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Impaired adipocyte glucose transport regulators in morbid obesity – Possible mechanisms contributing to metabolic dysfunction

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Abstract. – OBJECTIVE: Obesity is characterized by hypertrophy and pathological expansion of adipocytes with impaired insulin signaling causing insulin resistance (IR) and metabolic dysfunction. We recently reported decreased expression of glucose transporter-4 (GLUT4) in cultured adipocytes from visceral and abdominal subcutaneous fat depots from patients with morbid obesity and hyperinsulinemia (MOW) and with Type 2 diabetes (MODM). Subsequently, we wanted to study the molecular mechanisms of the glucose transport regulators, p85PI3K, Rab5 and Gapex5 in morbid obesity.

PATIENTS AND METHODS: Primary *in vitro* adipocyte cultures were developed from surgical biopsies from visceral (Visc) and abdominal (Sub) and gluteal subcutaneous (Glut) fat depots from 20 lean adults and 36 adults with morbid obesity divided into two groups: 20 with MOW and 16 MODM). mRNA and protein expression (P) of p85PI3K, Rab5 and Gapex5 were studied with RT-PCR and Western Immunoblotting (WI), respectively.

RESULTS: In Sub, the P of (1) p85PI3K and Gapex5 were increased in MODM and (2) Rab5 was decreased in MOW and MODM compared to the lean. In Glut, the P of p85PI3K, Rab5 and Gapex5 showed no difference between the lean and MODM.

CONCLUSIONS: In Sub of MODM (1) reduced RAB5 may possibly contribute to IR and glucose transport dysfunction, (2) increased Gapex5 may be a response to decreased Rab5 in an attempt to increase glucose transport and (3) increased p85PI3K may enhance IR mediating lipid accumulation in MODM. In Glut of MODM, though, the expression of p85PI3K, Rab5 and Gapex5 seems to be similar to that found in lean individuals.

Key Words:
Morbid obesity, Insulin resistance, Diabetes mellitus, p85PI3K, Rab5, Gapex5.

Introduction

The etiologic mechanisms involved in metabolic dysfunction associated with morbid obesity still remain a mystery despite new advancements in therapy. Obesity continues to be a major public health threat worldwide since the individuals with obesity have an extremely high risk for metabolic comorbidities[1]. These include insulin resistance (IR), the metabolic syndrome and Type 2 diabetes (T2D), leading to an increased risk for cardiovascular disease. Accepted plausible mechanisms involved in this mechanism are the pathological expansion of white adipose tissue (WAT), including the massive enlargement of existing adipocytes (hypertrophy), limited angiogenesis, ensuing hypoxia and an inflammatory phenotype that is strongly associated with systemic insulin resistance present in morbidly obese individuals[2]. These pathophysiological events seem to occur first in the subcutaneous fat depot, followed by the visceral adipose compartment[3]. Human primary adipocyte culture studies[4,5] played an important role in furthering our knowledge of

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normal and abnormal adipocyte metabolism in WAT, since it has been shown that specific characteristics present in the patients are retained in their cultured pre-adipocytes.

The underlying molecular mechanisms of T2D and its metabolic complications in obese adults have not yet been fully clarified, but the insulin and adiponectin signaling pathways in the adipocytes seem to play a crucial role in this disorder[6-8]. It has been reported that low GLUT4 expression in adipose tissue alone is capable of disruption of whole-body glucose homeostasis[9]. We previously reported that there was reduced protein expression of GLUT4 in cultured adipocytes from all three fat depots (Sub, Visc and Glut) in morbidly obese individuals with T2D whereas in morbidly obese individuals without T2D GLUT4 expression was found decreased only in Sub and Visc[10].

Postprandially, increased circulating concentrations of glucose can be utilized by the adipose tissue, due to the combined action of insulin and adiponectin[7,8]. Once insulin reaches the adipocyte, it interacts with its plasma membrane receptor, which activates the insulin receptor substrate 1/2 (IRS1/2). The activation of protein kinase B/Akt II, (AktII), a key molecule in the insulin signaling cascade, depends upon the presentation of the regulatory subunit, p85 of phosphoinositide 3-kinase (p85PI3K) to IRS1, *via* a small trafficking protein, Rab5. This leads to the translocation of the glucose transporter, GLUT4, to the plasma membrane (PM) facilitating glucose influx into the adipocyte[11]. Rab5 seems to play a pivotal role in insulin sensitivity[12] as it mediates the crosstalk between the insulin and adiponectin pathways, due to its interaction with the Adaptor Protein containing Pleckstrin homology domain, phosphotyrosine binding domain and Leucine zipper 1 (APPL1)[13].

The Rab5 protein possesses the endogenous ability to hydrolyse GTP to GDP, thus interacting both with GTP and GDP, which transform it into its active and inactive forms, respectively. Additionally, many factors regulate Rab5 function, such as: (1) the GDP/GTP Exchange Factors (GEF), which result in the enhancement of Rab5 function, due to their stabilization of the Rab5-GTP complex and (2) GTPase Activating Proteins (GAP), which hydrolyze GTP to GDP, thus deactivating Rab5. In addition, Gapex5, an important GEF of Rab5 which enhances insulin sensitivity in the adipocytes has also been recognised[14]. Furthermore, Gapex5 knockdown with siRNA, in a 3T3-L1 cell line, suppressed low dose insulin mediated glucose uptake, whereas with higher doses of insulin this effect was reversed[15].

Therefore, since there is a complex network of components involved in normal glucose transport, further investigation is warranted to elucidate whether some of the regulatory components of glucose transport trafficking, may be dysregulated in obese individuals.

The aim of our research was to study the expression of the glucose transport regulators, p85PI3K, Rab5 and Gapex5, that may play a role in the development of insulin resistance in the adipocytes of morbidly obese individuals.

Patients and Methods

Subjects

Our study population included 36 adults with morbid obesity (OB) with a BMI > 40 kg/m^2 and 20 lean (BMI ≤ 25 kg/m^2) adults. All OB underwent bariatric surgery for the first time and the lean individuals underwent surgery for non-inflammatory surgical conditions. The OB were divided into two subgroups as follows: a) MODM: 16 adults with morbid obesity and T2D; and b) MOW: 20 adults with morbid obesity and hyperinsulinemia (defined as fasting insulin ≥12.2 μU/mL in the presence of euglycemia)[16]. In the MODM group, the majority of adults had long-standing T2D with a mean duration of 3.69 years and were under treatment with metformin (13/14) or glimepiride (1/14). Two MODM patients were newly diagnosed during the presurgical oral glucose tolerance test (OGTT) with a plasma glucose of ≥200 mg/dl at 2 h and were not on any medication. Of note, none of the patients were under treatment with insulin.

The exclusion criteria included the following: (1) for the patients with morbid obesity: any patients who had previously undergone bariatric surgery were excluded and (2) for the lean individuals: any patients that were being treated for a medical condition were excluded.

Presurgically, a detailed medical background was obtained, and a physical exam was performed. The study was approved by the Ethical Committee of the University of Patras, Greece, an informed consent was obtained. All the investigations were carried out according to the rules of the Declaration of Helsinki of 1975 revised in 2013.

Primary Adipocyte Cultures

At the beginning of the surgical procedures, biopsies of the adipose tissue were obtained, under sterile conditions, from three different fat depots, abdominal visceral (Visc), abdominal subcutaneous (Sub) and gluteal subcutaneous tissue (Glut) and placed into a sterile vial with culture medium and taken immediately to the Research Laboratory of the Division of Pediatric Endocrinology and Diabetes so that primary adipocyte cultures could be developed.

At the laboratory, the adipose tissue was processed and the preadipocytes were cultured as previously described[17].

The preadipocyte cultures were replenished with fresh medium every 3 days, and then, subcultivated in a 1:2 split ratio upon reaching confluency by using a trypsin/EDTA solution (Invitrogen Corporation, Carlsbad, California, USA). Upon confluency, at the 3rd passage the differentiation of adipocytes was induced as we previously described[17].

Reverse Transcriptase PCR

Total RNA was extracted from the mature adipocyte cultured cells, using the Gen-Elute Mammalian Total RNA Miniprep Kit (Sigma, Aldrich Company Ltd., Dorset, UK) and complementary DNA (cDNA) was synthesized by Reverse Transcriptase Polymerase Chain Reaction (RT-PCR), using the Prime-Script 1st strand cDNA Synthesis Kit (TaKaRa Bio Inc., Otsu, Shiga, Japan), according to the manufacturer's instructions. Rab5, p85PI3K, mRNA were amplified from DNA using the primers: for Rab5: Forward 5' CAGAGGCAAGCAAGTCCTAACA 3' and Reverse 5' ACTGTGTGGGCCCAGACAGTCA 3', for p85PI3K: Forward 5' TGAAGAACAGTGCCAGACCC 3' and Reverse 5' TACAGAGCAGGCATAGCAGC 3'. Primers for β-actin, the housekeeping gene, were: Forward 5' AAGGCCAACCGTGAAAAGATGACC 3' and Reverse 5' ACCGCTCGTTGCCAATAGTGATGA 3'. The PCRs reactions were performed under the following conditions: a) 95°C for 1 min (denaturation), b) 30 sec at 51°C for Rab5 (annealing) 54°C for p85PI3K, and 56°C for β-actin c) 72°C for 1 min (extension) for 35 cycles. The products of the PCR were analyzed by electrophoresis in agarose gel using Ultrapure Agarose (Invitrogen Corporation, Carlsbad, CA, USA). Gel-Quant software 4.1 (Microsoft Windows) was used in order to quantify the PCR bands by densitometry. The relative mRNA expression was evaluated by comparison with the expression of the β-actin gene in the same samples.

Western Immunoblotting

Total protein was extracted from mature adipocytes using Laemnli Sample Buffer (63 mM Tris HCl, 10% glycerol, 2% SDS, 0.0025% Bromophenol Blue, pH 6.8). The protein content of each sample was quantified using electrophoresis and Coomassie Blue staining. 100 g of protein were used from each sample. Western immunoblotting was performed as described in our previous studies[17]. Signal was detected using chemiluminescence reagent ECL (GE Haelthcare, Buckinghamshire, UK), for immunodetection of the proteins.

The following dilutions and incubation at 4°C overnight were used for each individual IgG antibody: (1) Rab5 (25kDa) (rabbit polyclonal Anti-Rab5, ab18211, Abcam, Cambridge, UK, 1:200, overnight at 4°C), (2) Gapex5 (180kDa) [RAP6 (GAPex5) Antibody, NBP1-19156, Novus Biologicals, Cambridge, UK, 1:500 overnight at 4°C], (3) p85PI3K (85kDa) (anti-PI3-kinase p85a, B9: sc-1637, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA, 1:200, overnight at 4°C). The secondary antibodies used were anti-chicken IgG 1:1000 (Millipore), anti-mouse IgG 1:500 (A9044, Sigma-Aldrich) and anti-rabbit IgG 1:500 (12-348, Millipore). The measured protein expression was corrected with β-tubulin, detected at 50kDa with a specific antibody (mouse monoclonal anti-β-tubulin, clone AA2, 05-661, Cell Signaling Technology, Inc., Danvers, MA, USA; 1: 500, overnight at 4°C). For the quantification of the Western blotting signals, densitometry using scion image (version 4.0.3.2, Scion Corporation, Chicago, IL, USA) was used.

Blood Tests

Fasting blood samples were obtained the day before surgery for a complete biochemical and lipid profile [cholesterol, triglycerides, HDL (high-density lipoprotein), LDL (low-density lipoprotein)], and for serum insulin. ELISA was used for serum insulin measurements using the Human Insulin ELISA Kit (Millipore, Burlington, MA, USA). The lower limit of detection was 2 μIU/ml and the intra-assay coefficients of variation was 5.96% and the inter-assay coefficients of variation was 6.4%. For the assessment of Insulin Resistance, the HOMA-IR model was used using the formula: HOMA-IR = Fasting serum insulin (μU/ml) x Fasting plasma glucose (mmol/l)/22.5[18].

Statistical Analysis

A normality test was performed for each variable. *p*-values were calculated with the student *t*-test and the Mann-Whitney test. Relationships between parameters were assessed with Pearson's and Spearman's correlation coefficient tests. All values are presented as mean ± standard error (SE) of mean. The statistical analysis was performed using IBM SPSS Statistics 17 (SPSS, Chicago, IL, USA), and the significance level was set at 5%.

Results

Clinical Characteristics

The clinical characteristics of the adults studied are depicted in Table I. The lean individuals were older compared to the OB, MOW and MODM. Hypertension was observed in 11 of the OB, 3 of the MOW and 8 of the MODM group. Fatty liver was observed on the pre-surgical ultrasound in the majority of the adults with obesity (16/16 of MODM and 17/20 of MOW).

Serum Tests

Significantly higher fasting insulin, glucose concentrations and HOMA-IR were observed in the MODM compared to the lean and the OB overall. Also, significantly higher insulin concentrations and HOMA-IR were observed in the MOW in comparison to the lean and lower HOMA-IR, fasting glucose and glucose concentrations during the OGTT at 60, 90 and 120 min as compared to the MODM (Table I).

In addition, differences in the lipidemic profile and hepatic function markers between the groups are depicted in Table I.

p85PI3K Expression in Cultured Adipocytes

The mRNA expression of p85PI3K was found decreased in Visc of OB and of MOW (p=0.05

Table I. Real time PCR primers.

	Obesity group (OB) n = 36	Lean n = 20	Morbid obesity group without diabetes (MOW) n = 20	Morbid obesity group with diabetes (MODM) n = 16
Age	37.16 ± 13.3*	48.28 ± 14.92	36.29 ± 14.08*	38.31 ± 12.55*
Gender (F/M)	20/16	13/7	12/8	8/8
BMI(Kg/m^2)	57.59 ± 8.6*	23.26 ± 2.12	56.43 ± 7.32*	59.11 ± 10.08*
Weight (Kg)	166.99 ± 31.13*	68.91 ± 10.2	165.04 ± 29.39*	169.54 ± 34.08*
Fatty liver	33/36		17/20	16/16
γGT	27.21 ± 11.44	21.8 ± 5.63	23.53 ± 11.02†	32.91 ± 10*
SGOT	22.12 ± 8.32	23.8 ± 8.31	20.63 ± 7.73	24.14 ± 8.94
SGPT	31.2 ± 10.7*	22.67 ± 9.46	29.67 ± 12.48	33.5 ± 7.17*
LDH	267.63 ± 90.07*	181.56 ± 34.13	288.05 ± 87.32*	240.4 ± 89.25*
Cholesterol	191.76 ± 42.53	153.67 ± 24.5	190.48 ± 39.12	193.44 ± 47.9
HDL	42.63 ± 10.05	47.33 ± 14.5	45.07 ± 10.6	39.44 ± 8.56
LDL	125.55 ± 31.42*	87 ± 11.49	126.06 ± 29.31*	124.91 ± 34.86
TGs	134.23 ± 53.75	96.33 ± 21.94	134.71 ± 48.53	133.5 ± 62.71
Insulin (μIU/ml)	25.31 ± 15.01*	9.59 ± 4.49	23.28 ± 14.67*	28.15 ± 15.53*
Fasting Glucose (mg/dl)	106.2 ± 22.11*	87.7 ± 11.59	96.38 ± 10.46†	120.93 ± 26.85*
OGTT				
30'	157.29 ± 35.94		153 ± 27.56	165 ± 48.35
60'	165.28 ± 53.87		142.32 ± 36.84†	208.9 ± 55.56
90'	139.75 ± 49.93		114.89 ± 27.48†	192.22 ± 46.37
120'	120.7 ± 53.43		89.05 ± 19.53†	175.36 ± 49

Age was significantly higher in the lean compared to the OB (*p=0.004), and, more specifically, to MODM (*p=0.044) and MOW (*p=0.014). Insulin, fasting glucose and HOMA-IR were significantly higher in the MODM compared to the lean (*p<0.001, *p=0.003 and *p=0.002) and the OB overall, (*p<0.001 & *p=0.007 & *p=0.001). MOW had significantly higher Insulin (*p=0.001) and HOMA-IR (*p=0.015) compared to the lean, and lower HOMA-IR, fasting glucose and glucose values at 60, 90 and 120min during the OGTT as compared to MODM ($^\dagger p$=0.013, $^\dagger p$=0.017 and $^\dagger p$<0.001 for OGTT). MODM, MOW and the OB overall had higher levels of LDH (*p=0.027 and *p=0.001, *p=0.006) compared to the lean. MODM (*p=0.007) and OB overall (*p=0.038) had higher SGPT compared to the lean. MODM had significantly higher γGT when compared to the lean (*p=0.038) and MOW ($^\dagger p$=0,031). MOW (*p=0.036) and OB overall (*p=0.043) had higher LDL compared to the lean. Values are expressed in Mean ± Standard Deviation. The statistical test used was the Mann-Whitney test.

and p=0.025, respectively), compared to the lean group. OB exhibited the highest relative expression of p85PI3K mRNA in Glut, compared to the other fat depots (p=0.03 with Visc and p=0.012 with Sub). Also, the mRNA expression of p85PI3K in Glut was found increased in MOW (p=0.028) and MODM (p=0.05), when compared to Visc (data not shown).

The protein expression of p85PI3K showed no difference between the lean and obese groups in the Sub, Visc and Glut, but it was found increased in Sub of MODM, compared to the MOW(p=0.017) and their respective lean (p=0.009). In Glut there was no significant difference between the protein expression of p85PI3K between the lean, MOW and MODM (Figure 1).

Rab5 Expression in Cultured Adipocytes

Rab5 mRNA was increased in Glut, compared to Visc (p=0.04) in the lean group. In addition, MODM had reduced mRNA expression of Rab5 in Visc, compared to Sub (p=0.045) and Glut (p=0.005), and in Sub, compared to Glut (p=0.048). Therefore, the mRNA expression of Rab5 in MODM showed a decreasing trend, i.e., Glut > Sub > Visc (data not shown).

Rab5 protein expression was found reduced in Sub of OB (p=0.002) and MODM (p=0.004), compared to the lean and increased in Glut of MODM compared to Sub (**p=0.05). The protein expression of Rab5 was also decreased in Sub and Glut of MOW (p=0.014 and p=0.031, respectively), compared to the lean. In Glut, the protein expression of RAB5 was similar in the lean and MODM (Figure 2).

Gapex5 Protein Expression in Cultured Adipocytes

Gapex5 protein expression of OB was decreased in Sub compared to the Visc fat depot and was also found reduced in Sub of MOW compared to the MODM group. In Glut, Gapex5 had similar protein expression in the lean, MOW and MODM (Figure 3).

Correlations

There was a statistically significant positive correlation of the mRNA expression of p85PI3K in Visc with serum insulin (r=0.607, p=0.016) and HOMA-IR (r=0.671, p=0.004) of OB (Figure 3A and B). There was also a positive correlation of the mRNA expression of p85PI3K in Visc with serum insulin (r=0.738, p=0.037) and HOMA-IR (r=0.833, p=0.005) in the MODM group (Figure 4).

Discussion

The p85 subunit of PI3K has been reported to be a regulator of Rab5 with dual action. Insulin mediated phosphorylation of p85PI3K allows the translocation of Rab5 to the plasma mem-

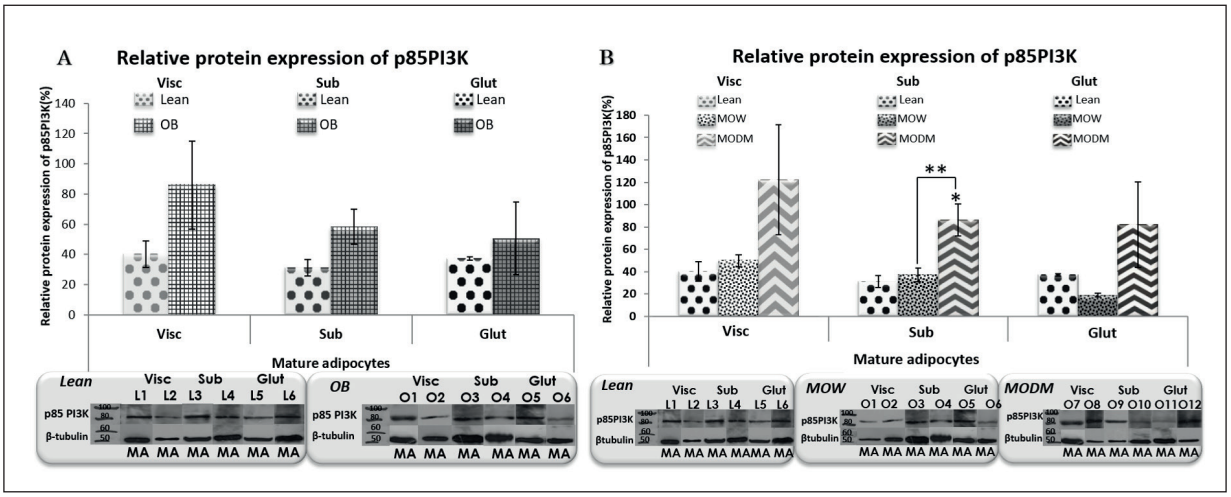

Figure 1. Relative protein expression of p85PI3K in mature cultured adipocytes in (**A**) OB and (**B**) subgroups. **A**, p85PI3K showed no statistically significant differences between the lean and obese groups. **B**, p85PI3K was increased in Sub of MODM, compared to the MOW (**p=0.017) and their respective lean (*p=0.009). In Glut there was no significant difference between the protein expression of p85PI3K between the lean, MOW and MODM.

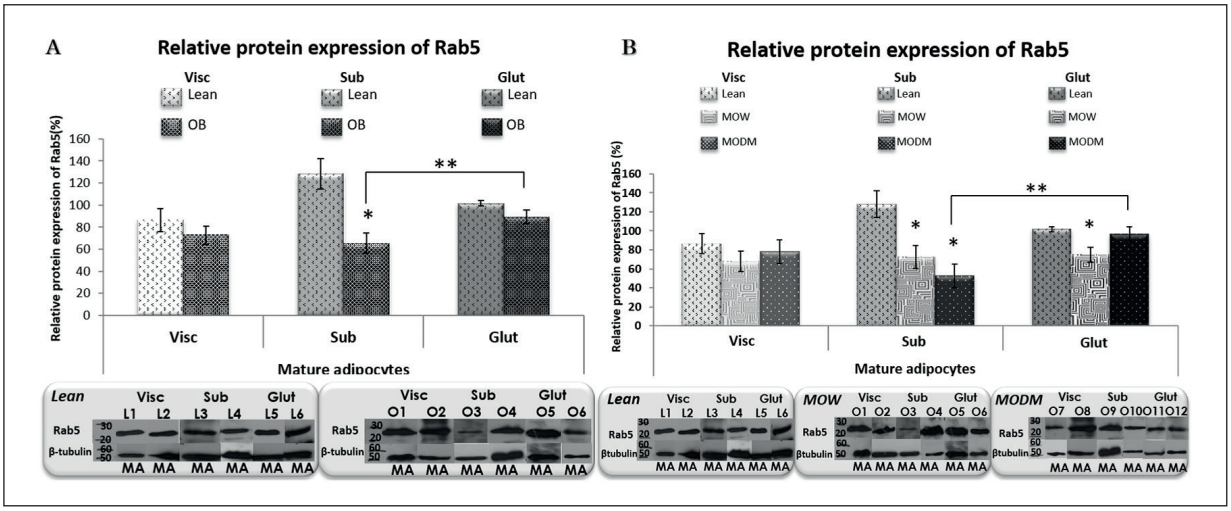

Figure 2. Relative protein expression of Rab5 in mature cultured adipocytes in (**A**) OB and (**B**) subgroups. **A**, The protein expression of Rab5 was decreased in Sub of OB compared to their respective lean (**p*=0.002) **B**, RAB5 was reduced in *Sub* MODM, compared to the lean (*p*=0.004) but increased in Glut of MODM compared to Sub (***p*=0.05). The protein expression of Rab5 was also, decreased in *Sub* and *Glut* of MOW (*p*=0.014 and *p*=0.031, respectively), compared to the lean. In Glut, the protein expression of RAB5 was similar in the lean and MODM.

brane (PM) in order to facilitate the presentation of p85PI3K to the catalytic subunit, p110PI3K and the subsequent activation of Akt II[19]. Afterwards, p85PI3K deactivates Rab5, indicating the involvement of p85PI3K in a second negative feedback mechanism of insulin signaling[20]. The PI3K/Akt pathway enhances lipogenesis, which dominates over lipolysis in the hypertrophic adipocytes of individuals with obesity[21]. More specifically, p85PI3K positively correlates with insulin resistance and T2D, since studies with deletion of p85PI3K resulted in enhancement of GLUT4 translocation to PM[22] and of Akt II phosphorylation[23,24]. Consistent with this is the positive correlation that we found in our study between the mRNA expression of p85PI3K in the Visc of MODM and the serum insulin levels and HOMA-IR.

Subcutaneous adipose tissue plays a vital role in maintaining a dynamic metabolic equilibrium in humans through its capability of storing surplus fat rather than permitting it to be stored in

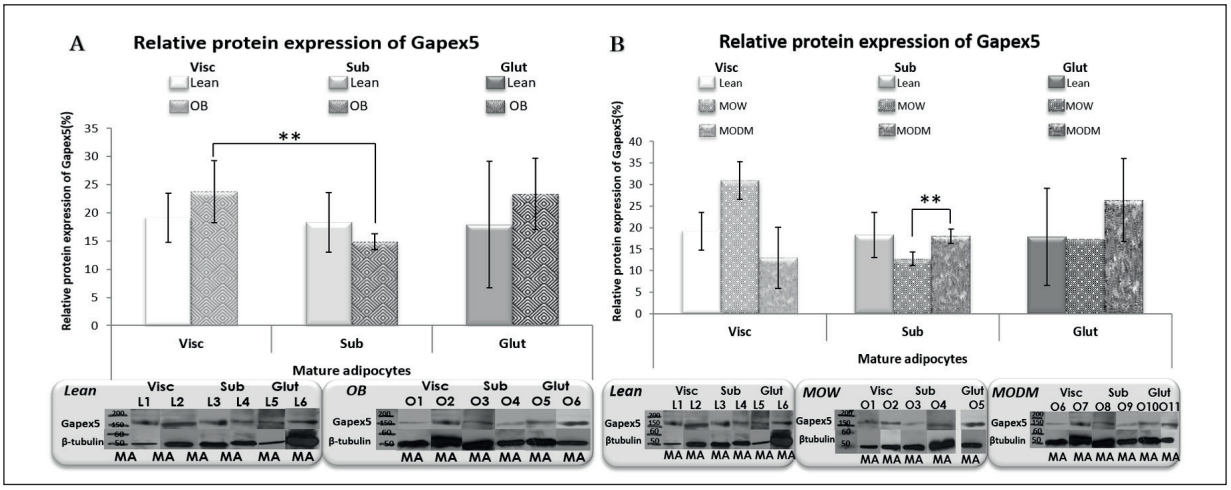

Figure 3. Relative protein expression of Gapex5 in mature cultured adipocytes in (**A**) OB and (**B**) subgroups. **A**, The protein expression of Gapex5 was decreased in Sub of OB, compared to the Visc (***p*=0.05). **B**, Gapex5 was also decreased in Sub of MOW, compared to the MODM group (***p*=0.05). Gapex5 had similar protein expression in the lean, MOW and MODM.

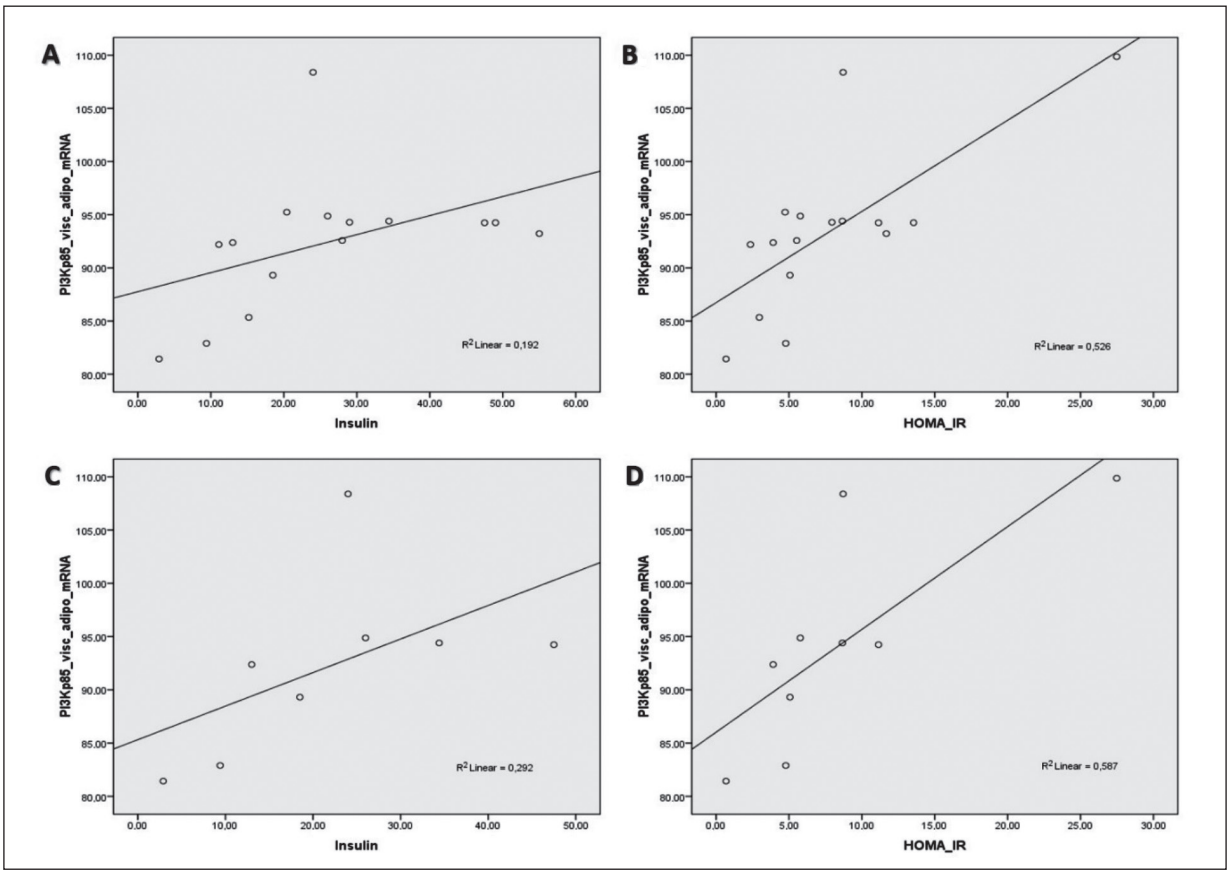

Figure 4. Correlation of the mRNA expression of p85PI3K of OB in Visc with (**A**) insulin and (**B**) HOMA-IR and of MODM with (**C**) insulin and (**D**) HOMA-IR. The mRNA expression of p85PI3K of OB in Visc exhibited positive correlation with (**A**) Insulin (r=0.607, *p*=0.016) and (**B**) HOMA-IR (r=0.671, *p*=0.004). The mRNA expression of p85PI3K of MODM in Visc exhibited positive correlation with (**C**) insulin (r=0.738, *p*=0.037) and (**D**) HOMA-IR (r=0.833, *p*=0.005).

ectopic depots, such as in visceral fat, the liver, within muscle fibers or around the heart[25]. It has also been shown to exhibit an early dysfunction in morbid obesity, before it is apparent in the other fat depots, followed by dysfunction of visceral adipose tissue[3,26]. The results of our present study support this in that they showed changes in the protein expression consistent with metabolic dysfunction in the cultured adipocytes from the subcutaneous abdominal adipose tissue as follows: (1) increased p85PI3K and Gapex5 in MODM compared to the MOW and (2) a decrease of Rab5 in OB, MOW and MODM, compared to the lean.

The decrease in Rab5 protein expression in the cultured adipocytes from Sub in MOW and MODM in our present study is in accordance with findings from our previous study[27] in obese children. Also, Rab5 has been reported to be decreased in the skeletal muscle of insulin resistant and T2D patients[12], but our study is the first to report decreased RAB5 in cultured adipocytes from subcutaneous abdominal tissue of patients with morbid obesity and hyperinsulinemia and patients with T2D. The reduction in RAB5 may perhaps play a role in the decreased GLUT4 that we found in the cultured adipocytes from the Sub and Visc of patients with morbid obesity and hyperinsulinemia and patients with T2D that we recently reported[10].

Also, it is possible that (1) the increased Gapex5 that we found in the cultured adipocytes of Sub in MODM in our present study may reflect a compensatory mechanism attempting to increase the low levels of Rab5 present in order to increase insulin sensitivity and glucose transport in the MODM adipocytes whereas (2) the increased p85PI3K may possibly be a cause of enhanced IR in MODM by mediating lipid accumulation and aggravating hypertrophy which may perhaps contribute to pathological expansion of Sub and the development of systemic IR in MODM.

The protective role of the gluteal subcutane-

ous adipose tissue which exhibits reduced inflammatory factors and an improved adipokine profile has been previously suggested. The Gluteofemoral adipose depot is positively associated with elevated adipokines such as adiponectin, increased insulin sensitivity and lower levels of inflammatory cells, together with a slower rate of lipolysis and free fatty acid release[28]. Consistent with this view, we previously reported that the protein expression of GLUT4 in the cultured adipocytes from the gluteal subcutaneous adipose tissue in morbidly obese individuals without T2D was similar to that found in the adipocytes from the same fat depot of lean adults[10]. Therefore, it is of particular interest that in our present study we found that in the cultured adipocytes from Glut: the protein expression of: (1) p85PI3K showed no significant differences between the lean, MOW and MODM, (2) Rab5 was similar in lean and MODM and (3) Gapex5 was similar in the lean, MOW and MODM. It is interesting to speculate whether these findings may infer that in the adipocytes of the gluteal subcutaneous tissue of individuals with obesity, insulin resistance and T2D the protein expression of the glucose transport regulators p85PI3K, Rab5 and Gapex5 may be "protected".

Strengths and Limitations

Of course, there are limitations to the findings of our present study as they need to be verified by further molecular studies in a larger population of individuals with obesity, hyperinsulinemia and T2D.

Conclusions

Our study is the first study, to our knowledge, to show that in the cultured adipocytes of individuals with morbid obesity there is possibly: (1) an earlier dysfunction of the glucose transport regulators, p85PI3K, RAB5 and Gapex5 in the abdominal subcutaneous tissue in comparison to the visceral adipose tissue depot, (2) a reduction in Sub of the protein expression of RAB5 in OB, MOW and MODM and an increase in the protein expression of Gapex5 and p85PI3K in MODM whereas (3) in cultured adipocytes from Glut, there is no significant difference in the protein expression of RAB5 in MODM with that found in lean individuals while the protein expression of Gapex5 and p85PI3K also show no significant differences between lean, MOW and MODM. The results of our present study may give some insight into: (1) the possible dysfunction of the expression of the glucose transport regulators p85PI3K, Rab5 and Gapex5 that may be present in the adipocytes in the subcutaneous abdominal tissue of patients with obesity, hyperinsulinemia and T2D and (2) the possible similarity of the expression of these proteins in the gluteal subcutaneous adipose tissue in patients with T2D with that found in lean individuals. These findings may be helpful in the formation of pharmacologic agents which may improve glucose transport regulator dysfunction in individuals with morbid obesity.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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