have been approved by the Ethic Committee of the Institute of Cellular Biology and Pathology ‘N. Simionescu’, Bucharest, Romania. Each patient or subject gave their informed consent after full explanation of the purpose, nature and risk of all procedures used.

Electron microscopy
Tissue processing for electron microscopy was performed essentially as described by Popov et al. [6]. Briefly, the dissected arterioles were fixed in a mixture made of 2.5% paraformaldehyde and 1.5% glutaraldehyde in 0.1 M sodium cacodylate/HCl buffer, supplemented with 2.5 mM CaCl2, pH 7.4, post-fixed in 1% OsO4, dehydrated in graded concentrations of ethanol and embedded in Epon 812. Thickly cut sections stained with 0.25% Toluidine Blue were examined by light microscopy and were used to locate the position where the thin sections were to be cut. The thin sections were stained with 7.6% uranyl acetate (in distilled water) and 0.4% lead citrate (in 0.1 M NaOH), and were finally examined by electron microscopy (FEI Tecnai F20 field emission gun TEM; Philips Electron Optics).

Isometric myography
Segments (1-mm long) of subcutaneous arterioles were dissected out from the periumbilical fat, and the small amount of connective tissue surrounding their thin walls was removed. Two stainless steel wires (φ = 40 μm) were threaded through the arteriole lumen, and the preparation
Measurement of endothelial NO production

We measured the NO concentration at the luminal side of longitudinally opened arterioles using the NO sensor technique (amino-700; Innovative Instruments) as described previously [10]. With the isolated arteriole bathed in 2 ml of Hepes salt solution [11], the NO sensor was placed perpendicularly to the luminal arteriole side (upwards), in the close vicinity of the EC (endothelial cell) layer, in order to minimize the diffusion path of NO and to obtain a high signal. The continuous monitoring of current (in pA) or of NO production (in nM) by the intact endothelium of the arterioles was also performed in the presence of $3 \times 10^{-5}$ M ACh or 6.4 units/ml insulin.

### Immunoblotting

Lysates of isolated arterioles explanted from human periumbilical fat were subjected to SDS/PAGE and transferred on to nitrocellulose membranes, as described previously [12]. The membranes were washed in PBS/0.05 % Tween 20, blocked in 5 % (w/v) BSA in PBS/0.05 % Tween 20 and incubated with the primary antibody at 4 °C. After 17 h, membranes were washed again and incubated for 1 h with the HRP-conjugated goat anti-(rabbit IgG) secondary antibody. The bound antibody was detected using the ECL (enhanced chemiluminescence) technique. The density of immunoreactive bands was measured using a microcomputer imaging system (Scion Image). The densitometric evaluation of protein expression of the studied proteins in obese patients with and without Type 2 diabetes was compared with the control group (considered as 100 %) and was calculated as a percentage (%).

### Data analysis

All values are means ± S.E.M. One-way ANOVA was employed to quantify the results, and the findings were considered significant when $P < 0.05$.

### RESULTS

#### Clinical characterization of the study subjects

The clinical parameters of middle-aged subjects in the healthy (control), obese and obese-Type 2 diabetes groups are shown in Table 1. In the control group,
the plasma glucose concentration was in the range of 70 to 105 mg/dl, the cholesterol concentration was between 120 and 190 mg/dl, BMI was <25 kg/m² and WC was <100 cm in men and <87.5 cm in women. In the obese group, the plasma glucose concentration exceeded 105 mg/dl, cholesterol concentration was increased approx. 10% compared with the control group, their BMI was >31 kg/m² and WC was >100 cm in men and >87.5 cm in women. The obese patients with Type 2 diabetes had an approx. 2.4-fold increase in glucose concentration and an approx. 15% increase in cholesterol concentration compared with the control group, their BMI was in the range of 37 to 42.6 kg/m² and WC was 87.5 cm in women. The obese patients with Type 2 diabetes, the media layer was thickened, and the lumen diameter was reduced compared with arteries in the control group (Figure 1A). In these samples, the ECs appeared as a secretor, containing abundant rough endoplasmic reticulum and an expanded subendothelial extracellular matrix (Figure 1B, b). The two to three layers of SMCs (smooth muscle cells) displayed abundant collagen deposits that separated nearby SMCs (Figure 1B, c). In contrast, the subcutaneous arterioles in the control group had a normal appearance, with a thin basal lamina separating the endothelium from the SMC layer (Figure 1B, a). These findings suggest that, in the human subcutaneous adipose tissue arterioles, obesity alone or associated with Type 2 diabetes activates the endothelium and enhances collagen accumulation within vascular wall.

**Vascular reactivity of human subcutaneous adipose tissue arterioles**

The isometric myograph technique was used to assess the effect of obesity alone and obesity associated with Type 2 diabetes on arteriole contractile and relaxant properties, in an attempt to correlate their vascular reactivity with morphological structure. To this purpose, the small amount of connective tissue surrounding their thin wall was removed, and the vascular reactivity of the wall was measured, without the contribution of the anti-contractile effect of the perivascular adipose tissue. The results showed that, in response to $3 \times 10^{-5}$ M NA, the subcutaneous arterioles explanted from lean subjects (control group) developed a maximal contractile force of $0.044 \pm 0.015$ mN/mm; at an identical NA concentration, arterioles in the obese group contracted to $0.11 \pm 0.02$ mN/mm, whereas in the obesity + Type 2 diabetes group the force of contractile response was even higher, measuring $0.24 \pm 0.027$ mN/mm (Figure 2). Analysis of the concentration-dependent curves of contractile responses to NA ($10^{-8}$–$10^{-4}$ M) showed that half-maximal NA concentrations ($EC_{50}$) were significantly different between the three groups, i.e. 5.15 ± 0.02 in the control group, 6.45 ± 0.02 in the obese group and 5.99 ± 0.03 in the obese–diabetics group (Figure 2). These findings suggest that obesity alone or in conjunction with Type 2 diabetes is associated with an increased sensitivity ($-\log EC_{50}$) of the contractile response of subcutaneous arterioles.

**Morphological examination of the human subcutaneous adipose tissue arterioles**

We investigated whether obesity and obesity associated with Type 2 diabetes influence the morphology of subcutaneous adipose tissue arterioles. Light microscopy examination of cross-sections of the arteriolar wall showed that, in obese patients with or without Type 2 diabetes, the media layer was thickened, and the lumen diameter was reduced compared with arteries in the control group (Figure 1A). In these samples, the ECs appeared as a secretor, containing abundant rough endoplasmic reticulum and an expanded subendothelial extracellular matrix (Figure 1B, b). The two to three layers of SMCs (smooth muscle cells) displayed abundant collagen deposits that separated nearby SMCs (Figure 1B, c). In contrast, the subcutaneous arterioles in the control group had a normal appearance, with a thin basal lamina separating the endothelium from the SMC layer (Figure 1B, a). These findings suggest that, in the human subcutaneous adipose tissue arterioles, obesity alone or associated with Type 2 diabetes activates the endothelium and enhances collagen accumulation within vascular wall.

**Effect of insulin on vascular reactivity of human subcutaneous adipose tissue arterioles**

As the metabolic syndrome is associated with elevated levels of insulin, we tested the effect of insulin (0.4–6.4 units/ml) on vascular reactivity of subcutaneous adipose fat arterioles explanted from the periumbilical fat of obese patients and obese patients with Type 2 diabetes (compared with arterioles from the lean control group). The results showed that arterioles in the control group relaxed with insulin (EC50: 0.72 ± 0.07), and the maximal vasodilatation measured 21.2 ± 1.7% of NA-induced contraction (at 6.4 units/ml insulin) (Figure 3b). Pre-contracted arterioles in the obese groups without or with Type 2 diabetes relaxed very little (2.72 ± 1.56% and 1.99 ± 0.95% respectively) in the presence of ACh (Figure 3a). Taken together, these findings suggest that obesity alone or when associated with Type 2 diabetes alters the vascular reactivity of subcutaneous adipose tissue arterioles by enhancing the sensitivity and adrenergic hyper-responsiveness and by desensitization to ACh-induced endothelium-dependent vasodilatation.
Arteriole dysfunction in obese patients without or with Type 2 diabetes

Figure 1  Morphological examination of human periumbilical subcutaneous adipose tissue arterioles
(A) Light microscopy aspect of the human periumbilical subcutaneous adipose tissue arterioles from (a) a control subject, (b) an obese patient, and (c) an obese patient with Type 2 diabetes. Sections were stained with 0.25 % Toluidine Blue, and the images are from a representative experiment done twice. (B) Ultrastructure of the human periumbilical subcutaneous adipose tissue arterioles. (a) Control group, (b) EC layer in arterioles explanted from an obese patient and (c) the SMC layer in arterioles explanted from an obese patient. Note that that arteriolar wall structure was similar in the obese and obese+Type 2 diabetes groups. A representative image from a single subject in each experimental group is given. l, vascular lumen; M, mitochondria; rER, rough endoplasmic reticulum; bl, basal lamina; ECM, extracellular matrix; C, collagen fibres; N, nucleus; MVB, multivesicular body.

and obesity associated with Type 2 diabetes, the arterioles respond very little to insulin.

Concentration of NO released by ECs of human subcutaneous fat arterioles
These experiments examined whether the suppression of vessel relaxation, induced by obesity, can be related to a decrease in NO production by the EC layer. To this purpose, endothelial NO production was continuously recorded at the lumen of human subcutaneous fat arterioles exposed to $3 \times 10^{-5}$ M ACh or 6.4 units/ml insulin, applied for 10 min. The results showed that, in the presence of ACh, the arterioles explanted from obese patients and obese patients with Type 2 diabetes produced approx. 5.7- and 11.6-fold less NO respectively compared
with the control group (Table 2). In the presence of insulin, the arterioles explanted from obese patients and obese patients with Type 2 diabetes produced approx. 8.3- and 13-fold less NO respectively compared with the control group (Table 2). These results demonstrate that obesity diminishes endothelial NO production in subcutaneous adipose tissue arterioles and that obesity associated with Type 2 diabetes induces an even more severe NO decrease.

**Protein expression of eNOS and insulin signalling intermediates in human subcutaneous adipose tissue arterioles**

To examine whether diminished endothelial NO production and the abolished vasodilatation (endothelium-dependent and insulin-induced) observed in obesity alone and obesity associated with Type 2 diabetes is related to a defect in eNOS expression, immunoblotting experiments were performed. The densitometric evaluation of both the phosphorylated and non-phosphorylated forms of eNOS showed that, in obesity, the levels of these were reduced approx. 2.51- and 5.20-fold respectively compared with the control group. Moreover, obesity associated with Type 2 diabetes produced the most attenuated phospho-eNOS and eNOS protein expression, which decreased by approx. 13.55- and 17.48-fold respectively (Figures 4A, a and 4B). To gain a deeper insight into the effects of insulin on human adipose tissue arterioles, the protein expression of signalling molecules in the insulin signalling pathway was investigated (Figure 4A, b). Densitometric analysis of the bands showed that, in the subcutaneous arterioles of obese patients, both IRS-1 and IRS-2 were decreased, i.e. IRS-1 by approx. 2.61-fold and IRS-2 by approx. 3.35-fold (compared with protein expression in control group) (Figure 4B). Obesity also impeded the expression of the downstream proteins in the insulin signalling pathway, such as PI3K (by approx. 1.43-fold), phospho-Akt (by approx. 2.14-fold) and Akt (by approx. 1.44-fold). Furthermore, obesity associated with Type 2 diabetes reduced the expression of all of the proteins examined (Figures 4A, b and 4B). Thus IRS-1 was reduced...
Table 2  NO concentration delivered at the luminal side of human subcutaneous fat arterioles explanted from lean subjects (control group), obese patients and obese patients with Type 2 diabetes

Values are means ± S.E.M. P values represent the statistical significance of comparisons: *differs compared with the control group.

<table>
<thead>
<tr>
<th>Stimulation</th>
<th>Control group (n = 10)</th>
<th>Obese group (n = 10)</th>
<th>Obese + Type 2 diabetes group (n = 10)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>In the presence of $3 \times 10^{-5}$ M ACh</td>
<td>34.68 ± 1.98</td>
<td>6.11 ± 1.54*</td>
<td>2.98 ± 0.7*</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>In the presence of 6.4 units/ml insulin</td>
<td>21.06 ± 1.89</td>
<td>2.55 ± 0.6*</td>
<td>1.62 ± 0.5*</td>
<td>&lt;0.0001*</td>
</tr>
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Figure 4  Protein expression of eNOS and insulin signalling intermediates in human periumbilical subcutaneous adipose tissue arterioles

(A) Representative Western blots of the arteries explanted from white adipose tissue of obese patients and obese patients with Type 2 diabetes compared with the control group. The expression of IRS-1, IRS-2, PI3K (PI3-kinase), Akt, phospho-Akt (p-Akt), eNOS and phospho-eNOS (p-eNOS) was also determined. The presence of β-actin on the same transfers was used to show equal loading of the samples. (B) Densitometric measurements from the Western blot analysis (n = 10 individual experiments).
by approx. 3.64-fold, IRS-2 by approx. 4.73-fold, PI3K by approx. 4.30-fold and both phospho-Akt and Akt by approx. 2-fold. These findings suggest that, in the subcutaneous adipose tissue arteriole, obesity decreases the protein expression of insulin signalling proteins, a process more severely impeded when obesity is associated with Type 2 diabetes.

**DISCUSSION**

The present study reports novel findings on the effect of obesity alone and obesity associated with Type 2 diabetes on arterioles isolated from human periumbilical subcutaneous adipose tissue. First, we emphasize the structural–functional correlations in endothelial vascular dysfunction, increased contractility and drastically reduced endothelium-dependent vasorelaxation. Secondly, we provide evidence on the decrease in insulin-induced vasorelaxation associated with obesity and the molecular defects in its signalling pathway. Thirdly, we show that Type 2 diabetes associated with obesity induces even more severe alterations, impeding NO release within arteriolar lumen and lowering the expression of insulin signalling proteins, such as IRS-1, IRS-2, PI3K, Akt and eNOS.

The present study was conducted on subcutaneous fat arterioles explanted from middle-aged patients, allowing comparisons between groups in the absence of significant co-morbidities. As expected, the middle-aged obese patients and obese patients with Type 2 diabetes, who provided subcutaneous fat arterioles, had higher BMIs and WCs compared with the control group. In our experiments, the small amount of fat and connective tissue surrounding the thin wall of arterioles were removed allowing the direct measurement of the endothelium and SMC layer response. In a different vascular bed (skeletal muscle), it was reported that the fat depot localized around arterioles may be involved in local TNF-α signalling, resulting in impaired tissue perfusion and insulin resistance [13,14].

In the present study, we provide morphological and functional evidence on the effect of obesity alone and obesity associated with Type 2 diabetes in human periumbilical subcutaneous fat arterioles. The ultrastructural examination of arterioles showed that obesity alone and obesity associated with Type 2 diabetes induced similar structural effects, i.e. activated the endothelium, thickened the media layer and apparently reduced the arteriole lumen diameter; the enhanced extracellular matrix accumulation within the vascular wall may account for the media layer thickening (Figure 1). These structural modifications suggest the presence of a remodelling process and a possible restriction in blood perfusion of subcutaneous fat in obesity. In another subcutaneous region, such as the gluteal fat, the hypertrophic remodelling of the small arteries wall was found to be associated with Type 1 diabetes [15]. Furthermore, it is generally acknowledged that impaired perfusion may underlie much of the tissue and organ dysfunction associated with hypertension, obesity and diabetes mellitus [16].

At the functional level, our present findings show that obesity alone or obesity associated with Type 2 diabetes produced an enhanced contractile response to NA and a decrease in endothelium-dependent vasodilatation to ACh in human periumbilical subcutaneous fat arterioles (Figure 2). We correlated the enhanced vasoconstriction, the thicker media layer and the drastic diminishment of relaxation to: (i) extracellular matrix accumulation within the vascular wall, (ii) decreased eNOS protein expression, and (iii) reduced NO production (Figure 4 and Table 2). Recent findings underline obesity-enhanced vasoconstriction of human gluteal subcutaneous small arteries and the fact that, in obesity, both the anti-contractile property of perivascular fat and the increase in NO bioavailability caused by adiponectin secreted by nearby adipocytes are lost [17]. Furthermore, in the subcutaneous microvascular ECs of patients with Type 2 diabetes, ET-1 (endothelin-1) mRNA levels were found to be significantly higher [18]. As for vasorelaxation, previous reports have shown that, in Type 1 diabetes, the vasodilatation of subcutaneous gluteal small arteries induced by exposure to ACh or BK (bradykinin) was reduced and diminished approx. 50% in the presence of eNOS inhibitors [15]. It was reported that NO contributes to reduced BK-mediated dilatation of subcutaneous fat microvessels especially in male subjects [19]. A study in diabetes has shown that microvascular blood flow abnormalities are evident and become more marked with the development of microvascular complications [20].

Although it is known that insulin stimulates the production and the release of NO [21], we show in the present study that this also applies to subcutaneous adipose tissue arterioles under physiological conditions; these arterioles display a robust protein expression of phosphorylated and total eNOS (Figures 3b and 4). However, obesity significantly reduced insulin-induced vasorelaxation in subcutaneous adipose tissue arterioles by decreasing of eNOS protein expression and NO production, and all of these processes were exacerbated in obesity associated with Type 2 diabetes (Figure 4 and Table 2). An explanation for these results has come from studies which showed that, in obesity, the enhanced production of circulating TNF (tumour necrosis factor) may reduce the insulin-mediated effects on microvascular function by impairing the balance between endothelial-derived vasodilator and vasoconstrictor substances. In addition, there is evidence which demonstrates that TNF down-regulates the expression of eNOS and up-regulates ET-1 expression in human ECs [22].
Examination of insulin signalling proteins in arterioles of lean subjects has shown the strong expression of IRS-1 and IRS-2, as well as of the downstream proteins PI3K and Akt (serine-phosphorylated Akt included) (Figure 4). In accordance with our findings, it was established that (i) the metabolic action of insulin to stimulate glucose uptake in skeletal muscle and adipose tissue is mediated through the stimulation of the insulin receptor, IRS-1, PI3K, PDK-1 (phosphoinositide-dependent kinase 1) and Akt [23], and (ii) insulin directly increased eNOS activity leading to augmented NO production [23,24]. Besides its vasodilator action, insulin also has the vasoconstrictor effects. These vasoconstrictor effects are mainly mediated by the vasoconstrictor peptide ET-1 [23]. ET-1 is produced in the vascular endothelium through stimulation of the intracellular MAPK (mitogen-activated protein kinase) signalling pathway and ERK1/2 (extracellular signal-regulated kinase-1/2) [25]. Thus insulin has opposing vasodilator and vasoconstrictor effects, the net effect being dependent on the balance between these two, and its imbalance causes the development of microvascular dysfunction.

In addition, our present results revealed that obesity alone or associated with Type 2 diabetes disrupted the proteins involved in the signalling pathway mediated by insulin (IRS-1, IRS-2, PI3K, phospho-Akt, Akt, phospho-eNOS and e-NOS), impaired endothelial NO production and generated an insulin-resistance condition. In the insulin signalling pathway, eNOS is activated through the Akt-mediated phosphorylation of Ser1179. The mechanisms regulating eNOS activity and endothelial NO production are complex and sometimes overlapping. For example, eNOS contains multiple phosphorylation sites in addition to Ser1179, and increasing evidence has implicated the participation of a variety of kinase and phosphatase activities in the regulation of eNOS activity. The phosphorylation events also participate in controlling the interactions between eNOS and other regulatory proteins [26]. Therefore we report in the present study that obesity and obesity associated with Type 2 diabetes impede the expression of proteins associated with insulin signalling and also blunt arteriole vasodilatation.

**Limitations of the study**

The present study was carried out on specimens from periumbilical subcutaneous adipose tissue routinely discarded during surgery. Although we know the clinical findings of the patients (Table 1), they were undergoing surgery for a variety of reasons and were probably taking a variety of medications. Therefore we cannot exclude the interference of the latter on the results presented. However, in the three groups studied, the intact arterioles always originated from the same body area (i.e. periumbilical) and from the deep compartment of subcutaneous fat (i.e. at 1.5–2.0 cm from the skin surface).

**Conclusions**

The results of the present study indicate that obesity alone or associated with Type 2 diabetes alters human periumbilical adipose tissue arterioles in terms of structure, function and biochemistry, including decreasing eNOS expression and reduced levels of IRS-1, IRS-2, PI3K and Akt in the insulin signalling pathway.

**AUTHOR CONTRIBUTION**

Adriana Georgescu designed the study, performed the experiments, analysed the data and wrote the manuscript. Doina Popov analysed the data and wrote the manuscript. Anamaria Constantin, Miruna Nemez and Nicoleta Alexandru performed the experiments. Daniel Cochior and Aura Tudor evaluated the patients and analysed the data.

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