Polo Like Kinase 1 (Plk1) Expression in Visceral Adipose Tissue of Morbidly Obese Non Diabetic Women

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Abstract

Dysregulation of glucose metabolism is a common association with obesity. However, not all types of obesity are complicated with diabetes. Obese animals can compensate for insulin resistance through β-Cell proliferation. Nevertheless in humans this mechanism remains to be elucidated. Here we evaluated the cell cycle related Polo like kinase-1 (Plk1) expression in omentum of morbidly obese non diabetics relative to circulating tumor necrosis factor alpha (TNF-α) and leptin plasma levels. This study included 15 morbidly obese non-diabetics, 12 morbidly obese diabetics, and 12 healthy controls. Omentum was obtained during bariatric surgery and other elective surgeries. Plk1 expression was evaluated by semiquantitative reverse transcriptase polymerase chain reaction and immunohistochemistry. Plasma TNF-α, leptin and fasting insulin were determined by immunoassays.

Plk1 expression and immunoreactivity were significantly higher in obese non diabetics than diabetics and controls and associated with hyperinsulinemia and hyperleptinemia. Plk1 expression negatively correlated with glycemic status and TNF-α levels in obese non diabetics. Our findings provide a possible role for Plk1 in visceral adipose tissue as an adaptive response to obesity and prevention of overt diabetes. This could be achieved by enhancing β-cell mass expansion and lowering TNF-α levels. Better understanding of the mechanistic role of Plk1 in obesity may lead to therapeutic strategies that target both obesity and diabetes.

Keywords: Plk1; FOXM1; VAT; Obesity; TNF-α; T2DM

Abbreviations:

Plk1: Polo like kinase 1

T2DM: Type 2 Diabetes Mellitus

FOXM1: Forkhead Box M1

VAT: Visceral Adipose Tissue

TNF-α: Tumour Necrosis Factor- alpha

RT-PCR: Reverse Transcriptase Polymerase Chain Reaction

NF-κB: Nuclear Factor Kappa- B

HOMA/IR: Homeostatic Model Assessment/ Insulin Resistance

LRb: Leptin Receptor b

Running title: Plk1 mRNA Expression in Omentum of Morbidly Obese Non Diabetic

Females

Introduction

Obesity, especially class III, or morbid obesity, has been recognized as an etiologic or

contributing factor for many health problems (Donahue and Abbott, 1987; Twells et al.,

2012). Large epidemiologic studies have revealed that insulin resistance risk increases from

the very lean to the very obese (Brancati et al., 1996). However, not all types of obesity are

associated with increased risk of metabolic complications (Folsom et al., 1999).

Individuals with peripheral fat distribution in the gluteofemoral regions are less prone to

develop type 2 diabetes mellitus (T2DM) than individuals with abdominal fat distribution

(Folsom et al., 1999). Furthermore, the intra-abdominal fat amount strongly correlates with

insulin resistance and can account for most of the variability in insulin sensitivity found in obese subjects (Gabriely et al., 2002).

Although insulin resistance occurs in most obese individuals, diabetes is generally forestalled through compensation with increased insulin (Folsom et al., 1999; Keller et al., 2008). This increase in insulin occurs through an expansion of β-cell mass and/or increased insulin secretion by β-cells. Failure to compensate for insulin resistance leads to development of T2DM (Frayn, 2005; Raz et al., 2005).

This response is more evident in rodents, in which β-cell replication is important for increasing β-cell mass (Dor et al., 2004; Hull et al., 2005).

However, the mechanistic basis of non diabetic obesity associated with human β-cell proliferation, remains largely elusive (Butler et al., 2003; Hanley et al., 2010).

The adipokine leptin is secreted mainly by adipocytes and acts via its receptors (LRb) to regulate appetite and energy balance (Konukoglu et al., 2006; Myers et al., 2008). Studies have demonstrated that leptin has a direct effect on insulin release through its effect on β-cell function (Date et al., 2002). Obesity has been characterized by hyperleptinemia, in that obese individuals are leptin resistant through a chronic low grade proinflammatory state. β-cell might be adversely affected by this hyperleptinemia and eventually ends in insulin resistance and diabetes (Hukshorn et al., 2004).

It has been recognized that adipose tissue is the site of expression of a large number of peptides related to metabolic regulation, including known cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin-6 that have been associated with obesity (Lumeng et al., 2007). Those inflammatory molecules associate with and account for insulin resistance and other complications of obesity (Greenberg and Obin, 2006).

The mammalian transcription factor Forkhead Box M1 (FOXM1) can regulate the expression of multiple cell cycle genes and is necessary for the maintenance of adult β-cell mass, proliferation, and glucose homeostasis (Davis et al., 2010). Gene profiling microarray studies of abdominal and subcutaneous adipose tissues have identified altered expression of some differentiation and cell cycle related genes in patients with morbid obesity (Rodríguez-Acebes et al., 2010).

Polo like kinase1 (Plk1), a cell cycle related serine/threonine kinase that regulates cellular growth (Gumireddy et al., 2005). Plk1 is under transcriptional control of FOXM1 and has a well characterized role during all phases of the cell cycle particularly M-phase (Dai et al., 2003; Fu et al., 2008). Plk1 phosphorylates and activates FOXM1 controlling mitotic progression and cellular proliferation (Fu et al., 2008).

One way to gain insight into diabetic pathophysiology is to examine the coordinate changes in gene expression which occur in obese subjects and compensate for insulin

resistance. In this study, we investigate the relative mRNA expression of Plk1 in visceral adipose tissue (VAT) of morbidly obese patients with and without T2DM in relation to their plasma TNF-α and leptin levels.

Material and Methods

Subjects

All patients have given their informed consents and the study was institutionally approved by the Research Ethical Committee of Tanta Faculty of Medicine. Morbidly obese patients admitted to the Department of General Surgery at Tanta University Hospital for bariatric surgery and open sleeve gastrectomy were recruited for this study.

The study included 15 morbidly obese non diabetic women, 12 morbidly obese diabetic women, in addition to 12 non obese non diabetic women served as population controls.

The omental fat tissues were obtained during bariatric surgery; VATs from controls were obtained during an elective abdominal surgical procedure (e.g., abdominal ventral hernia repair). All patients were morbidly obese as defined by the 1991 NIH consensus guidelines; body mass index >40 or a body mass index >35, (with at least one obesity related co-morbidity). Diabetic patients with type 2 DM were defined as those patients using oral hypoglycemic agents, and a fasting glucose > 150mg/dl.

All patients and controls were age matched women employing the following exclusion criteria: malignancy, pregnancy, acute cholecystitis and inflammatory bowel disease. During the surgical procedures, immediately after entering the abdominal cavity, samples of omentum were obtained, snap frozen and stored in liquid nitrogen or preserved in buffered paraformaldehyde (PFA) until furtherly processed.

Semiquantitative reverse transcriptase polymerase chain reaction

Total RNA was extracted from VAT using Isogene (Nippon Gene, Toyama, Japan) according to manufacturer's instructions. The integrity of total RNA was checked by electrophoresis through 1% agarose gel. RNA samples were then stored at -80°C.

cDNA synthesis was performed using the RevertAid H Minus First Strand cDNA Synthesis kit (#K1632, Thermo Scientific Fermentas, St. Leon-Ro, Germany) according to manufacturer's instructions.

To evaluate the Plk1 expression in omentum, cDNA synthesis was performed using the RevertAid H Minus First Strand cDNA Synthesis kit (#K1632,Thermo Scientific Fermentas, St. Leon-Ro, Germany) according to the manufacturer's instructions, where 800 ng RNA mixture were reverse transcribed in 20 µL reaction volume containing 4 µL of 5× reverse transcription buffer, 1 µL dNTP mix (0.5 mM final concentration), 100 pmol of Oligo (dT) 18 primer, 0.5 µL of RNase inhibitor and 1 µL RevertAid H Minus Reverse

Transcriptase. To evaluate the Plk1 expression in VAT, the resultant cDNA was amplified to yield 296 bp product using specific set of Plk1 primers (**No: NM_005030.3**) : **5` GAT TCC ACG GCT TTT TCG AG 3´ and 5`CCC ACA CAG GGT CTT CTT CC 3´**. β-actin (**No: NM_001101.3**) 153 bp product was amplified as internal control with the following primer set: **5`CCT CTA TGC CAA CAC AGT 3` and 5`AGC CAC CAA TCC ACA CAG 3'.**

All PCR reactions were carried out using Dream Taq polymerase (#EP0701,Thermo Scientific Fermentas, St. Leon-Ro,Germany) with an optimal number of cycles at 95°C for 3 minutes, 60 °C for 1 minute, and 72°C for 1 minute, followed by incubation at 72°C for 8 minutes in a thermal cycler (Biometra, Goettingen, Germany). PCR products were resolved on a 2% agarose gel along with 100 bp DNA marker (VC 100bp Plus DNA Ladder, Vivantis, Shah Alam, Malaysia) and visualized by ethidium bromide staining with ultraviolet transilluminator (Biometra, Goettingen, Germany).

The band intensities of ethidium bromide fluorescence were measured using Image J 1.4.3.67 image-analysis software package (National Institutes of Health, Bethesda, MD). The relative intensities of the bands were determined, and the ratios to β-actin were calculated. All experiments were conducted in triplicate.

Biochemical and immunoassays

An overnight fasting blood samples were obtained from each subject immediately

before the induction of general anesthesia and centrifuged at 3,000 rpm for 10 min. The extracted plasma was aliquoted and stored at -80°C till the assay time. Fasting Blood Sugar (FBS) was measured by the oxidase method (Biodiagnostic., Egypt), total Lipid profile including total cholesterol (TC), triglycerides (TG) and HDL-cholesterol (HDL-Ch) were measured by enzymatic-colorimetric methods (Biodiagnostic., Egypt). LDL cholesterol (LDL-Ch) concentration was calculated according to Friedewald equation (Friedewald et al., 1972) LDL- cholesterol= TC – (TG/5) – HDL- cholesterol (mg/dl).

Enzyme linked immunosorbent assay (ELISA) was used to detect plasma levels of fasting insulin using commercial kit (USCN Life Science Inc, Wuhan, China) according to manufacturer's instructions and read on microplate reader (Stat Fax®2100, Fisher Bioblock Scientific, France) at 450nm with correction wavelength set at 540nm.

Insulin resistance was assessed by the homeostatic model assessment (HOMA), calculated as: Fasting glycemia (mg/dl) * fasting insulinemia (µIU/mL) /405 (Chen et al., 2012; Demirbaş et al., 2002).

Circulating levels of plasma TNF-α were determined by quantitative sandwich enzyme linked immunosorbent assay technique (ELISA), (DTA00, Quantikine® ELISA, R&D Systems Inc., Minneapolis, MN, USA) according to the manufacturer's instructions, read at 450nm with correction wavelength set at 540nm. The minimum detectable

concentration by this assay was less than 4.8pg/ml with intra-assay and inter-assay CVs range

from (4.6-5.2 5.4-7.4) respectively.

Plasma leptin was assayed with a solid phase sandwich ELISA (DLP00, Quantikine®

ELISA, R&D Systems Inc., Minneapolis, USA), at 450nm with correction wavelength set at

570nm it detects up to 7.8 pg/ml with intra-assay and inter-assay CVs range from $(3.2 - 3.3\%$

and $3.5 - 5.4\%)$ respectively.

Immunohistochemistry

VAT was fixed in PFA (4%), dehydrated, and embedded in paraffin using standard

procedures. 4μm thick slices were prepared and either stained with H&E for histopathological

examination or incubated with a monoclonal antibody against Plk1 (clone 35-206) from

Upstate (Lake Placid, NY, USA). Peroxidase anti-peroxidase was used as secondary reagent

(Dako Cytomation) and 3,3-diaminobenzidine/H2O2 as substrate. The sections were lightly

counterstained with hematoxylin.

The number of Plk1 stained nuclei and the total number of other nuclei in VAT were

counted in 20 visual fields at 20 × magnifications (Leica Imaging System LTD., Cambridge,

England). The visual fields were randomly selected. Plk1 expression was expressed as

number of Plk1 immunoreactive cells related to the total number of other cells in the same

visual fields.

Statistical analysis

Results represented means± SD, multiple comparisons were performed by one-way analysis of variance (ANOVA) followed by Bonferroni correction. Comparison between any two groups was analyzed by the unpaired Student's *t*-test using GraphPad Prism 5.00 software (GraphPad Software, San Diego, USA). Correlations were analyzed using the Pearson test. Statistical significance was considered when the *P*-value was < 0.05.

Results

Demographic and biochemical characteristics of the studied subjects

This study included 39 female subjects aged <50 yrs. The demographic and clinical characteristics of all the studied subjects are depicted in Table 1. There was no significant difference in age between patients and controls. Obese non diabetics and diabetics had a significantly higher BMI (*p*< 0.0001) with respect to controls. Regarding the biochemical findings, FBG levels were significantly higher in obese patients with T2DM versus controls and obese non diabetics (*P*< 0.0001 for both).

Fasting insulin levels, insulin resistance (HOMA-IR), and leptin levels were significantly higher in obese non diabetics and diabetics compared to controls (*p*< 0.0001, *p*< 0.0001 and *p*= 0.0005 respectively).

Table 1: Demographic, clinical characteristics and measured biochemical parameters in all the studied subjects.

	Obese non diabetics (n=15)	Obese diabetics (n=12)	Non obese non diabetic (n=12)	Overall p value	Significance
Age (years)	46.41±2.56[a]	46.46±3.46[a]	47.5±2.31[a]	0.5492	NS
Diabetes Duration (years)	0	5.83± 3.40	0	-	-
(+ve) family history of DM	-	6/12	-	-	-
OHD drugs	-	100%	-	-	-
Systolic B.P.	130.69±5.58[a]	133.5±5.61[a]	122.3±13.26[b]	p= 0.0087	S
Diastolic B.P.	84.27±4.65[a]	83.25±5.49[a]	77.83±5.04[b]	p= 0.0057	S
BMI (kg/m^2)	41.9±6.7[a]	40.2±12.9[a]	24±2.1[b]	p< 0.0001	S
FBG (mg/dl)	102.09±11.50[a]	222.82±57.90[b]	97.48±11.03[a]	p< 0.0001	S
Insulin (uIU/ml)	18.84±3.83[a]	16.98±4.93[a]	5.106±1.63[b]	p< 0.0001	S
HOMA/IR	4.65354[a]	8.74981[b]	1.18958[c]	p< 0.0001	S
TC (mg/dl)	198.73±15.2[a]	203±13.25[a]	183.67±14.92[b]	p= 0.0059	S
TAG (mg/dl)	136.6±17.89[a]	144.67±14.15[a]	101.92±9.9596[b]	p< 0.0001	S
LDL (mg/dl)	123.15±16.34[a,,c]	133.73±11.03[a]	111.95±17.1[b,c]	p= 0.0049	S
HDL (mg/dl)	48.27±3.67[a]	40.33±4.68[b]	51.33±4.74[a]	p< 0.0001	S
Leptin (ng/ml)	23.69±8.95[a]	25.32±4.49[a]	6.95±2.74[b]	p= 0.0005	S
TNF-α (pg/ml)	112.44±26.67[a]	186.1±70.84[b]	30.62±8.04[c]	p< 0.0001	S

Data presented as means ± SD. (S) significant difference. (NS) Non-significant, p was calculated by one way ANOVA test followed by Bonferroni post-hoc test. Identical superscript letters indicate non significant differences while different superscript ones show statistically significant results.

There was no significant difference between obese diabetics and non diabetics regarding circulating leptin levels (Table 1). However, insulin resistance was significantly higher in obese diabetics when compared to obese non diabetic ones ($p< 0.0001$).

Table 1, also illustrates the biochemical abnormalities associated to obesity and insulin resistance (IR), showing low levels of HDL cholesterol in obese diabetics when compared to other groups (*p< 0.0001*). Only obese diabetics exhibited significantly higher LDL cholesterol relative to control ones (*p=0.0049*).

Both obese diabetic and non diabetic patients had significant increase in total cholesterol and triglycerides levels than control ones ($p= 0.0059$ and $p< 0.0001$ respectively). Since obesity is associated with a state of chronic, low-grade inflammation with elevated levels of inflammatory markers and proinflammatory cytokines (Palming et al., 2006).

We went further to detect plasma levels of TNF-α that were significantly increased in obese non diabetics and diabetics when compared to controls ($p< 0.0001$). Interestingly, the obese non diabetic patients exhibited significantly lower plasma levels of TNF-α relative to obese diabetics ($p= 0.0069$).

Plk1 expression in visceral adipose tissue (VAT)

Using a semiquantitative RT-PCR, we performed mRNA expression analysis of Plk1

in VAT samples from obese non diabetics and diabetics, and their allied controls. Plk1 gene expression was upregulated in VAT from obese non diabetics (by about 5 fold) as compared to obese diabetics and controls (using β-actin as internal control) (Fig. 1 A).

The calculated relative Plk1 mRNA expression levels were significantly higher in obese non diabetics when compared to obese diabetics and controls ($p < 0.0001$) (Fig. 1 B). Plk1 expression was noted to be lower in obese diabetics relative to controls although that was not of statistical significance ($p = 0.0803$).

Table 2 shows correlations of plk1 expression to various obesity related risk factors, where Plk1 mRNA expression negatively correlated with FBG levels in obese non diabetics (Fig. 2 A), diabetics and controls, that was statistically significant ($r = -0.9491$, $p < 0.0001$, $r = -0.9426$, $p < 0.0001$ and $r = -0.9414$, $p < 0.0001$ respectively).

Additionally, Plk1 expression showed statistically positive correlation with BMI, in obese non diabetics (Fig. 2 B) and negative one in controls ($r = 0.9052$, $p < 0.0001$, and $r = -0.8981$ and $p < 0.000$, respectively). For obese non diabetics (Fig. 2 C) and the entire cohort of subjects, there were significant positive correlations between levels of Plk1 expression and fasting insulin ($r = 0.9$ and $p < 0.0001$).

In obese non diabetics, Plk1 expression positively correlated with HOMA/IR and

leptin (Fig. 2 D, C) ($r= 0.714$, $p= 0.0028$ and $r= 0.9819$, $p< 0.0001$, respectively). Interestingly Plk1 expression negatively correlated with circulating levels of plasma TNF-α among all subjects particularly obese non diabetics (Fig. 2 E, Table 2) ($r= -0.8767$, $p< 0.0001$).

Although Plk1 expression had many positive and negative correlations with some obesity risk factors, a statistically significant correlation could not be observed as regard to lipid profile in the entire cohort of subjects enrolled in this current study. Histopathological examination of VAT from obese diabetics revealed increased cellular inflammatory infiltrations than observed in obese non diabetics or controls (Fig. 3 A, a-c).

Plk1 proteins were identified by immunohistochemistry where omental adipose tissue showed a strong immunostaining of Plk1 in obese non diabetics than obese diabetics or controls (Fig. 3 B, a-d). Plk1 exclusively localized at the nuclei of VAT, relative Plk1 stained cell number (%) was statistically higher in obese non diabetic VAT when compared to tissues from obese diabetic and control individuals ($p=0.0005$) (Fig. 3 C).

There was no statistically significant difference for Plk1 positive cell number in obese diabetic patients when compared to control ones.
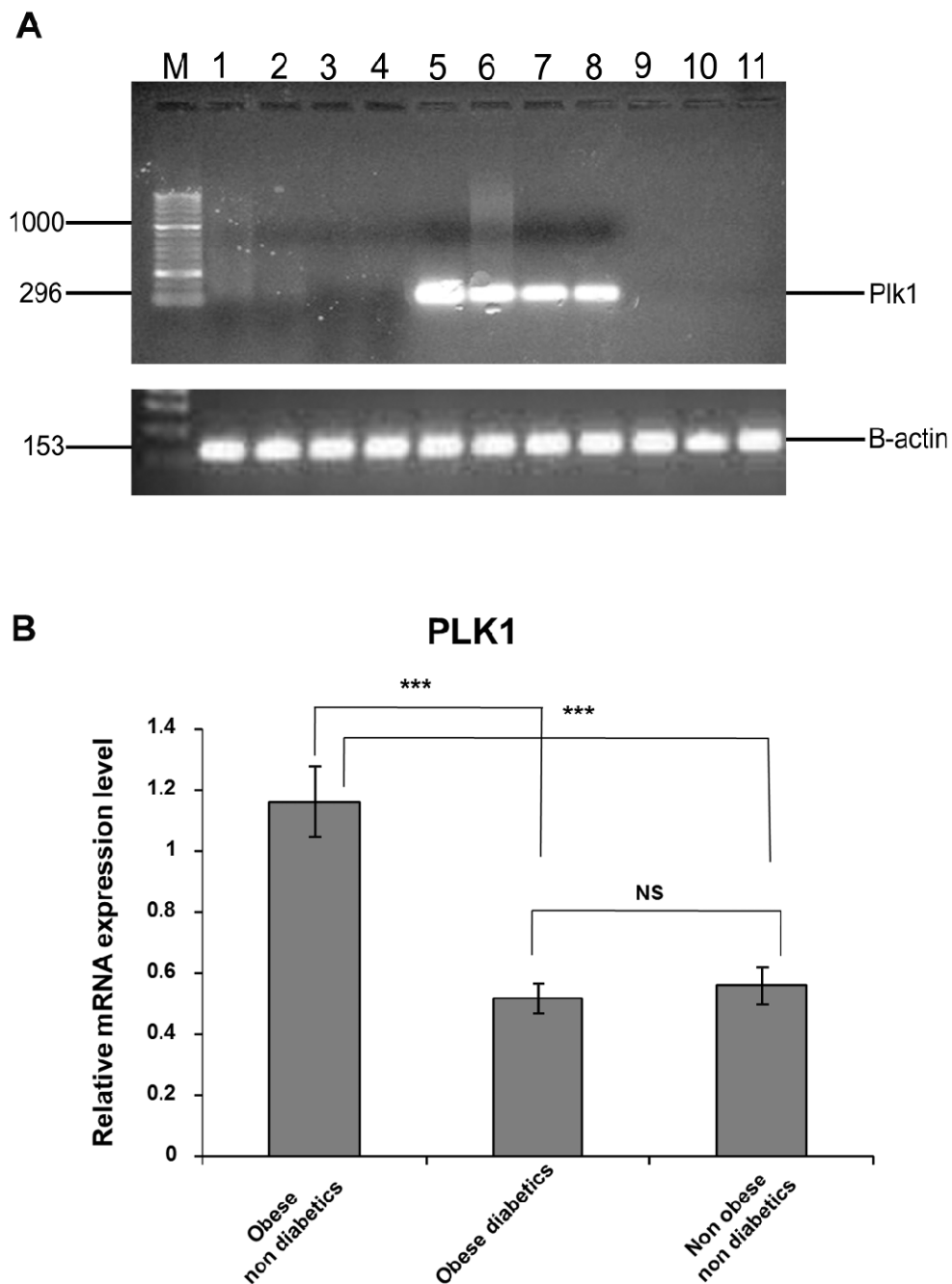
Figure (1) Plk1 expression is upregulated in VAT from morbidly obese non-diabetics.
(A) Semiquantitative RT-PCR of Plk1 was performed using cDNA from equal amounts of total RNA from VAT, lanes 1- 4: non obese, non diabetics, 5- 8 obese non-diabetics, and 9-11 obese diabetics. (Plk1: 296 bp, β-actin: 153 bp). (B) Comparison of relative Plk1 expression (%) (Plk1/β-actin ratio), among the indicated groups. Error bars indicate ± SD. (***) $p<$ 0.0001, NS; no-significance. (n= 12), data representative of three independent experiments.
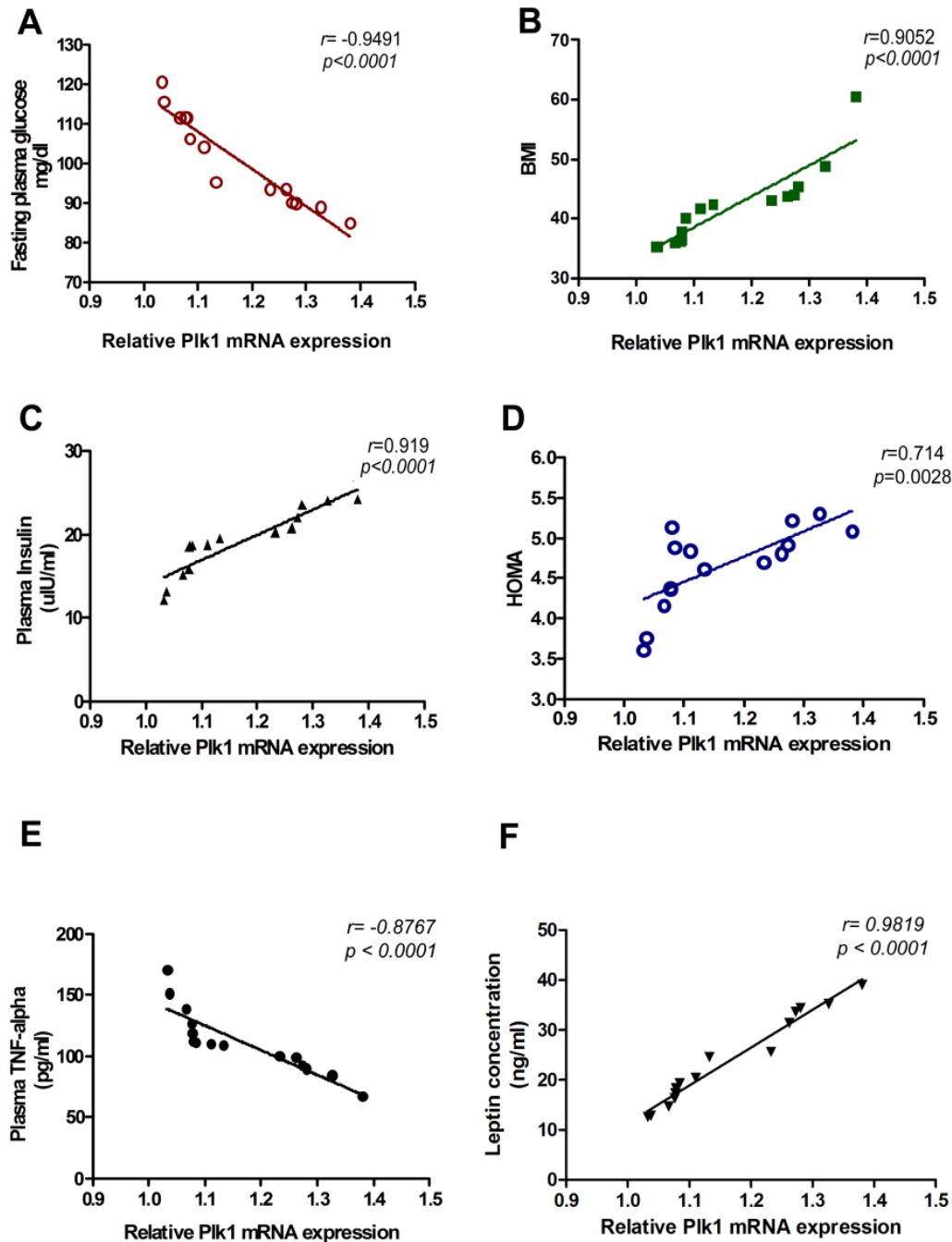
Figure (2) Plk1 is upregulated in morbidly obese non diabetics and negatively regulates TNF-α. Graphs representing Pearson correlations of relative omental Plk1 expression to FBG (A), BMI (B), fasting insulin (C), HOMA/IR (D), TNF-α (E) and leptin (F) in obese non diabetic patients.
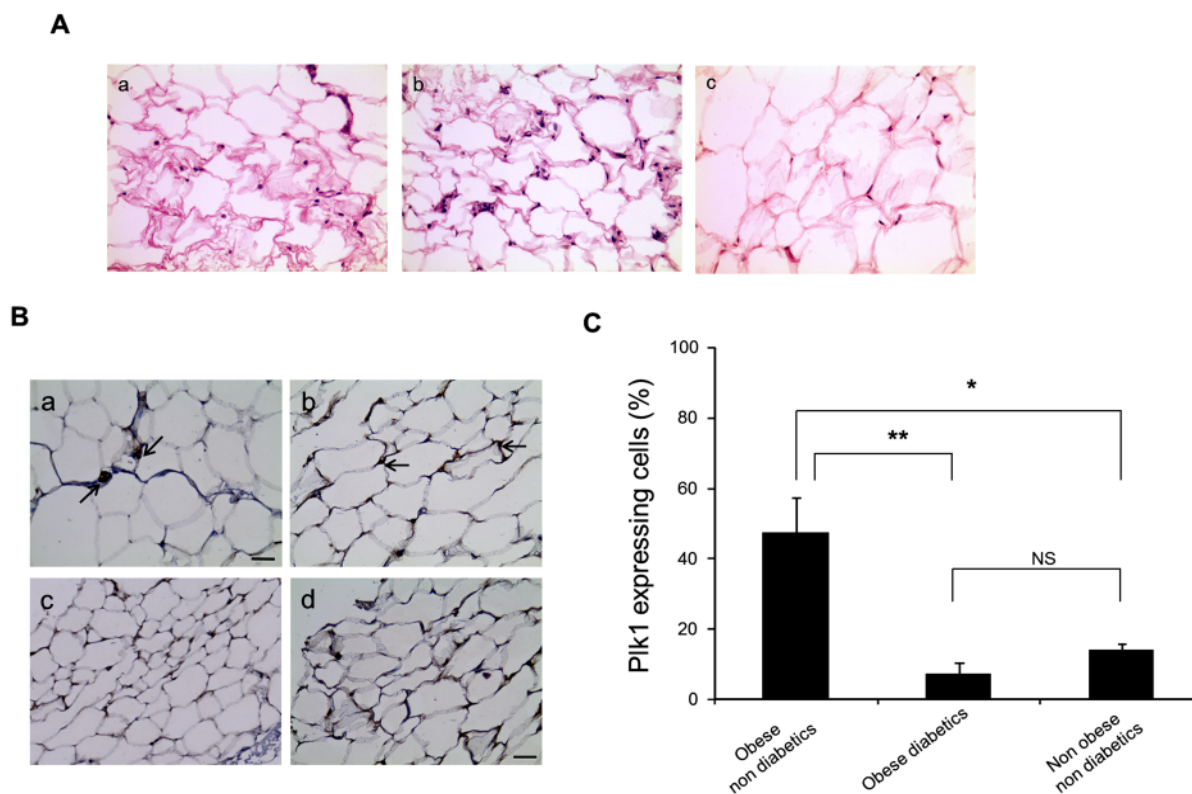
Figure (3) Increased Plk1 immunoreactivity in omentum of morbidly obese non diabetics.

Plk1 immunoreactivity in VAT (A) Photomicrographs showing histopathological H&E stained paraffin sections of VAT from a; obese non-diabetics, b; obese diabetics and c; non obese, non-diabetics; 200x magnification.   (B) Representative paraffin sections of VAT to demonstrate immunoreactivity of Plk1 (dark brown), counterstained by hematoxylin, arrows indicate nuclear Plk1. a,b; Obese non-diabetics, c; obese diabetics and d; non obese non diabetics., scale bar: 100μm. (C) Comparison of Plk1 expressing cells (%) in VAT among the indicated groups. Error bars indicate ± SD. (**); $P= 0.0249$, (*); $P=0.0367$, NS; no-significance. (n= 50) data representative of three independent experiments.

Table 2: Correlation between omental Plk1 transcription and some obesity risk factors

	Obese non diabetics		Obese diabetics		Non obese non diabetics	
	r	*p*	*r*	*p*	*r*	*p*
Fasting blood glucose (mg/dl)	**-0.9491**	**< 0.0001**	**-0.9426**	**< 0.0001**	**-0.9414**	**< 0.0001**
Insulin (uIU/ml)	**0.919**	**< 0.0001**	**0.9706**	**< 0.0001**	**0.9743**	**< 0.0001**
HOMA/IR	**0.714**	**0.0028**	0.209	0.5145	**0.9746**	**< 0.0001**
BMI (kg/m^2)	**0.9052**	**< 0.0001**	-0.00763	0.9812	**-0.8981**	**< 0.0001**
TNF-α (pg/ml)	**-0.8767**	**< 0.0001**	**-0.9845**	**< 0.0001**	**-0.9114**	**< 0.0001**
Leptin (ng/ml)	**0.9819**	**< 0.0001**	0.3333	0.2898	0.2828	0.3731
TC (mg/dl)	-0.1198	0.6708	0.3799	0.2232	0.1607	0.6178
TAG (mg/dl)	-0.3991	0.1406	0.0907	0.7792	0.1691	0.5993
LDL (mg/dl)	-0.07472	0.7913	0.2734	0.3899	0.2064	0.5198
HDL (mg/dl)	0.2257	0.4186	0.3764	0.2279	-0.3096	0.3274

Pearson correlation method was used, $P < 0.05$ was considered significant. Values highlighted

in bold indicate significant results.

Discussion

Insufficient insulin production and β-cell mass are key contributors to type 1 and

type 2 diabetes (Prentki and Nolan, 2006; Wajchenberg, 2007; Weir and Bonner-Weir, 2004).

During the transition from obesity into to diabetes, tissues undergo coordinated changes in

gene expression.

What is the link between obesity and an increase in islet cellular proliferation? And

why not all obese patients become diabetic, is still a matter of debate. Our data clearly show that Plk1 mRNA expression is selectively upregulated in the VAT of obese non diabetics relative to obese diabetic and control subjects.

Since Plk1 plays a role in normal cell cycle progression and proliferation, we find our data consistent with published microarray analysis of adipose tissue from morbidly obese women that reported altered expressions of some cell cycle related genes like cyclin-dependent kinase inhibitor genes (Rodríguez-Acebes et al., 2010). Plk1 has been implicated in DNA damage response.

Previous studies described that GADD 45B (growth arrest and DNA damage-inducible beta), was overexpressed in the adipose tissues of morbidly obese subjects (Rodríguez-Acebes et al., 2010). Prior animal studies estimated increased Plk1 expression in pancreatic tissues from obese non diabetic mice than obese diabetic ones (Davis et al., 2010).

Other pancreatic islet microarray studies on animal models of obesity have found that non diabetic mice islets have a robust, obesity dependent increase in islet cell replication and cell cycle genes, in contrast islets from diabetic prone mice failed to increase proliferation in response to obesity. Thus, the islet cell cycle gene profiling can predict diabetic transformation (Keller et al., 2008).

Our data described a statistically significant but negative correlation between VAT Plk1 expression and FBG in all entire cohorts of study. A similar finding has been reported with FOXM1 the transcriptional regulator of Plk1 in obese non diabetic mice (Davis et al., 2010). It is of interest that Plk1 can regulate its own transcription via phosphorylation of FOXM1 (Fu et al., 2008).

Intriguingly, FOXM1 expression in pancreatic beta cells correlates positively with BMI in obese non diabetic mice (Davis et al., 2010). We similarly detected a statistically positive correlation between VAT Plk1 expression and BMI in obese non diabetics. Our data suggest that obesity-stimulated expression of Plk1 could be a protective tool for β-cell to proliferate and prevent metabolic decompensation.

This could be strengthened by the increased fasting insulin levels we observed in obese non diabetics that positively correlated with Plk1 expression. The pattern of regulation of insulin signalling proteins in β-cells of obese patients remained to be elucidated (Muscelli et al., 2001).

Expansion of β-cell mass is one way that an obese individual may generate an adequate insulin supply to meet the demands imposed by insulin resistance, where we observed that Plk1 expression positively correlated with HOMA/IR in obese non diabetics.

In agreement with this notion, cultured human pancreatic islets from donors

increased their FOXM1 expression and its cell cycle-regulated targets such as Plk1 and Aurora kinase in response to obesity (Davis et al., 2010). Plk1 has been well characterized in the context of cancer (Reagan-Shaw and Ahmad, 2005). However, a role of Plk1 in control of innate immune response has been reported (Vitour et al., 2009).

In the current work both obese individuals have significantly higher levels of TNF-α relative to controls, a finding that has been previously reported where several proinflammatory factors such as TNF-α, IL-6 and inducible nitric oxide synthase are highly expressed in adipose tissue with their known ability to promote insulin resistance and impaired glucose metabolism (Bergmann and Sypniewska, 2013; Greenberg and Obin, 2006; Hotamisligil et al., 1993).

Adipose tissue TNF-α concentrations correlated with obesity and insulin resistance in patients with and without T2DM (Hotamisligil et al., 1995). In obese women, adipose tissue TNF-α expression correlated with FBG, insulin, and TAG concentrations (Hotamisligil et al., 1993) promoting insulin resistance in peripheral tissues (Greenberg and Obin, 2006).

Interestingly, in our data TNF-α levels in obese non diabetic women are significantly lower than in obese diabetic ones. This finding is consistent with the previous reports which stated that TNF-α is coupled with diabetes rather than obesity and insulin resistance and is increased before the onset of diabetes and further increase was not associated with insulin

resistance (Miyazaki et al., 2003; Mol et al., 1997).

In contrast, Demirbas et al (Demirbaş et al., 2002) showed that in patients with hypertension serum TNF-α concentration increased together with increased insulin, and HOMA IR.

In our obese non diabetic patients Plk1 upregulation strongly associated with decreased TNF-α levels. One possible explanation is that Plk1 expression may suppress TNF-α expression. This is consistent with previous report describing that Plk1 inhibited NF-κB transcriptional activation induced by TNF-α (Zhang et al., 2010). NF-κB-inducing kinase (NIK) promotes hyperglycemia and glucose intolerance in obesity by augmenting glucagon action (Sheng et al., 2012).

So we could speculate that morbidly obese non diabetics are protected from development of overt diabetes through increased Plk1 expression that in turn down regulate TNF-α/NF-κB pathway which has been well characterized in development of inflammation and obesity-induced insulin resistance (Arkan et al., 2005).

Serum leptin levels of obese diabetic and non-diabetic subjects have been reported to be higher as compared with lean diabetic and non-diabetic ones ($p < 0.005$) (Haque and Rahman, 2003). This is in line with our current findings, where leptin levels significantly increased in obese diabetics and non diabetics relative to control. However, only in obese non

diabetics Plk1 expression positively correlated with leptin levels. This correlation can be explained by similar subcellular distribution of leptin and Plk1 in VAT (Bornstein et al., 2000) where Plk1 via its polo binding domain interacts and enhances the Rho-associated protein kinase 2 (ROCK 2) activity (Lowery et al., 2007) which is a downstream mediator in leptin and leptin receptor signalling pathway (Schram et al., 2011).

Conclusively, our attained data unravel a novel role for Plk1 in visceral adipose tissue of morbidly obese diabetic patients in delaying impairment of glucose metabolism and T2DM. This is could be mediated partly by increasing pancreatic β-cell proliferation and hyperinsulinaemia to compensate for increased metabolic demands and partly be interfering with the expression of TNF-α and impairing NF-κB signalling pathway (s).

It might be concluded that obesity is the main driving force for Plk1 expression and the transcriptome of the VAT may govern the β-cell behavior to proliferate or to develop DM, however, when and why morbidly obese patients may develop diabetes remains to be furtherly investigated.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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