Subcutaneous Fat Shows Higher Thyroid Hormone Receptor-α1 Gene Expression Than Omental Fat

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The aims of this work were to evaluate thyroid hormone receptor-α (TRα), TRα1, and TRα2 mRNA gene expression and TRα1:TRα2 ratio, identified as candidate factors for explaining regional differences between human adipose tissue depots. TRα, TRα1, and TRα2 mRNA levels, and the gene expressions of arginine–serine-rich, splicing factor 2 (SF2), heterogeneous nuclear ribonucleoprotein H1 (hnRNP H1), heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1), and Spot 14 (S14) were evaluated in 76 paired adipose tissue samples obtained from a population of 38 women who varied widely in terms of obesity and body fat distribution. Gene expression for these factors was also studied in stromal-vascular cells (SVCs) and mature adipocytes (MAs) from eight paired fat depots. *TRα* gene and TRα1 mRNA expression were increased 1.46-fold ($P = 0.006$) and 1.80-fold ($P < 0.0001$), respectively, in subcutaneous (SC) vs. visceral fat. These differences in gene expression levels were most significant in the obese group, in which the TRα1:TRα2 ratio was 2.24-fold ($P < 0.0001$) higher in SC vs. visceral fat. *S14* gene expression was also increased by 2.42-fold ($P < 0.0001$) and correlated significantly with *TRα* and *TRα1* gene expression and with the TRα1:TRα2 ratio. In agreement with these findings, hnRNP A1:SF2 ratio was decreased by 1.39-fold ($P = 0.001$). TRα and S14 levels were 2.1-fold ($P < 0.0001$) and 112.4-fold ($P < 0.0001$), respectively, higher in MAs than in SVCs from both fat depots. In summary, genes for TR-α, their upstream regulators, and downstream effectors were differentially expressed in SC vs. omental (OM) adipose tissue. Our findings suggest that TRα1 could contribute to SC adipose tissue expandability in obese subjects.

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INTRODUCTION

Thyroid hormone plays a central role in normal development, differentiation, and metabolic homeostasis. It is well known that thyroid hormone stimulates basal metabolic rate and adaptive thermogenesis. In fact, the relationship between body fat, thyroidal state, and metabolism became firmly established with the measurement of basal metabolic rates.

Some insights into the thyroidal regulation of fat tissue have been obtained from animal studies. Both stimulations and inhibitions of fatty acid synthesis or lipogenic enzymes by thyroid hormones have been described (1–3). Although some studies reported a stimulation of lipogenesis by hypothyroidism, others showed an inhibition under the same circumstances (3,4). Careful review of these animal studies reveals no consistent differences in rat strain, sex, treatment, or adipose site to explain these discrepant results.

However, it seems clear that thyroid hormones are linked to lipogenesis in lipogenic tissues such as the liver (5) and adipose tissue (2,3) through activated thyroid hormone receptor (TR)–induced gene expression. The two major TR isoforms, TRα and TRβ, are differentially expressed during development and are differentially distributed in adult tissues (6). TRβ plays an essential role in inner ear, cerebellar, and retinal development, thyrotropic hormone regulation, and mediating the metabolic actions of T_3 in the liver (7). TRα has specific roles in the brain and heart and mediating adaptive thermogenesis in brown adipose tissue (8–10). TRβ1 and, specially, TRα1 are the most prevalent isoforms of TRs (11).

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In mammals, transcripts from the *c-erbA-α* gene (*NR1A1*; Nuclear Receptors Committee, 1999) code for two major TR isoforms, TRα1 (*NR1A1a*) and TRα2 (*NR1A1b*), which are functionally antagonistic (for a review, see ref. 12). TRα1 is an authentic TR that binds thyroid hormone (T_3) and mediates its activity in many cells and tissues. Interestingly, impaired adipogenesis caused by a mutated TRα1 has been described (3). TRα2 (also known to be expressed in human adipose tissue) is a variant receptor that lacks a functional hormone-binding site and antagonizes the ability of TRα1 to activate gene transcription in the presence of T_3 (ref. 13). Although both isoforms are expressed in most mammalian cells, the levels of each vary widely in different tissues and at different stages of development. Thus, the regulation of TRα alternative processing is important for determining cellular levels of TRα1 and TRα2 mRNAs, which, in turn, are critical for modulating the response to T_3.

The balance of the TRα1:TRα2 ratio may be responsible for variations in thyroid hormone sensitivity between different tissues. The TRα1:TRα2 ratio is tissue-specific and depends on cell differentiation status (11,14). The TRα1:TRα2 ratio also depends on certain components of the general splicing mechanism, such as the family of serine–arginine proteins and heterogeneous nuclear ribonucleoprotein (hnRNP) related proteins (15,16) and the balance between them. These proteins bind the RNA on specific sequences and thereby regulate the splicing direction and the expression pattern of proteins encoded by a single gene in a dose-dependent manner (15). Among these splicing factors, hnRNP H1 seems to enhance the arginine–serine-rich, splicing factor 2 (SF2) splicing effect (17). While hnRNP A1 mediates silencing, hnRNP H1 antagonizes this silencing and hnRNP A1 competes with SF2 in the binding to some G-rich pre-mRNA tracts (18). This phenomenon has been previously described with β-tropomyosin exon 6B alternative splicing (19) and in the c-src exon N1 (ref. 20). The presence of an splicing enhancer element (SEα2), a purine-rich sequence with several G-rich motifs in the final intronic sequences of *c-erbA-α* gene, supports the idea that these splicing factors could be involved in the tissue TRα1:TRα2 ratio (16). SEα2 would stimulate TRα2 production interacting with serine–arginine and hnRNP proteins.

It is commonly assumed that omental (OM) and subcutaneous (SC) adipocytes are different at lipogenic and lipolytic activities. Although findings on this subject are not at all unanimous, these differences include differential GLUT4, RBP4, peroxisome proliferator–activated receptor-γ, and insulin receptor gene expression (21), lipoprotein lipase activity, lipolytic rate under maximal stimulation by β-adrenergic agonist, and adipocyte size (22,23), among others (24). To our knowledge, none of these studies have linked these documented differences in both human fat depots to thyroid hormone-induced effects through TRα1:TRα2 ratio. We thus aimed to examine the expression of TRα1 and TRα2 mRNA in human adipose tissue samples representing the two largest fat mass depots, OM and SC adipose tissue, and to test whether this expression is different in obese and nonobese subjects. We found that the TRα expression was significantly higher in human SC than in OM adipose tissue and that the TRα1:TRα2 ratio was also higher in SC than in OM adipose tissue. To further substantiate our results, we analyzed Spot 14 (S14) gene expression. The most striking aspect of S14 gene expression is its T_3 dependence, very useful in the study of thyroid hormone action in lipogenic tissues, at least in the liver (25). Finally, we also evaluated SF2, hnRNP H1 and hnRNP A1 gene expression levels, and hnRNP A1:SF2 ratio as a possible determinant factor for alternative *c-erbA-α* gene 5′-splice-site selection *in vivo* as well as *in vitro* (26).

METHODS AND PROCEDURES

Subjects

A group of 76 samples, 38 OM, and 38 SC adipose tissue fragments, from 38 women, with a BMI between 20 and 68 kg/m^2, who were recruited at the Endocrinology Service of the Hospital Universitari Dr Josep Trueta (Girona, Spain), were analyzed. All subjects were of white origin, euthyroid, and reported that their body weight had been stable for at least 3 months before the study. Liver and renal diseases were specifically excluded by biochemical work-up. All subjects gave written informed consent after the purpose of the study was explained to them. The institutional review board approved the protocol, so we certify that all applicable institutional regulations concerning the ethical use of information and samples from human volunteers were followed during this research.

Anthropometric measurements

BMI was calculated as weight (in kilograms) divided by height (in meters) squared. The subjects' waist was measured with a soft tape midway between the lowest rib and the iliac crest. The hip circumference was measured at the widest part of the gluteal region. The waist-to-hip ratio was then calculated. Deurenberg formula (27) was used to estimate body fat composition in those subjects. According to these anthropometric parameters, subjects were classified on nonobese (BMI <30 kg/m^2) and obese (BMI ≥30 kg/m^2) following World Health Organization guidelines.

Samples treatment

Adipose tissue samples were obtained from SC and visceral depots during elective surgical procedures (cholecystectomy, surgery of abdominal hernia, and gastric by-pass surgery). All samples were washed, fragmented, and immediately flash-frozen in liquid nitrogen before be stored at −80 °C.

Approximately 5 g of SC and OM adipose tissue samples from eight subjects were aseptically isolated and all visible connective tissue was removed. Tissues were finely minced and subjected to a 1-h digestion at 37 °C in a shaking water bath. The digestion buffer included 100 mmol/l HEPES (Sigma-Aldrich, St Louis, MO) buffer containing 120 mmol/l NaCl, 50 mmol/l KCl, 5 mmol/l D-glucose, 1 mmol/l $CaCl_2$, 1.5% type-V bovine serum albumin (Sigma-Aldrich), 2% P/S, and 0.075% collagenase type I (Sigma-Aldrich) solution. The collagenase type I solution used to isolate stromal-vascular cells (SVCs) and mature adipocytes (MAs) from fat samples contained ~1.5 mg collagenase type I/ml. The remaining procedure was similar to a previously described method for isolating SVCs from adipose tissue (28). Briefly, upon disintegration of the adipose tissue aggregates, digested tissue was centrifuged and two cellular fractions, a pellet of SVCs and a supernatant of MAs, were placed in 20 ml of phosphate buffered saline 2% P/S and passed through sterile nylon mesh filters (autoclaved metal screen) to isolate digested cells. Finally, both SVCs and MAs filtered fractions were washed and centrifuged for 1 min at 400*g* before being stored at −80 °C.

RNA was prepared from all these samples (both fat biopsies and cellular debris) using RNeasy Lipid Tissue Mini Kit (Qiagen, Gaithersburg, MD).

The integrity of each RNA sample was checked by either agarose gel electrophoresis or with an Agilent Bioanalyzer (Agilent Technologies, Palo Alto, CA). Total RNA was quantified by means of spectrophotometer (GeneQuant; GE Healthcare, Piscataway NJ) or with the bioanalyzer and reverse transcribed to cDNA using High Capacity cDNA Archive Kit (Applied Biosystems, Darmstadt, Germany) according to the manufacturer's protocol.

Cell culture

SVCs from both visceral and SC fat samples (two paired samples) were resuspended in 2 ml of red cell lysis buffer (ammonium chloride) for 10 min on ice, washed two times with 20 ml of phosphate buffered saline 2% P/S and centrifuged at 400*g* for 5 min at 37 °C. SVCs ($n = 4$) were then plated on T-75 cell culture flasks and cultured at 37 °C and 5% CO_2 in Dulbecco's modified Eagle's medium/Nutrient Mix F-12 medium (1:1, v/v) supplemented with 10 U/ml P/S, fetal bovine serum 10%, HEPES 1% and glutamine 1% (all from Gibco BRL, Grand Island, NY). One week later, human visceral and SC preadipocytes were resuspended and cultured (~40,000 cells/cm^2) in 12-well plates in preadipocytes medium (Zen-Bio, Research Triangle Park, NC) composed of Dulbecco's modified Eagle's medium/Nutrient Mix F-12 medium (1:1, v/v), HEPES, fetal bovine serum, penicillin, and streptomycin in a humidified 37 °C incubator with 5% CO_2. At 24 h after plating, cells were checked for complete confluence (day 0) and differentiation was induced using differentiation medium (Zen-Bio) composed of preadipocytes medium, human insulin, dexamethasone, isobutylmethylxanthine, and peroxisome proliferator–activated receptor-γ agonists (rosiglitazone). After 7 days, differentiation medium was replaced with fresh adipocyte medium (Zen-Bio) composed of Dulbecco's modified Eagle's medium/Nutrient Mix F-12 medium (1:1, v/v), HEPES, fetal bovine serum, biotin, pantothenate, human insulin, dexamethasone, penicillin, streptomycin, and amphotericin. Two weeks after initiation of differentiation (day 14), cells appeared rounded with large lipid droplets apparent in the cytoplasm. Cells were then considered MAs, harvested, and stored at −80 °C for RNA extraction following the aforementioned methods.

Analytical determinations

The serum glucose concentrations were measured in duplicate by the glucose oxidase method with the use of a Beckman Glucose Analyzer II (Beckman Instruments, Brea, CA). Total serum cholesterol was measured through the reaction of cholesterol esterase/oxidase/peroxidase, using a BM/Hitachi 747. High-density lipoprotein–cholesterol was quantified after precipitation with polyethylene glycol at room temperature. Total serum triglycerides were measured through the reaction of glycerol-phosphate-oxidase and peroxidase. Free T_4, free T_3, and thyrotropic hormone were measured by electro-chemiluminescence (Roche Diagnostics, Basel, Switzerland) with intra- and interassay coefficients of variation <5%.

Gene expression analyses

Gene expression was assessed by real-time PCR using an ABI Prism 7000 Sequence Detection System (Applied Biosystems), using SybrGreen and TaqMan technology suitable for relative genetic expression quantification. The human *Cyclophilin A* (peptidylprolyl isomerase A (PPIA)) was used as endogenous control and gene expression results are expressed as expression ratio relative to PPIA gene expression, ratio relative to TRα2 in TRα1:TRα2 or to SF2 in hnRNP A1:SF2 ratio, using the delta threshold cycle (ΔC_t) method with SybrGreen primer and TaqMan primer/probes (Applied Biosystems) according to the manufacturer's protocol.

The SybrGreen primer sets used were previously validated to give an optimal amplification over serial dilutions of target, and analysis of melting curves demonstrated specific single product for each gene primer. Primer sequences were as follows: PPIA forward/reverse primer sequences were 5′-CAAATGCTGGACCCAACACAA/CCTCCACAA TATTCATGCCTTCTT-3′; TRα1 and TRα2 forward/reverse primer sequences were 5′-GTTCCCAGGACCCCATCCT/GGGTGAGTTG AGGGCATCTTC-3′ and 5′-GGCCCCAACTCAAGTGTCAC/CTT GGGAAACAGACTCATGCC-3′, respectively. *TRα* gene expression was assessed by adding up relative TRα1 and TRα2 mRNA values in each sample.

The commercially available and prevalidated TaqMan primer/probe sets used were as follows: endogenous control PPIA (4333763, RefSeq. NM_002046.3, cyclophilin A) and target genes THRSP14 (Hs00930058_m1, RefSeq. NM_003251.2, Spot 14), HNRPH1 (Hs00800662_sH, RefSeq. NM_005520.1, heterogeneous nuclear ribonucleoprotein H1), HNRPA1 (Hs01656228_s1, RefSeq. NM_031157.2 and NM_002136.2, heterogeneous nuclear ribonucleoprotein A1), and SF2 (Hs00199471_m1, RefSeq. NM_006924.4 and NM_001078166.1, arginine–serine-rich, splicing factor 2).

In both RT-PCR techniques, SybrGreen and TaqMan, the reaction was performed in a final volume of 25 µl. The cycle program consisted of an initial denaturing of 10 min at 95 °C then 40 cycles of 15-s denaturizing phase at 95 °C and 1-min annealing and extension phase at 60 °C. A C_t value was obtained for each amplification curve and a ΔC_t value was first calculated by subtracting the C_t value for human *Cyclophilin A* (PPIA) RNA from the C_t value for each sample. Fold changes compared with the endogenous control were then determined by calculating $2^{-\Delta Ct}$, so gene expression results are expressed as expression ratio relative to PPIA gene expression according to manufacturers' guidelines. The TRα1:TRα2 ratio was performed using TRα2 mRNA gene expression as reference and TRα1 mRNA as target gene. The same analysis was performed to establish hnRNP A1:SF2 ratio. Positive and negative controls were included in all reactions.

Statistical analyses

Descriptive results of continuous variables are expressed as mean ± s.d. Before statistical analysis, normal distribution and homogeneity of the variances were evaluated using Levene's test. Student's paired two-sample *t*-test was used to compare groups of adipose tissue samples according to its origin, SC or visceral. All data from human samples are expressed as mean ± s.d. Relation between quantitative variables were tested using Pearson's test. The statistical analyses and graphics were performed using the program SPSS (version 13.0; SPSS, Chicago, IL).

RESULTS

TRα gene expression and TRα1:TRα2 ratio in both adipose tissue samples

The anthropometric and metabolic characteristics of the study subjects and the relative gene expression levels in both fat depots are summarized in **Tables 1** and **2**, respectively, for both nonobese (BMI <30, $n = 15$) and obese (BMI ≥30, $n = 23$) subjects. Analysis of these 76 samples (38 pairs) of OM and SC fat depots revealed that TRα gene expression (the sum of TRα1 and TRα2 relative gene expression levels) was significantly increased in the SC vs. OM adipose tissue of obese women (1.61-fold, $P = 0.010$) (**Table 2**). TRα1 gene expression was also significantly increased only in SC fat depot from obese women ($P < 0.0001$, **Figure 1a**, **Table 2**).

TRα2 gene expression was not significantly different in the SC vs. OM fat depots. In all subjects as a whole, the TRα1:TRα2 ratio was 2.18-fold higher ($P < 0.0001$) (**Figure 1b**) in SC than in OM fat depots (2.07-fold higher ($P = 0.002$) in nonobese and 2.39-fold higher ($P < 0.0001$) in obese women) (**Table 2**). We calculated that ~35% of the TRα gene is spliced into the orphan receptor isoform, TRα2, in OM adipose tissue but only ~21% in the SC fat depot (**Figure 1c,d**). Thyroid function was not significantly associated with the expression of any of these factors.

Table 1 Anthropometric and metabolic characteristics of the study group

	Lean (BMI < 30)	Obese (BMI ≥ 30)	
	Mean ± s.d.		*P*
N (Women)	15	23	
Age (years)	47 ± 11	45 ± 13	0.581
BMI (kg/m^2)	**26.1 ± 2.7**	**42.9 ± 9.1**	**<0.0001**
Blood glucose (mg/dl)	101.9 ± 53.9	95.0 ± 18.5	0.675
Free T_3 (pg/ml)	3.0 ± 0.7	2.6 ± 0.7	0.270
Free T_4 (ng/dl)	1.5 ± 0.7	1.3 ± 0.3	0.519
TSH (nU/l)	**1.2 ± 0.3**	**2.2 ± 1.3**	**0.044**
Total cholesterol (mg/dl)	205.4 ± 45.5	195.3 ± 31.6	0.504
HDL-cholesterol (mg/dl)	69.0 ± 18.3	64.7 ± 68.4	0.781
LDL-cholesterol (mg/dl)	113.0 ± 48.3	119.5 ± 31.0	0.683
Triglycerides (mg/dl)	117.5 ± 72.5	121.1 ± 43.6	0.881

Values are means ± s.d. Significative differences ($P < 0.05$) are shown in bold. HDL, high-densiy lipoprotein; LDL, low-density lipoprotein; TSH, thyrotropic hormone.

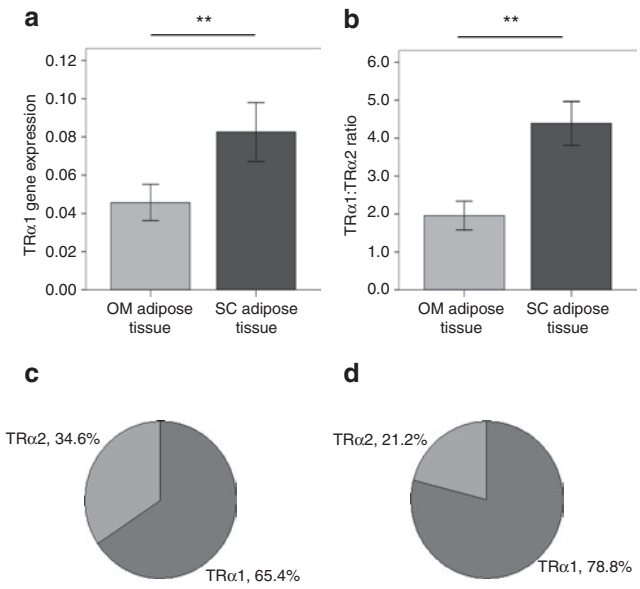

Figure 1 Regional differences in TRα gene expression levels and TRα1:TRα2 ratios: (**a**) relative TRα1 mRNA gene expression and (**b**) TRα1:TRα2 ratio in omental (OM) and subcutaneous (SC) adipose tissue samples from both nonobese ($n = 15$) and obese ($n = 23$) women. The mean relative prevalence for each TRα isoform (TRα1 and TRα2) is represented in **c** for OM and in **d** for SC fat depot. Values are mean and 95% confidence interval for the mean. $^{**}P < 0.001$ for comparisons between fat depots in all subjects as a whole. TR, thyroid hormone receptor.

S14 gene expression analyses

To gain further insight in the detected gene expression differences, we analyzed S14 gene expression and the splicing factors involved in this process. S14 gene expression was also increased by 2.42-fold ($P < 0.0001$) in SC vs. visceral adipose tissue depots (**Figure 2a**) in both groups. In fact, S14 gene expression correlated significantly with TRα ($P = 0.001$), with TRα1 gene expression ($P < 0.0001$), and with the TRα1:TRα2 ratio ($P = 0.001$) in both obese and nonobese subjects.

Table 2 Differences in relative gene expression levels and ratios between omental (OM) and subcutaneous (SC) fat depots for nonobese (upper panel) and obese (lower panel) women

	OM	SC	
	Mean ± s.d.		*P*
Nonobese women ($n = 15$)			
TRα	0.076 ± 0.053	0.098 ± 0.050	0.263
TRα1	0.052 ± 0.040	0.077 ± 0.033	0.081
TRα2	0.024 ± 0.016	0.021 ± 0.019	0.689
TRα1:TRα2	**2.275 ± 1.477**	**4.708 ± 2.279**	**0.002**
hnRNP H1	1.213 ± 0.425	0.861 ± 0.358	0.064
hnRNP A1	0.026 ± 0.007	0.021 ± 0.016	0.469
SF2	0.151 ± 0.047	0.141 ± 0.058	0.675
hnRNP A1:SF2	**0.160 ± 0.043**	**0.103 ± 0.030**	**0.003**
S14	**0.228 ± 0.116**	**0.573 ± 0.336**	**0.005**
Obese women ($n = 23$)			
TRα	0.067 ± 0.027	0.108 ± 0.068	**0.010**
TRα1	0.041 ± 0.019	0.086 ± 0.054	**<0.0001**
TRα2	0.025 ± 0.012	0.022 ± 0.015	0.326
TRα1:TRα2	**1.749 ± 0.853**	**4.183 ± 1.339**	**<0.0001**
hnRNP H1	1.144 ± 0.424	0.923 ± 0.516	0.203
hnRNP A1	0.025 ± 0.009	0.018 ± 0.008	**0.017**
SF2	0.163 ± 0.063	0.146 ± 0.064	0.439
hnRNP A1:SF2	**0.163 ± 0.061**	**0.123 ± 0.029**	**0.030**
S14	**0.219 ± 0.132**	**0.518 ± 0.165**	**<0.0001**

Expression values (means ± s.d.) are the ratio relative to endogenous control PPIA (cyclophilin A). Significative differences ($P < 0.05$) are shown in bold. hnRNP, heterogeneous nuclear ribonucleoprotein; PPIA, peptidylprolyl isomerase A; SF2, arginine–serine-rich, splicing factor 2; S14, Spot 14; TR, thyroid hormone receptor.

Differential hnRNP A1:SF2 ratio between adipose tissues

Trying to analyze more in depth the molecular processes involved, we analyzed the expression of three splicing proteins, hnRNP H1, hnRNP A1, and SF2, the levels of which are supposed to determine TRα gene alternative and constitutive splicing. We found no differences in hnRNP H1 gene expression in OM vs. SC adipose tissue from nonobese subjects. Interestingly, hnRNP A1 expression was significantly higher in OM vs. SC adipose tissue from obese subjects (**Table 2**). In fact, in all subjects as a whole, hnRNP A1 gene expression was significantly higher in OM vs. SC fat depots (1.32-fold, $P = 0.025$) (**Figure 2b**). Because SF2 gene expression levels were not different between fat depots (**Table 2**), hnRNP A1:SF2 ratio was 1.39-fold ($P = 0.001$) higher in OM vs. SC fat in all subjects (1.55-fold, $P = 0.003$) and 1.33-fold ($P = 0.030$) in nonobese and obese women, respectively) (**Figure 2c**).

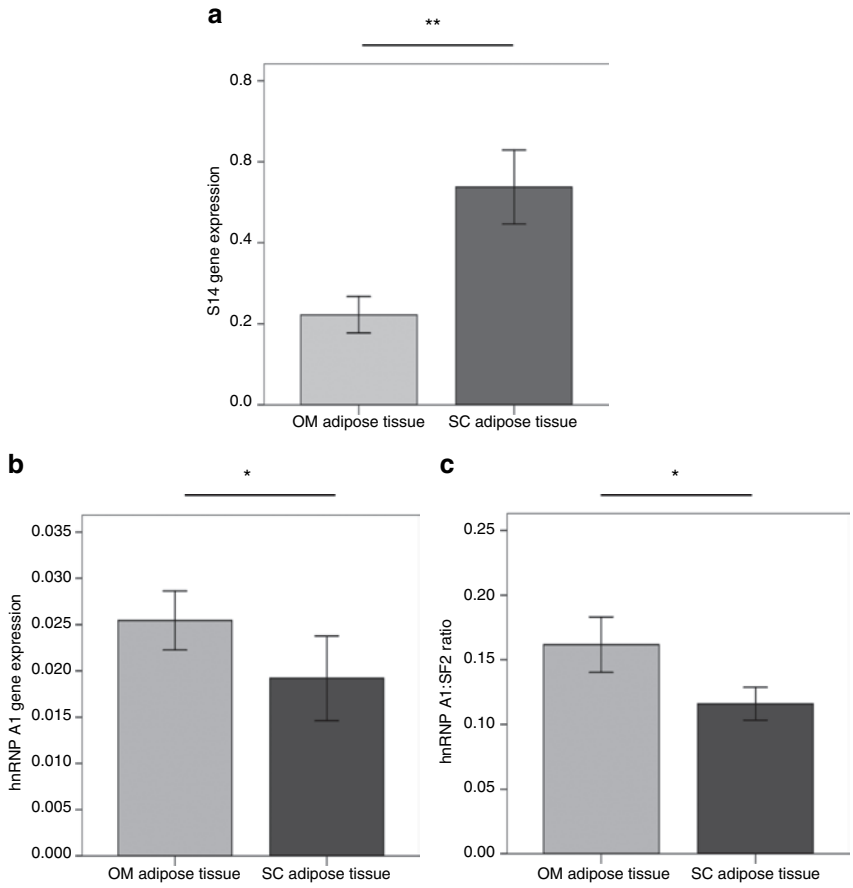

Figure 2 Regional differences in S14 and hnRNP A1 gene expression levels and hnRNP A1:SF2 ratio: (**a**) relative Spot 14 and (**b**) hnRNP A1 gene expression levels and hnRNP A1:SF2 ratio (**c**) in omental (OM) and subcutaneous (SC) adipose tissue samples from both nonobese ($n = 15$) and obese ($n = 23$) women. Values are mean and 95% confidence interval for the mean. $^{**}P < 0.001$ and $^{*}P < 0.05$ for comparisons between fat depots in all subjects as a whole. hnRNP, heterogeneous nuclear ribonucleoprotein; SF2, arginine–serine-rich, splicing factor 2; S14, Spot 14.

Table 3 Differences in relative gene expression levels and ratios between stromal-vascular cells (SVCs, upper panel) and mature adipocytes (MA, lower panel) from omental (OM) and subcutaneous (SC) fat depots

	OM	SC	
	Mean ± s.d.		*P*
SVCs ($n = 8$)			
TRα1:TRα2	1.71 ± 1.01	2.58 ± 1.51	0.201
hnRNP A1:SF2	0.195 ± 0.263	0.143 ± 0.070	0.599
S14	**0.0020 ± 0.0013**	**0.0075 ± 0.0039**	**0.002**
MAs ($n = 8$)			
TRα1:TRα2	1.99 ± 0.45	2.56 ± 1.06	0.182
hnRNP A1:SF2	0.617 ± 0.584	0.590 ± 0.702	0.943
S14	0.532 ± 0.131	0.692 ± 0.338	0.243

Expression values (means ± s.d.) are the ratio relative to endogenous control PPIA (cyclophilin A). Significative differences ($P < 0.05$) are shown in bold.
hnRNP, heterogeneous nuclear ribonucleoprotein; PPIA, peptidylprolyl isomerase A; SF2, arginine–serine-rich, splicing factor 2; S14, Spot 14; TR, thyroid hormone receptor.

In fact, hnRNP A1 gene expression correlated significantly with TRα2 gene expression ($r = 0.78$, $P < 0.0001$; Figure 3 upper panel) and inversely with TRα1:TRα2 ratio ($r = -0.39$, $P = 0.05$; Figure 3 lower panel). This suggests that the relative presence of these splicing factors in human adipose tissue from different depots could determine *c-erbA-α* gene processing.

TRα, S14, hnRNP A1, and SF2 levels in SVCs and MAs from OM and SC fat samples

Adipose tissue represents a pool of adipocytes, preadipocytes, and macrophages. Analysis of these 32 samples (16 pairs) of SVCs and MAs obtained from OM (eight pairs) and SC (eight pairs) fat depots revealed that TRα gene expression was 2.1-fold ($P < 0.0001$) higher in MAs than in SVCs from both fat depots (Figure 4a). On the other hand, S14 levels were 112-fold ($P < 0.0001$) higher in MAs (Figure 4b). It should be noted that also hnRNP A1 (but not SF2) levels were higher in MAs than in SVCs (Figure 4c,d).

The expression of these factors was similar in cells from SC or OM adipose tissue (Table 3). Interestingly, under the same culture conditions (free $T_3 = 1.68$ pg/ml), preadipocytes from SC fat depots differentiated earlier (lipid droplets were detected at ~5th day vs. ~7th day) and "better" (Figure 5) than those from OM fat. Furthermore, S14 gene expression levels were significantly increased in preadipocytes from SC

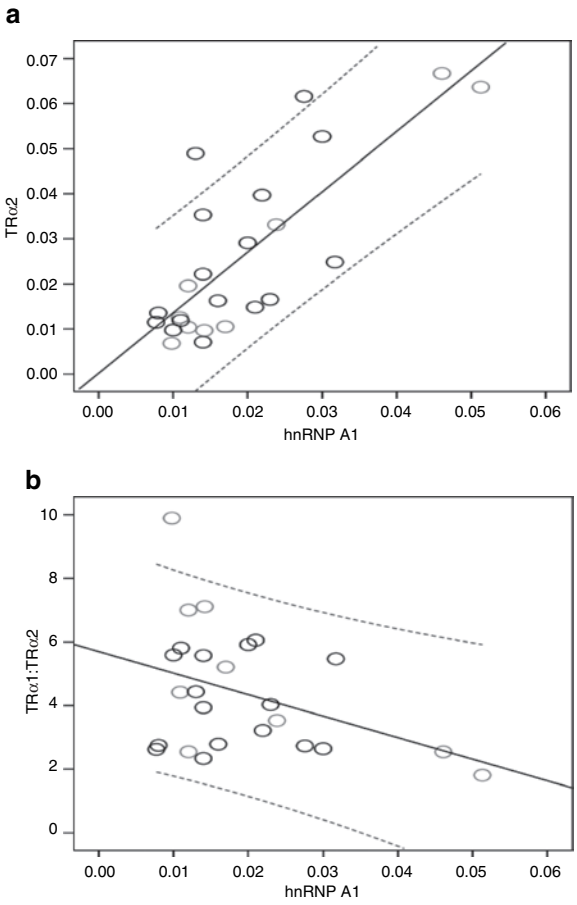

Figure 3 Linear relationship between hnRNP A1 expression and (**a**) thyroid hormone receptor α2 ($r = 0.78$, $P < 0.0001$) and (**b**) thyroid hormone receptor α1/α2 ratio ($r = -0.39$, $P = 0.05$) in subcutaneous adipose tissue from nonobese (gray circles) and obese (black circles) women. hnRNP, heterogeneous nuclear ribonucleoprotein.

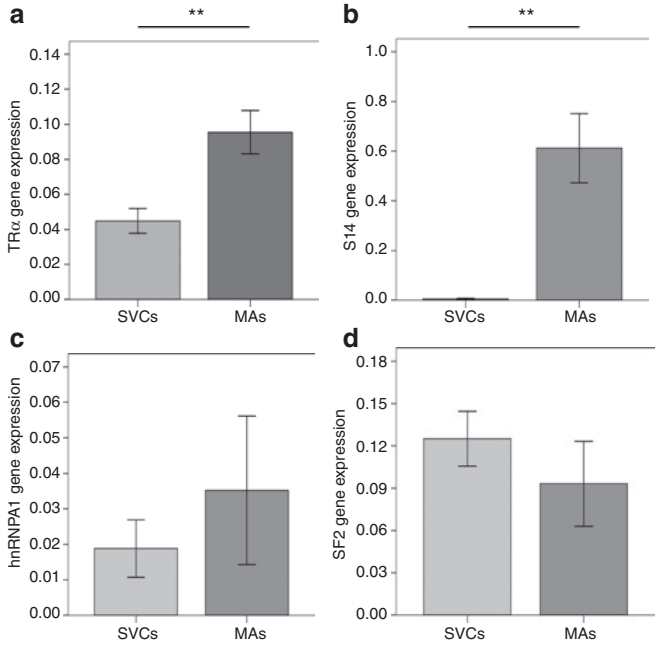

Figure 4 TRα, S14, hnRNP A1, and SF2 gene expression levels in isolated fat cells: (**a**) *TR*α gene expression and (**b**) Spot 14, (**c**) hnRNP A1, and (**d**) SF2 levels in stromal-vascular cells (SVCs) and mature adipocytes (MAs) isolated from 16 adipose tissue biopsies. Values are mean and 95% confidence interval for the mean. $^{**}P < 0.001$ for comparisons between cells from both SC and OM fat depots. hnRNP, heterogeneous nuclear ribonucleoprotein; PPIA, peptidylprolyl isomerase A; SF2, arginine–serine-rich, splicing factor 2; S14, Spot 14; TR, thyroid hormone receptor.

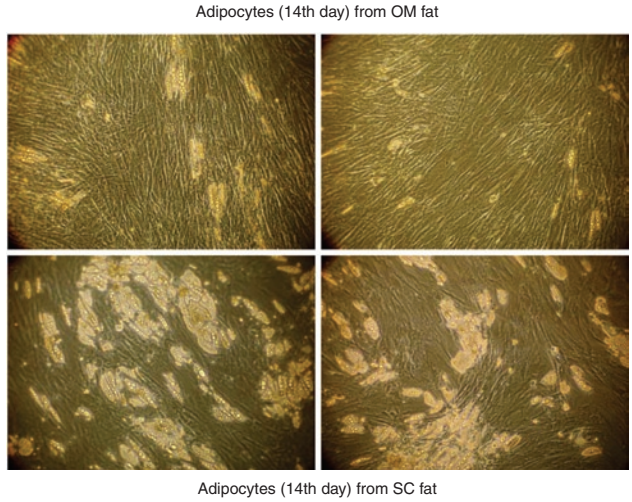

Figure 5 Appearance of human preadipocytes isolated from visceral (upper panels) and subcutaneous (lower panels) fat depots, differentiated to mature adipocytes (MAs) during 14 days under the same conditions.

samples at the 1st day (~300%), and remained higher (~33%) at the 14th day in MAs from SC fat depots than in those from OM samples.

DISCUSSION

We here describe that TRα and TRα1 gene expressions were significantly increased in SC vs. OM fat depot from obese women ($P < 0.0001$) but not in nonobese subjects. According to these findings, human SC adipose tissue from obese subjects is supposed to be more T_3-responsive than OM adipose tissue. In this regard, S14 gene expression was also higher in SC than in OM fat depots. Furthermore, we also found that MA is the cell responsible for TRα gene expression levels (**Figure 4a**), for both TRα1 and TRα2, and for S14 expression levels (**Figure 4b**). These data could help to understand the role of thyroid hormone in the development of the obesity.

TRα2 is an alternative product of the *c-erbA-α* gene expression that lacks the intact ninth intronic sequence and acquires a unique carboxyl-terminal region. Given its inability to bind T_3, the TRα2 capacity to act as a transcription factor is abolished (12) but not to bind thyroid hormone response elements. As a result, TRα2 exerts a dominant negative effect by competing for TR binding to thyroid hormone response elements on DNA (29). The balance between TRα1 and TRα2 is thus important given their clearly opposite effects on T_3-responsive gene expression (5). In this regard, the balance of the TRα1:TRα2 ratio could be responsible for development changes through

the control of growth and homeostasis (30). The present results showed higher TRα gene expression in SC fat, mainly due to increased TRα1 mRNA isoform levels, in detriment to TRα2, in SC adipose tissue. Considering the sum of TRα1 and TRα2 values as the total *c-erbA-α* gene expression, TRα1 constituted the 78.8% of the total expression in SC but only the 65.4% in OM adipose tissue depot.

Additionally, our results imply the existence of differential splicing events of *c-erbA-α* pre-mRNA according to adipose depots. To further study this differential pre-mRNA processing, we aimed to analyze the involved splicing factors, such as hnRNP A1, hnRNP H1, and SF2. We found that hnRNP H1 and hnRNP A1 (but not SF2) tended to be under-expressed in SC samples. Importantly, in parallel to TRα and TRα1 changes, hnRNP A1 was significantly downregulated in the SC adipose tissue depot of obese subjects. Our findings suggest that hnRNP A1:SF2 ratio could be responsible for tissue-specific TRα1:TRα2 ratio differences detected between SC and OM adipose tissue depots. The distal splicing site leading to TRα2 is chosen over polyadenylation of TRα1. This relationship has been previously reported in *in vitro* studies (31) but, to our knowledge, this phenomenon has not been observed *in vivo*.

Serine–arginine-rich protein factors are important mediators of splicing enhancement in both constitutive and alternative splicing (32). hnRNPs constitute a set of polypeptides that bind heterogeneous nuclear RNA with a full rage of functions, most of them presently unknown (33). Among these splicing factors, hnRNP A1 seems to be implicated in a variety of cellular splicing silencing mechanism, through its cooperative recognition of UAGGG[U/A] and related motifs, in a manner that is opposite to the effects exerted by serine–arginine proteins (18). On the other hand, hnRNP H acts as dose-dependent splicing enhancer cooperating with serine–arginine proteins (17). The relative abundance of these spliceosome-related factors and, in particular, the ratio of SF2 or hnRNP H1 to its antagonist, hnRNP A1, could be important in regulating the patterns of alternative splicing in a tissue-specific or developmentally regulated manner (19).

Thyroid status influences specifically for each tissue the expression of a number of genes involved in lipid and glucose metabolism. Examples of T_3-regulated genes include S14 (2), peroxisome proliferator–activated receptor, malic enzyme, and adenosine triphosphate citrate liase (34), fatty acid synthase and glucose transporters (35), acetyl-CoA carboxylase I, and β-adrenoceptor genes (36). S14, commonly related to lipogenic pathways, and β_2-adrenergic receptor, associated to lipolytic processes (37), are especially prominent among the most T_3-responsive genes in lipogenic tissues.

Others studies have previously focused on TRα1:TRα2 ratio as a reflection of T_3-sensitivity. This ratio increases with age and severity of illness in liver samples of critically ill patients (38) but decreases in the liver of patients with chronic liver disease (39) or in fasted rats (30). On the other hand, many studies have investigated the differences between OM and SC adipose depots. Although findings of these studies are not at all unambiguous, most of them showed higher basal lipolysis rates per cell but lower absolute response to lipolytic stimuli above basal levels to isoproterenol or other catecholamines in SC fat (40), in parallel to increased adipocyte size (22).

In summary, SC fat was characterized by higher TRα gene expression and TRα1:TRα2 ratio. These differences are associated with differential S14 gene expression. Regional differences in TRα1:TRα2 ratio between both adipose depots can be explained thought differential hnRNP A1:SF2 ratios, also detected between both fat depots. The significantly higher expression of the TRα1 in SC vs. OM fat in obese subjects suggests that TRα1 could contribute to SC adipose tissue expandability in obese subjects. Further investigations will be required to evaluate the functional consequences of these findings.

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DISCLOSURE

The authors declared no conflict of interest.

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