*Obesity Surgery, **17**, 934-940*

Adipose Tissue Distribution and Quantification of PPARβ/δ and PPARγ1-3 mRNAs: Discordant Gene Expression in Subcutaneous, Retroperitoneal and Visceral Adipose Tissue of Morbidly Obese Patients

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Background: Adipose tissue (AT) metabolism is altered in obese subjects, and the reestablishment of energy homeostasis requires the identification and regulation of genes with altered patterns. The aim of this study was to compare mRNA expression of PPARβ/δ and PPARγ1-3 in morbidly obese and non-obese patients. The expression pattern of these receptors in various abdominal adipose tissues, subcutaneous (SAT), retroperitoneal (RAT) and visceral (VAT), was also evaluated.

Methods: The AT depots were obtained by surgery. Total RNAs were extracted using TRIzol. PPARs reverse transcripts were determined by quantitative polymerase chain reaction (qRT-PCR).

Results: The amounts of PPARβ/δ mRNA in different depots of morbidly obese AT showed a significant decrease in VAT (*P*<0.05). In the non-obese group, the level of PPARβ/δ was higher in SAT (*P*<0.05), but PPARγ1-3 was not differentially expressed in obese and non-obese depots. When comparing obese and non-obese, the results revealed a decrease in PPARβ/δ expression in SAT (*P*=0.058) and VAT (*P*=0.094) of the morbidly obese. PPARγ1-3 mRNA expression was increased significantly in SAT (*P*=0.022) and decreased in RAT (*P*=0.034) in morbidly obese subjects. PPARβ/δ expression in SAT and VAT correlated negatively with hip size and insulin serum respectively. PPARγ1-3 expression in RAT correlated negatively with waist and hip circumference and in VAT correlated positively with waist size.

Conclusions: The present study demonstrates that PPARβ/δ and PPARγ1-3 mRNAs are quantitatively different in AT of morbidly obese individuals compared to non-obese, and that PPARβ/δ mRNA levels are characteristic for each AT depot.

Key words: Adipose tissue depots, PPARβ/δ, PPARγ1-3, morbid obesity, non-obese

Abbreviations
PPAR = peroxisome proliferator-activated receptors
AT = adipose tissue
SAT = subcutaneous adipose tissue
RAT = retroperitoneal adipose tissue
VAT = visceral adipose tissue
mRNA = messenger RNA
qRT-PCR = quantitative real-time polymerase chain reaction
TZD = thiazolidinedione

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Introduction

Obesity is defined as a state of pathologically excessive adipose tissue (AT) mass, conferring a higher risk of cardiovascular and metabolic disorders.[1] Morbid obesity is an important public health problem,[2-4] and is associated with serious co-morbid effects, including hypertension, diabetes, peripheral insulin resistance and dyslipidemia.[2]

There is growing evidence that the distribution of body fat influences the metabolic consequences of obesity. Abdominal AT depots – intra-abdominal AT

(visceral or intraperitoneal and retroperitoneal) and SAT – are metabolically active, and appear to be important for the pathogenesis of insulin resistance, dyslipidemia, glucose intolerance, hypertension, hypercoagulable state and cardiovascular risk.[5] These depots are metabolically different and have profound differences in their gene expression profile.[6]

The *peroxisome proliferator-activated receptors* (PPARs) are members of the nuclear receptor superfamily,[7] and there are three related PPAR isoforms: PPARα, PPARγ, and PPARβ/δ, with different ligand specificities and tissue distribution.[8] PPARα is mainly expressed in liver, brown AT, small intestine, skeletal muscle and heart. PPARγ is expressed in white and brown AT, placenta, large intestine and macrophages. PPARβ/δ is the most widely expressed gene but has higher levels in skeletal and cardiac muscle and white AT.[9]

The PPARγ gene contains three promoters that yield three isoforms, namely PPARγ1, PPARγ2 and PPARγ3. PPARγ1 and PPARγ3 transcripts translate into the identical PPARγ1 protein,[10] while PPARγ2 protein contains an additional N-terminal 28 amino acid exon.[11] PPARγ1 is found in a broad range of tissues, whereas PPARγ2 is restricted to AT. PPARγ3 is abundant in macrophages, large intestine and white AT.[10] PPARγ regulates the formation and function of fat cells. The effect of PPARγ activation is seen in all aspects of the mature cell phenotype, including morphological changes, lipid accumulation and acquisition of insulin sensitivity.[12] Their ligands include dietary fatty acids, prostaglandins of the J series, including 15d-PGJ_2,[13] and antidiabetic compounds of the thiazolidinedione (TZD) class.[9]

PPARβ/δ initially received much less attention than the other PPARs. However, genetic studies and the recent development of synthetic PPARβ/δ agonist have helped to reveal its role as a powerful regulator of fatty acid catabolism and energy homeostasis. The PPARβ/δ agonist GW501516 was shown to lower plasma triglyceride levels in obese monkeys while raising high-density lipoprotein levels (HDL), prompting the initiation of clinical trials to assess its efficacy in hyperlipidemic patients.[14] Transgenic expression of an activated form of PPARβ/δ in AT produces lean mice that are resistant to obesity, hyperlipidemia and tissue steatosis, induced genetically or by a high fat diet.[15] The activated receptor induces genes required for fatty acid catabolism and adaptive thermogenesis.[14]

Morbid obesity is associated with disturbances in lipid and glucose metabolism. We investigated whether the expression of PPARβ/δ and PPARγ1-3 was modulated in morbidly obesity. The aim of this work was thus to evaluate the mRNA levels of these receptors in morbidly obese patients and compare them with normal-weight subjects. Because AT depots have metabolic variables and play a yet unknown role in obesity, we compared PPARβ/δ and PPARγ1-3 in different abdominal AT: subcutaneous, retroperitoneal and visceral.

Materials and Methods

Samples

Samples of VAT (omentum), RAT and SAT were obtained from 10 morbidly obese patients who underwent bariatric surgery. The extensive clinical and laboratory data routinely collected for each patient is shown in Table 1. Apart from obesity, all subjects were in good health and were not taking any medication affecting adipocyte metabolism. Similar samples of VAT, RAT and SAT were obtained from 10 non-obese patients who underwent elective surgery; their age range was from 23 to 52 years, with body mass index (BMI) 17.9 to 29.0 kg/m^2. Samples were collected during surgery and were immediately immersed in TRIzol reagent.

Table 1. Anthropometric and biological parameters of morbidly obese patients (n=10)

	Mean (±SE)	Range
Age (years)	33.9 (±1.6)	(24-39)
BMI (kg/m^2)	50.3 (±2.3)	(40.7-63.6)
Waist (cm)	132.4 (±4.6)	(108-149)
Hip (cm)	144.7 (±4.4)	(125-170)
Glycemia (mg/dL)	104.2 (±7.6)	(77-153)
Total cholesterol (mg/dL)	199.3 (±12.0)	(141-273)
HDL cholesterol (mg/dL)	50.0 (±5.0)	(22-82)
LDL cholesterol (mg/dL)	115.2 (±6.8)	(70-151)
Triglycerides (mg/dL)	195.2 (±20.6)	(104-289)
Insulin (μU/mL)	26.2 (±3.2)	(14.0-43.4)
ALT (U/L)	32.9 (±2.7)	(21-43)
AST (U/L)	27.4 (±6.2)	(13-79)

The study was approved by the Ethics Committee of the University Federal of Rio Grande do Sul. All subjects gave written informed consent.

Analysis of Human PPARs Gene Expression

Approximately 2 µg of total RNA were added to each cDNA synthesis reaction using the SuperScript-II RT preamplication system (Invitrogen). Reactions were performed at 42˚C for 1 h using the primer T23V (5' TTT TTT TTT TTT TTT TTT TTT TTV). Quantitative polymerase chain reaction (qRT-PCR) amplification was carried out using specific primer pairs designed with Oligo Calculator version 3.02 (http://www.basic.nwu.edu/biotools/oligocalc.html) and synthesized by RW-Genes (RJ, Brazil). The sequences of the primers used are listed in Table 2. qRT-PCRs were carried out in an Applied-Biosystem 7500 real-time cycler. Reaction settings were composed of an initial denaturation step of 5 min at 94˚C, followed by 40 cycles of 10 s at 94˚C, 15 s at 60˚C, 15 s at 72˚C and 35 s at 60˚C; samples were held for 2 min at 40˚C for annealing and then heated from 55 to 99˚C with a ramp of 0.1˚C/s to acquire data to produce the denaturing curve of the amplified products. qRT-PCRs were carried out in 20 µl final volume composed of 10 µl of each reverse transcription sample diluted 50 to 100 times, 2 µl of 10 times PCR buffer, 1.2 µl of 50 mM MgCl2, 0.1 µl of 5 mM dNTPs, 0.4 µl of 10 µM primer pairs, 4.25 µl of water, 2.0 µl of SYBR green (1:10.000 Molecular Probe), and 0.05 µl of Platinum Taq DNA polymerase (5 U/µl) (Invitrogen).

Data Analysis

We quantified gene expression using the $2^{-\Delta\Delta Ct}$ (threshold cycle) method.[16] For each sample, analyzed in triplicate, a ΔC_T value was obtained by subtracting the β-actin C_T value from the C_T of the gene of interest. Relative PPARβ/δ and PPARγ1-3 mRNA intra-tissue expression levels were normalized to PPARβ/δ and PPARγ1-3 mean expression of VAT. To compare morbidly obese and non-obese expression, PPARβ/δ and PPARγ1-3 levels were normalized to the average expression values of non-obese AT for each tissue depot.

Statistical Analysis

Data is shown as mean ± SE. Mean values for PPARs expression in SAT, RAT and VAT in the obese and non-obese group were compared by the non-parametric Friedman test. PPARs expression in obese and non-obese patients in the same AT depot was assessed using the Mann-Whitney U and correlation coefficients determined using the Spearman statistical package. Differences between groups were considered statistically significant at $P \leq 0.05$.

Results

The expression of PPARβ/δ and PPARγ1-3 mRNA in SAT, RAT and VAT of morbidly obese and non-obese subjects was assessed by qRT-PCR. Table 3 reflects the expression profile of three distinct AT depots (SAT, RAT and VAT) in morbidly obese and non-obese tissues. The morbidly obese group showed significant differences in the pattern of PPARβ/δ mRNA expression in AT depots. We observed a decreased expression of this receptor in the VAT of obese subjects ($P = 0.025$). The PPARγ1-3 mRNA distribution in various AT depots in this group showed no differences in mRNA expression.

The non-obese group presented a different expression profile of PPARβ/δ mRNA in the AT depots analyzed. In this group, SAT revealed a more pronounced expression ($P=0.021$) of the receptor than RAT and VAT. However, the PPARγ1-3 mRNA

Table 2. Oligonucleotides used in qRT-PCR reactions, 5' to 3'

Gene	Forward primer	Reverse primer
PPARβ/δ	AATGCCTACCTGAAAAACTTCAAC	GTGCACGCTGATTCCTTGT
PPARγ1-3	AGGCCATTTTCTCAAAC	AGAAATGCTGGAGAAGTCAACA
β-actin	CCACGAAACTACCTTCAACTCC	TCATACTCCTGCTGCTGCTTGCTGATCC

Table 3. Expression of PPARβ/δ and PPARγ1-3 mRNA in various abdominal AT in obese and non-obese subjects

Adipose tissue	SAT	RAT	VAT	*P*
PPARβ/δ (Mean±SE)				
Obese	5.21±1.58	5.24±1.80	1.85±0.80	0.025
Non-obese	7.60±2.97	1.58±0.66	0.72±0.18	0.021
PPARγ1-3 (Mean±SE)				
Obese	6.48±2,93	4.07±0.97	3.36±1.25	NS
Non-obese	2.58±1.23	4.90±1.76	2.02±0.66	NS

To compare the expression pattern of PPARβ/δ and PPARγ1-3 mRNA in different abdominal AT: subcutaneous (SAT), retroperitoneal (RAT) and visceral (VAT) adipose tissues of morbidly obese or non-obese subjects, relative expressions were normalized against β-actin (ΔCt) and the mean of visceral adipose tissue ($2^{-\Delta\Delta Ct}$). NS = Not Significant.

expression profile in non-obese subjects did not differ in three AT depots.

Table 4 represents the mRNA expression of PPARβ/δ and PPARγ1-3 in different depots of AT in morbidly obese compared to non-obese patients. There was a PPARβ/δ decrease in SAT of morbidly obese subjects (*P*=0.058) and a tendency for decreased expression of this receptor in the VAT of the obese group (*P*=0.094). However, no significant differential expression of this nuclear receptor was observed when RAT from morbidly obese was compared to non-obese subjects.

We also compared PPARγ1-3 in the same depots of AT from morbidly obese and non-obese patients. PPARγ1-3 mRNA expression was significantly increased (*P*=0.022) in SAT of morbidly obese compared to non-obese subjects. In contrast, RAT presented a significant decrease in PPARγ1-3 mRNA levels in the morbidly obese (*P*=0.034). The expression was not different in VAT from both groups of patients.

Table 4: PPARβ/δ and PPARγ1-3 expression in morbidly obese compared to non-obese subjects in different depots of AT

	Obese	Non-obese	*P*
PPARβ/δ	**(Mean±SE)**	**(Mean±SE)**	
SAT	0.61±0.20	3.60±2.03	0.058
RAT	4.37±1.43	3.58±1.62	NS
VAT	1.27±0.50	8.68±4.18	0.094
PPARγ1-3	**(Mean±SE)**	**(Mean±SE)**	
SAT	5.60±2.45	1.19±0.27	0.022
RAT	0.34±0.08	2.59±1.03	0.034
VAT	1.31±0.51	1.62±0.57	NS

To obtain the relative quantity of PPARβ/δ and PPARγ1-3 mRNA in different abdominal AT: subcutaneous (SAT), retroperitoneal (RAT) and visceral (VAT) of morbidly obese compared to non-obese, relative expressions were normalized against β-actin (ΔCt) and the mean of each respective non-obese adipose tissue ($2^{-\Delta\Delta Ct}$). NS = Not Significant.

In obese AT depots with anthropometric and biochemical variables, there was a significant negative correlation between PPARβ/δ SAT expression with hip circumference (r=-0.886, *P*=0.019), and a tendency for negative correlation with waist size (r =-0.771, *P*=0.072). There was also a significant negative correlation between PPARβ/δ VAT expression with insulin serum level (r =-0.900, *P*=0.037). No correlation was found with PPARβ/δ RAT expression. PPARγ1-3 expression correlated with anthropometric variables. A negative correlation was found between PPARγ1-3 RAT expression and waist (r = -1.000, *P*<0.0001) and hip size (r = -0.900, *P*=0.037). However, a positive correlation was found between PPARγ1-3 VAT expression and waist size (r =0.900, *P*= 0.037).

Discussion

Obesity is a heterogeneous disorder and a knowledge of the regional distribution of AT is important to understand the relationship between obesity and disturbances in glucose and lipid metabolism.[17] PPARs transcriptional factors are nuclear receptors and the major gene regulators of lipid and glucose metabolism, allowing adaptation to the prevailing

nutritional environment.[18] Information on site-related gene expression of PPARs in different human AT depots is limited.[19]

PPARβ/δ enhances fatty acid catabolism and energy uncoupling in AT and muscle and may potentially be used to control weight gain.[20] Surprisingly, no studies have examined PPARβ/δ mRNA expression in different depots of human AT. Our results showed that morbidly obese subjects had difference in PPARβ/δ mRNA expression pattern when analyzed in three different depots. VAT of individuals from this group showed a minor expression of PPARβ/δ, and there was no difference between SAT and RAT. These could be contributing to the visceral obesity typical of the morbidly obese. Non-obese patients had a higher expression of PPARβ/δ mRNA in SAT. The SAT of normal weight subjects may have a more pronounced up-regulation of genes involved in fatty acid oxidation and energy dissipation, but further investigations are needed to clarify this.

PPARγ is an adipocyte master transcription factor that, when activated by natural or synthetic agonists,[13] triggers expression of terminal differentiation-related genes and adipogenesis.[21] Unlike PPARβ/δ expression, PPARγ1-3 receptor transcripts did not differ significantly between the three AT of morbidly obese, or of non-obese, patients. These results agree with a previous study of total PPARγ expression in SAT and VAT of non-diabetic subjects. Normolipidemic morbidly obese men revealed a similar intensity of this receptor.[22] Lefebvre et al[23] and Montague et al[24] also found no difference between total PPARγ expression in omental and SAT.

A different scenario appeared when the two groups of individuals, normal weight and morbidly obese, were compared for amounts of PPARγ1-3 and PPARβ/δ mRNA isoforms in the same depot-specific AT. For the first time, PPARs expression in RAT has been analyzed and no significant difference in PPARβ/δ expression found between obese and non-obese subjects. However, PPARγ1-3 expression was down-regulated in obese subjects in this tissue. In corroboration, correlation analysis revealed a negative coefficient between PPARγ1-3 RAT and waist and hip circumference. Studies in men with a wide range of adiposity showed that intra-abdominal AT mass was changed in obesity. Lean subjects had 58% visceral mass and 42% retroperitoneal mass, while these tissues in obese subjects were 69% and 31%, respectively.[25] This could be explained by down-regulation of PPARγ1-3, adipogenic transcriptional factor, and the lack of difference in PPARβ/δ in RAT found in our obese study group.

Our results revealed reduced PPARβ/δ expression in SAT and a tendency for decreased expression in VAT of morbidly obese patients compared to non-obese. Activation of PPARβ/δ in AT specifically induces expression of genes required for FA oxidation and energy dissipation, which reduces adiposity.[15] Down-regulation of PPARβ/δ SAT could be implicated in obesity, because it was correlated negatively with hip and waist circumference in morbidly obese patients. PPARβ/δ expression in VAT could be associated with visceral obesity and metabolic syndrome, because it was correlated negatively with serum insulin levels.

PPARγ1-3 expression was increased in obese SAT, but no difference was found in VAT for either group. Previous studies compared nuclear receptor expression in obese and non-obese humans, and results are still controversial.[21,22,26] These studies used semi-quantitative RT-PCR or Northern blots to measure mRNAs, and there was a large individual variation in PPARγ expression. In the present study, PPARβ/δ and PPARγ1-3 mRNA levels were quantified using a highly sensitive quantitative Polymerase Chain Reaction (qRT-PCR) assay.

The increase of PPARγ1-3 mRNA expression found in SAT of the morbidly obese group is in agreement with the paradox of TZD.[27] TZD are agonists of PPARγ, commonly used in the treatment of diabetes mellitus, improving insulin sensitivity while simultaneously causing weight gain. This effect is markedly enhanced in subcutaneous fat, with less effect in visceral fat.[28,29] However, there is relationship between this receptor expression in VAT and anthropometric data, waist circumference. It is important to remember that we used PPARγ1-3 to understand its relationship with obesity. In view of this, we examined the assembly of PPARγ1 and PPARγ3 expressions (PPARγ1-3) that differ in their 5' non-translated region but encode the same protein sequence.[10]

Our correlation data suggest an effective participation of SAT, VAT and PPARβ/δ expression in obesity. At this time, GW501516, a selective activator of PPARβ/δ, has been proposed as a pharmacological target for the treatment of dyslipidemia, obesity, insulin

resistance and vascular inflammation, and is now in clinical development.[30] Connecting the possible function of this agonist with the low level of PPARβ/δ mRNA found in our research in the obese group, it is possible to suggest that this pharmacological target could be effective in the treatment of obesity.

Obesity could be a result of imbalance of many genes related to metabolism. Microarray studies have shown a deregulated expression of genes in human obesity.[31-33] A study in morbidly obese subjects revealed that 85.5% of genes were up-regulated and 14.5% down-regulated.[31] Our data suggest a probable imbalance in PPARβ/δ and PPARγ1-3 expression regulating adipocyte development: down-regulated genes involved in fatty acid oxidation and energy dissipation and increased genes related to adipogenesis.

In conclusion, the present study demonstrates that PPARβ/δ and PPARγ1-3 are differentially expressed in the AT of morbidly obese individuals compared to non-obese. These patterns of gene expression will contribute to the understanding of the pathogenesis of obesity-related disorders and provide potential targets for future therapy. A knowledge of the distribution of the nuclear receptors in different AT depots may be important to implement therapeutic researches in a specific manner.

The authors thank Doctors José F. Bortolotto and Pedro Furian for non-obese AT for the analyses. We thank Nurses in the hospitals cited above and Dr. Maria Luisa Pereira for lending Real-time PCR apparatus. This work was supported by research grants from Conselho Nacional de Pesquisa (CNPq) and Fundação de Apoio a Pesquisa do Rio Grande do Sul (FAPERGS). Dr. R. Margis is the recipient of a research fellowship from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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(Received January 9, 2007; accepted March 21, 2007)