Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 59 (2010) 1106 – 1114
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Adipose tissue lamin A/C messenger RNA expression in women

Mélanie Nadeau[a], Suzanne Noël[b], Philippe Y. Laberge[b], Johanne Hurtubise[b], André Tchernof[a,c,*]

[a]Molecular Endocrinology and Genomics Center, Laval University Medical Research Center, Quebec City, Quebec, Canada G1V 4G2
[b]Gynecology Unit, Laval University Medical Research Center, Quebec City, Quebec, Canada G1V 4G2
[c]Department of Nutrition, Laval University, Quebec City, Quebec, Canada G1V 0A6

Received 28 April 2008; accepted 9 September 2009

Abstract

Mutations in the lamin A/C gene (*LMNA*) cause lipodystrophy. However, little data are available on lamin A/C expression in various fat depots in women. We recruited 34 women scheduled for gynecologic surgery. Blood samples were collected on the morning of surgery to obtain a detailed lipid profile. Radiological examinations were performed to measure total body fat mass and abdominal fat accumulation. Fat samples were taken from the subcutaneous (SC) fat depot and from the greater omentum (OM) during the surgical procedure. Whole adipose tissue samples were used for total messenger RNA (mRNA) extraction and real-time polymerase chain reaction quantification of the *LMNA* transcript. No association was observed between lamin A/C mRNA expression, either in SC or OM fat tissue, and adiposity measures. Women with low SC lamin A/C expression, identified on the basis of the median value of SC lamin A/C mRNA expression, had a significantly altered lipid profile including lower levels of high-density lipoprotein (HDL) cholesterol and HDL_2 cholesterol and reduced HDL_2 cholesterol to HDL_3 cholesterol ratio ($P < .05$ for all). These women were also characterized by higher cholesterol to HDL cholesterol, low-density lipoprotein–triglycerides, very low-density lipoprotein–apolipoprotein B, and low-density lipoprotein cholesterol to HDL cholesterol ($P < .05$ for all). Low SC lamin A/C mRNA expression levels were also associated with significantly increased lipolysis in isolated fat cells from this fat depot. Specifically, the response to lipolytic agent isoproterenol was significantly increased at doses ranging from 10^{-5} to 10^{-10} mol/L ($P < .05$). A similar trend was observed in OM fat cells but did not reach significance. In conclusion, low lamin A/C expression in SC adipose tissue is associated with significant alterations in the lipid profile and increased fat cell lipolysis, independent of the level of total or abdominal adiposity.
© 2010 Elsevier Inc. All rights reserved.

1. Introduction

Lamins are structural proteins and represent the main component of the nuclear lamina network located at the inner layer of the nuclear membrane [1,2]. They are members of the intermediate filament protein family. In mammals, 3 genes code for 7 different proteins of which 5 are expressed in somatic tissue. Lamin B1 and lamin B2 are encoded, respectively, by *LMNB1* and *LMNB2* and are expressed in all somatic cells. The lamin A type includes lamin A, lamin C, and lamin ΔA10, which are generated from alternate splicing of the same gene, *LMNA*. They are present in most differentiated somatic cells.

Proteins generated from the lamin A gene transcripts were first known to be involved in the structure maintenance of the nucleus and have recently been shown to interact with several cellular pathways [2-4]. LAP1, LAP2α, LAP2β, and emerin are some examples of architectural partners for lamins at the inner layer of the nuclear membrane, providing functions such as mechanical strength, nuclear shape, and anchor [5,6]. Interestingly, lamins also bind DNA and histones in vitro, which suggests a role in scaffolding protein complexes for gene expression [5-8]. Lamins can bind several proteins involved in gene expression regulation such as Rb, SREBP1, MOK2, and BAF [9-12]. Lamin A can also bind to signaling proteins and provide a structural basis for several cell pathways. For example, the PKCα signalization pathway might involve

* Corresponding author. Endocrinology and Genomics and Department of Nutrition, Laval University Medical Research Center, and Laval University, Québec City, Québec, Canada G1V 4G2. Tel.: +1 418 654 2296; fax: +1 418 654 2761.
E-mail address: andre.tchernof@crchul.ulaval.ca (A. Tchernof).

0026-0495/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2009.09.034

lamin A attachment [13]; and 12(s)-LOX and E1B 19K also bind to lamin A [14,15].

Mutations in the lamin A/C (*LMNA*) gene have been associated with disorders that primarily affect muscle, adipose, bone, or neuronal tissues and also result in premature aging [16]. Several *LMNA* mutations cause Dunningan-type familial partial lipodystrophy [17], an autosomal dominant disorder characterized by anomalous distribution of adipose tissue, insulin resistance, and altered lipid metabolism [18]. Another laminopathy with a lipodystrophic component is the mandibuloacral dystrophy; an extremely rare autosomal recessive disorder characterized by musculoskeletal abnormalities and lipodystrophy, concomitant with altered blood lipids, insulin resistance, and impaired glucose tolerance. Mechanisms involved in such lipodystrophic phenotypes remain incompletely defined.

Several models have been proposed to explain the molecular basis of laminopathies [19]. The "structural model" proposes a defect in lamin protein structure leading to more fragile cells and nuclei. As muscles are exposed to mechanic stress, this model could explain laminopathies affecting muscle tissue such as Emery-Dreifuss muscular dystrophy. The "ER-retention model" suggests that mutated lamins fail to retain nuclear membrane proteins in the nuclear envelope. Mutations in LMNA could also impair the control of cell proliferation. Such "cell cycle model" could explain the premature aging types of laminopathies like the Hutchinson-Gilford progeria syndrome. The "gene expression model" suggests that mutated lamin A/C may affect gene expression through different mechanisms. Finally, a proposed "tissue regeneration model" could integrate all previous models, with impaired tissue regeneration or altered cell-type plasticity as the common denominator [19].

In adipose tissue, lamin A can interact with the adipogenic transcription factor SREBP1. In cases of *LMNA* mutations, prelamin A accumulates and sequesters SREBP1 at the nuclear periphery, thereby blocking this pathway in vitro [9,20]. In vivo, the nature of the lamin A/SREBP1 interaction is not yet clear. Lamin A may facilitate the import and localization of SREBP1 in the nucleus or may be involved in the formation, stabilization, or regulation of transcription complexes involving SREBP1 [9]. Recent studies have shown that, in vitro, lamins may act as inhibitors of adipocyte differentiation [20], whereas others indicated that they have no effect on this process [21].

Very little data are available on lamin A/C expression in human fat tissues. A study by Lelliot et al [22] examined lamin expression patterns in preadipocytes and mature adipocytes and demonstrated that lamin A/C expression increases with ex vivo differentiation. Another study by Miranda et al [23] showed increased lamin A/C mRNA expression in subcutaneous (SC) adipose tissue of obese and type 2 diabetes mellitus patients. It is not clear, however, whether lamin A/C expression in abdominal fat compartments is related to fat tissue distribution, adipocyte morphology, and metabolism, or to variables of the lipid profile. The aim of the present study was to investigate the associations between the expression of lamin A/C mRNA in omental and SC fat tissues in women and measures of adiposity, blood lipid profile, and adipocyte lipolysis. Based on its role in lipodystrophy and its postulated impact on adipocyte differentiation, we tested the hypothesis that low lamin A/C expression would relate to alterations in blood lipids and increased adipocyte lipolysis.

2. Methods

2.1. Subjects

Thirty-four women were recruited through the elective surgery schedule of the Gynecology Unit of Laval University Medical Center. Women were between 39.6 and 61.7 years old and elected for total (n = 33) or subtotal (n = 1) abdominal hysterectomies, some with salpingo-oophorectomy of 1 (n = 5) or 2 (n = 14) ovaries. Reasons for surgery included one or more of the following: menorrhagia/menometrorrhagia (n = 18), myoma/fibroids (n = 24), incapacitating dysmenorrhea (n = 5), pelvic pain (n = 1), benign ovarian cyst (n = 7), endometriosis (n = 4), adenomyosis (n = 1), pelvic adhesions (n = 3), benign cystadenoma (n = 1), endometrial hyperplasia (n = 1) or polyp (n = 2), and ovarian thecoma (n = 1). This study was approved by the medical ethics committee of Laval University Medical Center. All subjects provided written informed consent before their inclusion in the study.

2.2. Body fatness and body fat distribution measurements

These tests were performed on the morning of or within a few days before or after the surgery. Measures of total body fat mass, fat percentage, and fat-free mass were determined by dual-energy x-ray absorptiometry using a Hologic QDR-2000 densitometer and the enhanced array whole-body software V5.73A (Hologic, Bedford, MA). Measurement of cross-sectional abdominal SC and visceral adipose tissue areas was performed by computed tomography as previously described [24] using a GE Light Speed 1.1 CT scanner (General Electric Medical Systems, Milwaukee, WI). The scan was performed at the L4 through L5 vertebrae level, and the quantification of visceral adipose tissue area was done by delineating the intraabdominal cavity at the internal-most aspect of the abdominal and oblique muscle walls surrounding the cavity and the posterior aspect of the vertebral body using the ImageJ 1.33u software (National Institutes of Health, Bethesda, MD). Adipose tissue was highlighted and computed using an attenuation range of −190 to −30 Hounsfield units. The coefficient of variation between 2 measures from the same observer (n = 10) were 0.0%, 0.2% and 0.5% for total, SC, and visceral adipose tissue areas, respectively.

2.3. Plasma lipid-lipoprotein measurements

Blood samples were obtained after a 12-hour fast on the morning of surgery. Cholesterol and triglyceride level measurements in plasma and lipoprotein fractions were performed with a Technicon RA analyzer (Bayer, Etobicoke, Canada) using enzymatic methods, as previously described [25]. Plasma very low-density lipoproteins (VLDLs) were isolated by ultracentrifugation, and the high-density lipoprotein (HDL) fraction was obtained by precipitation of the low-density lipoproteins (LDLs) from the infranatant with heparin and $MnCl_2$ [25,26]. The cholesterol and triglyceride content of the infranatant of each fraction was measured before and after precipitation, and the concentration of LDL cholesterol was obtained by difference. The cholesterol content of the HDL_2 and HDL_3 subfractions was also determined after further precipitation of HDL_2 with dextran sulfate. Apolipoprotein (apo) B or A-I concentrations were measured in plasma or relevant lipoprotein fractions by the rocket immunoelectrophoretic method of Laurell [27].

2.4. Adipose tissue sampling

Subcutaneous adipose tissue was collected at the site of surgical incision (lower abdomen), and omental adipose tissue was collected from the distal portion of the greater omentum (OM). Samples were collected during the surgical procedure and immediately carried to the laboratory in 0.9% saline preheated at 37°C. Adipocyte isolation was performed with a portion of the fresh biopsy, and the remaining tissue was immediately frozen in liquid nitrogen and stored at −80°C for subsequent analyses.

2.5. Adipocyte isolation, lipolysis, and lipoprotein lipase activity

Tissue samples were digested for 45 minutes at 37°C with collagenase type I in Krebs-Ringer-Henseleit buffer (25 mmol/L HEPES, 125 mmol/L NaCl, 3.7 mmol/L KCl, 5 mmol/L $CaCl_2$, 2.5 mmol/L $MgCl_2$, and 1 mmol/L K_2HPO_4, pH 7.4) supplemented with 5 mmol/L glucose, 0.1 μmol/L adenosine, 0.1 mg/mL ascorbic acid, and 4% electrophoresis-grade delipidated bovine serum albumin according to a modified version of the Rodbell [28] method. Adipocyte suspensions were filtered through nylon mesh and washed 3 times with the buffer. For cell size measurements, mature adipocyte suspensions were visualized using a contrast microscope attached to a camera and computer interface. Pictures were taken, and the Scion Image software (Scion, Frederick, MA) was used to measure the size of 250 adipocytes from each sample.

Lipolysis experiments were performed by incubating isolated cell suspensions for 2 hours at 37°C in Krebs-Ringer Henseleit buffer, with or without β-adrenergic receptor agonist isoproterenol in concentrations ranging from 10^{-10} to 10^{-5} mol/L or postreceptor-acting agents dibutyryl cyclic adenosine monophosphate (cAMP) (10^{-3} mol/L) and forskolin (10^{-5} mol/L). Cell suspensions were diluted to 5000 cells per condition in 30 μL. Glycerol release in the medium was measured by bioluminescence using the nicotinamide adenine dinucleotide–linked bacterial luciferase assay [29], a Berthold Microlumat plus bioluminometer (LB 96 V), and the WinGlow software (EG&G, Bad Wildbad, Germany). Intra- and interassay coefficients of variation for glycerol release measurements were 5.9% and 13.9%, respectively. Lipid weight of the cell suspension used in lipolysis experiments was measured by performing the Dole extraction. Average adipocyte weight and cell number in the suspensions were calculated using lipid weight, average cell volume, and the density of triolein. Lipolysis results were expressed in micromoles per 10^6 cells per 2 hours.

Heparin-releasable lipoprotein lipase (LPL) activity was determined in 30- to 50-mg frozen adipose tissue samples by the method of Taskinen et al [30]. Tissue eluates were obtained by incubating the sample in Krebs-Ringer phosphate buffer and heparin at 37°C for 90 minutes. The eluates were then incubated with excess concentrations of unlabeled and ^{14}C-labeled triolein in a Tris-albumin buffer emulsified with ultrasound. The reaction was carried out at 37°C for 60 minutes with agitation. The resulting free fatty acids liberated from triolein by the LPL reaction were isolated by the Belfrage extraction procedure. Porcine plasma was used as a source of apo C-II to stimulate LPL activity and unpasteurized cow's milk as an internal LPL activity standard for interassay variations. Activity results were expressed in nanomoles oleate per 10^6 cells per hour.

2.6. Real-time reverse transcriptase polymerase chain reaction

Total RNA was isolated from each sample using the Rneasy method according to the manufacturer's protocol (Qiagen, Mississauga, Ontario, Canada) and then digested with deoxyribonuclease to remove any contaminating genomic DNA (Turbo DNA-free from Ambion, Austin, TX). RNA quantity and quality were assessed using an Agilent Technologies 2100 Bioanalyzer and RNA 6000 Nano LabChip kit (Agilent, Mountain View, CA). Complementary DNA (cDNA) was generated from 500 ng of total RNA using a random primer hexamer following the protocol for Superscript II (Invitrogen, Carlsbad, CA). Equal amounts of cDNA were run in triplicate and amplified in a 15-μL reaction containing 7.5-μL of 2× Universal PCR Master Mix (Applied Biosystems, Foster City, CA), 10 nmol/L of *Z*-tailed forward primer, 100 nmol/L of reverse primer, 250 nmol/L of Amplifluor Uniprimer probe (Chemicon, Temecula, CA), and 1 μL of cDNA target. Appropriate no-template controls were used. The mixture was incubated at 50°C for 2 minutes and at 95°C for 4 minutes, and then cycled at 95°C for 15 seconds and at 55°C for 30 seconds 55 times using the Applied Biosystems Prism 7900 Sequence Detector. Abundance of the target transcript was calculated according to a

standard curve and normalized to 18S RNA expression. Primers were designed using Primer Express 2.0 (Applied Biosystems, Foster City, CA) with the following sequence: 18S gene: 5-CGGTACAGTGAAACTGCGAATG-3 and 5-CCAAAGGAACCATAACTGATTTAATGA-3; hLMNA gene (from exons 3 and 4): 5-TCCAGAAGAACATCTA-CAGTGAGGA-3 and 5-CATGACGGCGCTTGGTCT-3. Amplicons were detected using the Amplifuor UniPrimer system and the 5' Z sequence ACTGAACCTGACCGTACA added to forward primers.

2.7. Statistical analyses

Variables that were not normally distributed based on a significant Shapiro-Wilk test ($P < .05$) were $\log_{10}$-transformed or Box-Cox–transformed in parametric statistical analyses. Subgroups of low and high LMNA expression were identified on the basis of the median value of SC or OM lamin A/C mRNA abundance. Pearson correlation coefficients were computed to assess associations among LMNA mRNA expression, adiposity measures, and variables of the lipid profile or measures of adipocyte metabolism. Nonparametric Spearman ρ correlation coefficients were computed for variables that could not be normalized. Student t tests were used to compare means between women with low and high SC or OM LMNA mRNA expression.

3. Results

Average values and ranges of anthropometric data, adiposity measures, variables of the lipid profile, adipocyte size, LPL activity, and adipose tissue *LMNA* expression are shown in Table 1. Women were 47.9 years old and were slightly overweight according to mean body mass index (BMI) (26.7 kg/m^2). Average values of the lipid profile fell within the reference range. One woman was characterized by elevated plasma cholesterol, 1 by elevated plasma LDL cholesterol, 4 by low plasma HDL cholesterol, and 9 by elevated plasma triglycerides [31].

Table 2 shows the correlations between OM or SC LMNA mRNA expression and age, adiposity, variables of the lipid profile, as well as measures of adipocyte metabolism. No association was observed between lamin A/C mRNA expression, either in SC or OM fat tissue, and adiposity. Lamin A/C mRNA expression was not associated with adipocyte diameter. We also observed that lamin A/C mRNA expression was not significantly correlated with LPL activity. However, significant correlations were observed between SC lamin A/C mRNA expression levels and several parameters of the blood lipid profile. A positive correlation was observed between SC lamin A/C mRNA expression and plasma HDL cholesterol, whereas negative correlations were obtained with the cholesterol to HDL cholesterol ratio, LDL cholesterol to HDL cholesterol ratio, apo B to non–HDL cholesterol ratio, and LDL–apo B to LDL cholesterol. We observed a negative correlation between HDL–apo A-1 and lamin A/C mRNA expression in OM fat tissue. All these results were statistically significant ($P < .05$).

Table 1
Characteristics of women of the study (N = 34)

	Mean ± SD	Range
Age (y)	47.9 ± 5.2	39.6-61.7
Weight (kg)	69.7 ± 14.2	49.5-110.5
BMI (kg/m^2)	26.7 ± 5.0	19.1-41.3
Fat mass (kg)	24.4 ± 8.4	11.2-45.0
Lean body mass (kg)	43.0 ± 6.7	32.5-63.0
Computed tomography adipose tissue areas (cm^2)		
Total	406 ± 154	150-737
Visceral[a]	94 ± 43	38-219
SC[a]	313 ± 120	108-575
Cholesterol content (mmol/L)		
Total	4.87 ± 0.66	3.61-6.22
VLDL	0.44 ± 0.31	0.11-1.24
LDL	3.03 ± 0.60	1.75-4.17
HDL	1.40 ± 0.31	0.79-2.28
HDL_2/HDL_3[a]	0.70 ± 0.28	0.18-1.41
HDL_3 cholesterol[a]	0.83 ± 0.17	0.57-1.14
HDL_2 cholesterol[a]	0.57 ± 0.23	0.13-1.17
Total/HDL	3.63 ± 0.90	2.04-5.75
LDL/HDL	2.29 ± 0.79	0.98-4.59
Triglyceride content (mmol/L)		
Total	1.27 ± 0.67	0.53-2.91
VLDL	0.77 ± 0.60	0.14-2.05
LDL	0.23 ± 0.06	0.13-0.42
HDL	0.26 ± 0.07	0.13-0.44
Apo B and A-1 content (g/L)		
Total apo B	0.92 ± 0.19	0.60-1.28
VLDL–apo B	0.12 ± 0.06	0.04-0.29
LDL–apo B	0.80 ± 0.16	0.42-1.07
HDL–apo A-1	1.36 ± 0.21	0.88-1.70
Apo B/non-HDL cholesterol	0.27 ± 0.01	0.24-0.29
LDL–apo B/LDL cholesterol	0.27 ± 0.02	0.24-0.31
OM adipocyte diameter (μm)	79 ± 15	52-101
SC adidocyte diameter (μm)	98 ± 13	67-122
OM LPL activity[b]	10.0 ± 5.1	4.2-25.1
SC LPL activity[b]	15.3 ± 6.3	2.3-29.7
OM LMNA mRNA[c]	0.48 ± 0.34	0.06-1.63
SC LMNA mRNA[c]	0.55 ± 0.41	0.11-1.76

[a] n = 33 for these measures.
[b] Lipoprotein lipase activity in nanomoles per hour per 10^6 cells.
[c] Expression levels in arbitrary units.

Comparison of the subgroups with high vs low lamin A/C mRNA expression did not reveal any difference in total or visceral adiposity (Table 3). Women with low SC lamin A/C expression had a significantly altered lipid profile including lower levels of HDL cholesterol ($P < .05$) and HDL_2 cholesterol ($P < .005$) and a reduced HDL_2 cholesterol to HDL_3 cholesterol ratio ($P < .05$). These women were also characterized by higher cholesterol to HDL cholesterol, LDL-triglycerides, VLDL–apo B, and LDL cholesterol to HDL cholesterol ($P < .05$) (Figs. 1 and 2). Low lamin A/C mRNA expression in OM adipose tissue was associated with lower HDL–apo A-1 and higher VLDL–apo B ($P < .005$).

Low SC lamin A/C mRNA expression levels were also associated with significantly increased lipolysis in isolated fat cells from the SC fat depot ($P < .05$) (Fig. 3). Specifically,

Table 2
Pearson correlation coefficients between OM and SC LMNA mRNA expression, age, adiposity measures, variables of the lipid profile, and adipocyte metabolism (N = 34)

	SC LMNA[b]	OM LMNA[b]
Age	0.02	−0.12
Weight	0.13	−0.01
BMI	0.06	0.06
Fat mass	0.12	0.03
Lean body mass	0.07	−0.05
Computed tomography adipose tissue areas		
Total	0.06	0.06
Visceral[a]	−0.09	0.17
SC[a]	0.11	0.03
Cholesterol content		
Total	0.04	−0.03
VLDL[d]	−0.03	−0.12
LDL	−0.09	0.16
HDL	0.34*	−0.26
HDL_2/HDL_3[a]	0.15	−0.1
HDL_3 cholesterol[a,b]	0.29	−0.24
HDL_2 cholesterol[a]	0.27	−0.19
Total/HDL	−0.34*	0.13
LDL/HDL	−0.35*	0.16
Triglyceride content		
Total[c]	0.00	−0.03
VLDL[c]	0.04	−0.02
LDL	−0.19	−0.04
HDL	−0.02	−0.12
Apo B and A-1 content		
Total apo B	−0.21	−0.07
VLDL–apo B	−0.07	−0.11
LDL–apo B	−0.21	0.11
HDL–apo A-1	0.27	−0.34*
Apo B/non-HDL cholesterol	−0.37*	−0.07
LDL–apo B/LDL cholesterol	−0.41*	−0.06
OM adipocyte diameter	−0.08	0.12
SC adidocyte diameter	−0.07	0.12
OM LPL activity[b]	−0.03	0.06
SC LPL activity[b]	−0.27	0.02
OM LMNA mRNA expression[b]	−0.17	–
SC LMNA mRNA expression[b]	–	−0.17

[a] n = 33 for these measures.
[b] Analyses performed on $\log_{10}$-transformed data.
[c] Analyses performed on Box- Cox–transformed data.
[d] Spearman ρ correlation.
* $P \leq .05$.

the response to isoproterenol was significantly increased at all doses tested (10^{-5} to 10^{-10} mol/L). A similar trend was observed in OM fat cells but did not reach significance. Differences between the subgroups with high and low lamin A/C mRNA expression for basal, forskolin-, and dibutyryl cAMP–stimulated lipolysis were not significant (Fig. 3).

4. Discussion

The aim of the present study was to investigate lamin A/C mRNA expression in OM and SC adipose tissue in women and their relation with adiposity, blood lipids, and adipocyte metabolism. Based on the role of lamin A/C in lipodystrophy and its postulated impact on adipocyte differentiation, we tested the hypothesis that low lamin A/C expression would relate to alterations in blood lipids and increased adipocyte lipolysis. We found no association between lamin A/C mRNA expression and detailed measures of total or visceral adiposity. However, low lamin A/C expression was associated with an impaired lipid profile and increased lipolytic response in the SC fat depot. It has been shown that *LMNA* mutations can lead to lipodystrophy [17] and several other diseases [16]; but this is the first study to report that low lamin A/C expression in adipose tissue is associated with increased adipocyte lipolysis and altered blood lipid levels, independent of adiposity or fat cell size.

As mentioned, we found several alterations of the lipid profile in patients with low SC adipose tissue lamin A/C mRNA expression. The associations with HDL cholesterol levels and cholesterol to HDL cholesterol ratio show that patients with low lamin A/C expression have a reduced proportion of their blood cholesterol in the HDL fraction, which is suggestive of altered net movement of cholesterol from extrahepatic tissues back to the liver [32]. The finding of lower HDL_2 cholesterol and HDL_2 cholesterol to HDL_3 cholesterol ratio in subjects with low SC lamin A/C mRNA expression suggests a reduced proportion of cholesterol in the larger lipoprotein moiety of the HDL fraction (HDL_2 particles). This finding would be consistent with increased HDL particle clearance [32] in patients with low adipose

Table 3
Comparison of adiposity measures in women with either low or high LMNA mRNA expression in SC and OM fat samples

	SC adipose tissue			OM adipose tissue		
	Low LMNA	High LMNA	*P* value	Low LMNA	High LMNA	*P* value
Weight (kg)[b]	70.2 ± 15.4	69.3 ± 13.5	.89	70.8 ± 16.5	68.7 ± 12.0	.77
BMI (kg/m^2)	25.3 ± 5.4	26.1 ± 4.6	.50	27.1 ± 5.8	26.4 ± 4.1	.72
Fat mass (kg)	24.7 ± 8.4	24.2 ± 8.7	.88	25.0 ± 9.8	23.8 ± 7.0	.69
Lean body mass (kg)	43.5 ± 7.3	42.6 ± 6.2	.72	43.4 ± 7.3	42.7 ± 6.3	.75
Total AT area (cm^2)[a]	416 ± 153	396 ± 158	.72	412 ± 182	399 ± 123	.81
Visceral AT area (cm^2)[a,b]	107 ± 51	81 ± 30	.13	95 ± 42	93 ± 45	.96
SC AT area (cm^2)[a]	311 ± 112	315 ± 132	.93	320 ± 147	306 ± 88	.75[c]

Subgroups were identified on the basis of median LMNA expression in each fat depot. AT indicates adipose tissue.
[a] n = 33 for these measures.
[b] Analyses performed on $\log_{10}$-transformed data.
[c] Welch analysis of variance allowing unequal variances.

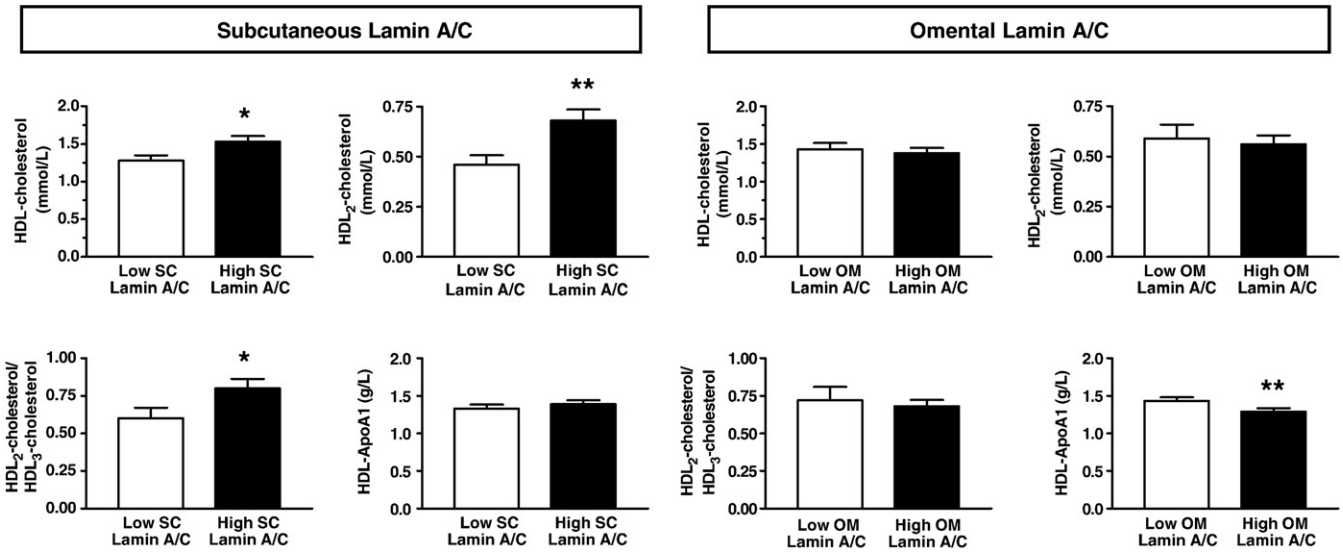

Fig. 1. Comparison of plasma HDL composition measures in women with either low or high lamin A/C mRNA expression in SC or OM fat samples. Subgroups were identified on the basis of median lamin A/C expression in each fat depot. *$P < .05$ and **$P < .005$.

tissue lamin A/C expression. On the other hand, our finding of increased VLDL–apo B, apo B to non–HDL cholesterol, and LDL–apo B to LDL cholesterol ratios in subjects with low SC lamin A/C expression may reflect increased number and reduced size of apo B–containing lipoproteins. This phenotype may result from increased hepatic release of triglyceride-rich lipoproteins, reduced triglyceride hydrolysis, and triglyceride enrichment of these lipoproteins. Accordingly, we also found significantly higher LDL-triglyceride content in subjects with low SC adipose tissue lamin A/C expression. Overall, these findings are concordant with observations in lipodystrophic individuals. Indeed, other studies have shown that *LMNA* mutations causing lipodystrophy are clearly linked to increased plasma triglyceride levels and decreased HDL cholesterol [33,34].

Our study is based on the postulate that quantitative alterations in the expression level of the LMNA gene cause or may be associated with alterations in adipocyte differentiation, development, life span, or function [35]. Worman and Courvalin [1] suggested that because Lmna-null mice do not develop lipodystrophy [36,37], this phenotype may not result from loss of lamin A/C function. They rather suggested that adipocyte loss in lipodystrophies caused by lamin A/C mutations may result from dominant interference with a specific lamin-mediated activity necessary for the survival of differentiated adipocytes [1]. We propose that such activity may also possibly be altered in the presence of lower lamin A/

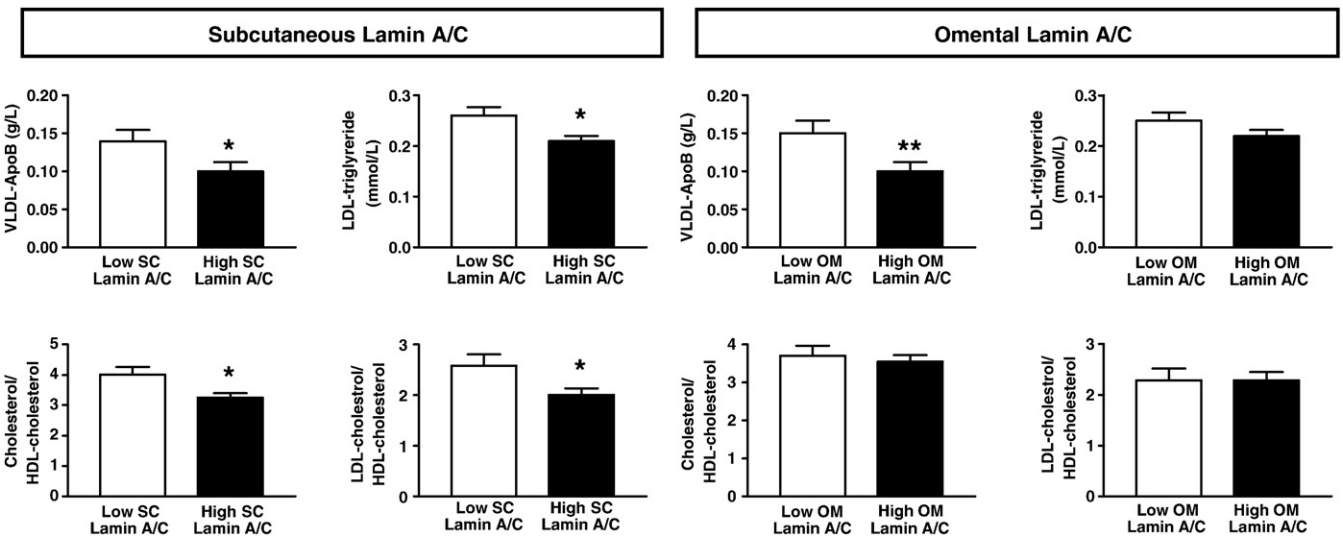

Fig. 2. Comparison of plasma VLDL–apo B, LDL-triglyceride, and lipid ratios in women with either low or high lamin A/C mRNA expression in SC or OM fat samples. Subgroups were identified on the basis of median lamin A/C expression in each fat depot. *$P < .05$ and **$P < .005$.

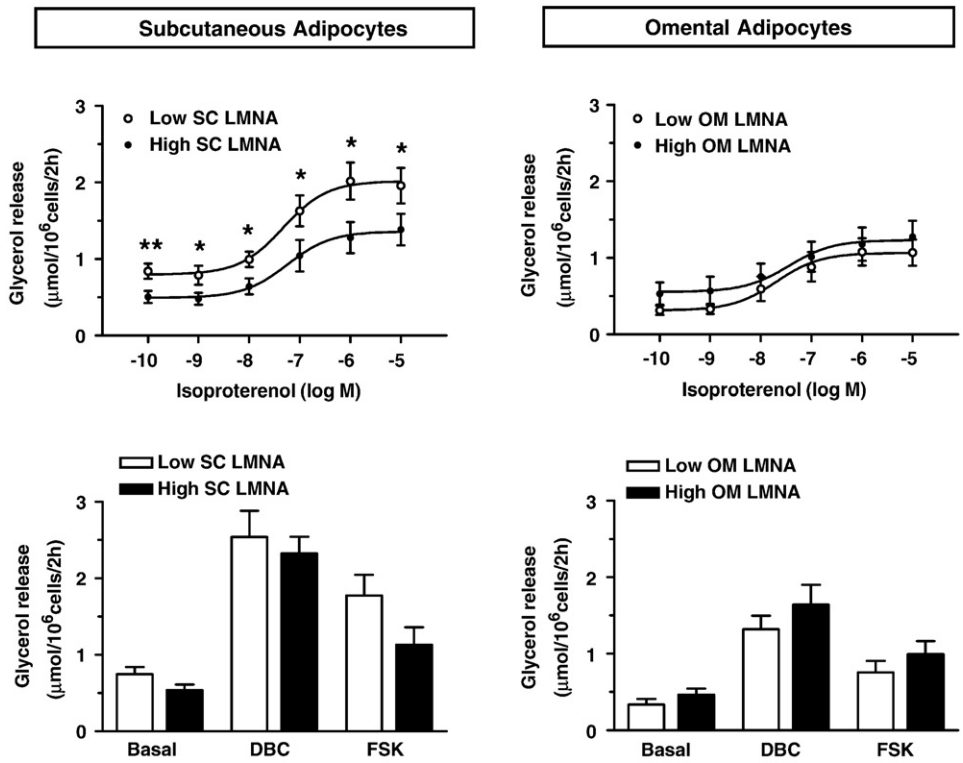

Fig. 3. Comparison of isolated SC or OM adipocyte lipolysis in women with either low or high lamin A/C mRNA expression in SC or OM fat samples, respectively. Subgroups were identified on the basis of median lamin A/C expression in each fat depot. DBC indicates dibutyryl cAMP; FSK, forskolin. *$P < .05$ and **$P < .005$.

C expression levels. Of note, lamins A and C have been hypothesized as essential factors in the time-specific expression of certain genes [1]. According to this hypothesis, mutations in the LMNA gene could alter chromatin compartmentalization or protein scaffolding of certain genes with regulatory functions, including SREBP1 [1]. Whether low lamin A/C mRNA expression acts through these mechanisms is unknown and warrants further investigation.

Interestingly, we found no association between lamin A/C expression and adiposity measures or adipocyte size. Moreover, subjects with either low or high lamin A/C expression had identical adiposity values, including total fatness or visceral adipose tissue accumulation. Although this finding is at variance with a previous investigation [23], we suggest that it is unlikely to be related to low statistical power or methodological limitations because criterion measures of adiposity were used and most adiposity values were very close in the subgroup analysis. We cannot exclude that examining a larger sample or more obese individuals could lead to significant differences in lamin A/C expression between obese and lean individuals. However, our results suggest that adiposity, either total or regional, is not a strong correlate of lamin A/C expression.

In the presence of a positive energy imbalance, adipose tissue accumulates the excess energy in the form of triglycerides; and when the storage capacity of adipose tissue is reached, metabolic disturbances are observed, including insulin resistance and hypertriglyceridemia [38,39]. PPARγ and SREBP1 play crucial roles in adipogenesis and lipogenesis in adipose tissue, and their activity increases the expandability of adipose tissue [40]. Gray and Vidal-Puig [39] have suggested that it is not the absolute amount of adipose tissue but rather the capacity of adipose tissue to expand that affects metabolic homeostasis. As mentioned, lamin A/C may be involved in the activation of the SREBP1/PPARγ pathway. Accordingly, increased stimulation of adipogenesis would provide better buffering of lipid fluxes by adipose tissue. Although our study cannot identify the precise mechanism of the lamin-adipogenesis interaction, our results suggest that low lamin A/C, by being correlated with an altered lipid profile independent of adiposity, possibly reflects a reduced fat storage capacity, especially in the SC depot.

Women with low lamin A/C expression had increased lipolysis in SC adipose tissue, a phenomenon suggestive of a high free fatty acid release that could possibly lead to the dyslipidemic state observed. Lipoprotein lipase plays a key role in the clearance of plasma triglycerides and free fatty acids by adipocytes. It has been reported that overexpression of LPL in a given tissue is associated with intracellular accumulation of fatty acid–derived metabolites in this tissue [41]. Even if we did not observe a correlation between adipose tissue lamin A/C expression and LPL activity, increased lipolysis in the face of unaltered LPL activity

would still be indicative of a net efflux of lipids from adipose tissue. However, examination of other indicators of lipogenesis including insulin-stimulated triglyceride synthesis is needed to rule out a link between lamin A/C expression and this process. In our lipolysis experiments, in addition to testing the responsiveness of fat cells to isoproterenol, a β_1/β_2 adrenergic agonist, we also tested the effects of postreceptor-acting agents including dibutyryl cAMP and forskolin. Interestingly, despite clear differences in the responsiveness to isoproterenol, no significant difference was found in the responsiveness to postreceptor-acting agents. These findings suggest that low lamin A/C expression is specifically associated with β-adrenergic receptor–mediated lipolytic alterations.

Limitations of this study need to be acknowledged. The cross-sectional nature of our study prevents us from reaching conclusions on causality. Thus, it is not possible to clearly conclude that low lamin A/C expression is indeed the cause of the blood lipid alterations that we observed. In addition, the limited size of adipose tissue biopsies did not allow us to measure protein levels of lamin A/C and prelamin A. Posttranscriptional and translational modifications may also affect the function of lamin A/C. Further in vitro studies are required to address these issues.

In conclusion, low lamin A/C mRNA expression in SC adipose tissue is associated with significant alterations in the lipid profile and increased fat cell lipolysis, independent of the level of obesity or fat cell size. Low lamin A/C may reflect a lower fat storage capacity associated with high lipolytic rates and dyslipidemia.

Acknowledgment

This study was supported by operating funds from the Canadian Institutes of Health Research Institute of Gender and Health to André Tchernof (MOP-64182). The contribution of Drs Marleen Daris, Céline Bouchard, Caroline Rhéaume, Jacques Mailloux, Céline Huot, and Michel Fortier is gratefully acknowledged. We acknowledge the invaluable help of nurses from the outpatient care unit and of radiology technicians. Expression measurements were performed by the Gene Quantification core laboratory of the Centre de Génomique de Québec. We thank all women who participated in the study for their excellent collaboration. AT is the recipient of a senior scholarship from the Fonds de la recherche en santé du Québec.

References

[1] Worman HJ, Courvalin JC. Nuclear envelope, nuclear lamina, and inherited disease. Int Rev Cytol 2005;246:231-79.
[2] Gruenbaum Y, Margalit A, Goldman RD, et al. The nuclear lamina comes of age. Nat Rev Mol Cell Biol 2005;6:21-31.
[3] Goldman RD, Gruenbaum Y, Moir RD, et al. Nuclear lamins: building blocks of nuclear architecture. Genes Dev 2002;16:533-47.
[4] Zastrow MS, Vlcek S, Wilson KL. Proteins that bind A-type lamins: integrating isolated clues. J Cell Sci 2004;117:979-87.
[5] Foisner R, Gerace L. Integral membrane proteins of the nuclear envelope interact with lamins and chromosomes, and binding is modulated by mitotic phosphorylation. Cell 1993;73:1267-79.
[6] Ostlund C, Ellenberg J, Hallberg E, et al. Intracellular trafficking of emerin, the Emery-Dreifuss muscular dystrophy protein. J Cell Sci 1999;112(Pt 11):1709-19.
[7] Luderus ME, den Blaauwen JL, de Smit OJ, et al. Binding of matrix attachment regions to lamin polymers involves single-stranded regions and the minor groove. Mol Cell Biol 1994;14:6297-305.
[8] Taniura H, Glass C, Gerace L. A chromatin binding site in the tail domain of nuclear lamins that interacts with core histones. J Cell Biol 1995;131:33-44.
[9] Lloyd DJ, Trembath RC, Shackleton S. A novel interaction between lamin A and SREBP1: implications for partial lipodystrophy and other laminopathies. Hum Mol Genet 2002;11:769-77.
[10] Mancini MA, Shan B, Nickerson JA, et al. The retinoblastoma gene product is a cell cycle–dependent, nuclear matrix–associated protein. Proc Natl Acad Sci U S A 1994;91:418-22.
[11] Dreuillet C, Tillit J, Kress M, et al. In vivo and in vitro interaction between human transcription factor MOK2 and nuclear lamin A/C. Nucleic Acids Res 2002;30:4634-42.
[12] Holaska JM, Lee KK, Kowalski AK, et al. Transcriptional repressor germ cell-less (GCL) and barrier to autointegration factor (BAF) compete for binding to emerin in vitro. J Biol Chem 2003;278: 6969-75.
[13] Martelli AM, Bortul R, Tabellini G, et al. Molecular characterization of protein kinase C–alpha binding to lamin A. J Cell Biochem 2002;86:320-30.
[14] Tang K, Finley Jr RL, Nie D, et al. Identification of 12-lipoxygenase interaction with cellular proteins by yeast two-hybrid screening. Biochemistry 2000;39:3185-91.
[15] Rao L, Modha D, White E. The E1B 19K protein associates with lamins in vivo and its proper localization is required for inhibition of apoptosis. Oncogene 1997;15:1587-97.
[16] Parnaik VK, Manju K. Laminopathies: multiple disorders arising from defects in nuclear architecture. J Biosci 2006;31:405-21.
[17] Shackleton S, Lloyd DJ, Jackson SN, et al. LMNA, encoding lamin A/C, is mutated in partial lipodystrophy. Nat Genet 2000;24:153-6.
[18] Dunnigan MG, Cochrane MA, Kelly A, et al. Familial lipoatrophic diabetes with dominant transmission. A new syndrome. Q J Med 1974;43:33-48.
[19] Gotzmann J, Foisner R. A-type lamin complexes and regenerative potential: a step towards understanding laminopathic diseases? Histochem Cell Biol 2006;125:33-41.
[20] Boguslavsky RL, Stewart CL, Worman HJ. Nuclear lamin A inhibits adipocyte differentiation: implications for Dunnigan-type familial partial lipodystrophy. Hum Mol Genet 2006;15:653-63.
[21] Kudlow BA, Jameson SA, Kennedy BK. HIV protease inhibitors block adipocyte differentiation independently of lamin A/C. AIDS 2005;19:1565-73.
[22] Lelliott CJ, Logie L, Sewter CP, et al. Lamin expression in human adipose cells in relation to anatomical site and differentiation state. J Clin Endocrinol Metab 2002;87:728-34.
[23] Miranda M, Chacon MR, Gutierrez C, et al. LMNA mRNA expression is altered in human obesity and type 2 diabetes. Obesity (SilverSpring) 2008;16:1742-8.
[24] Deschenes D, Couture P, Dupont P, et al. Subdivision of the subcutaneous adipose tissue compartment and lipid-lipoprotein levels in women. Obes Res 2003;11:469-76.
[25] Moorjani S, Dupont A, Labrie F, et al. Increase in plasma high density lipoprotein concentration following complete androgen blockage in men with prostatic carcinoma. Metabolism 1987;36:244-50.
[26] Gidez LI, Miller GJ, Burstein M, et al. Separation and quantitation of subclasses of human plasma high density lipoproteins by a simple precipitation procedure. J Lipid Res 1982;23:1206-23.
[27] Laurell CB. Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. Anal Biochem 1966;15:45-52.

[28] Rodbell N. Metabolism of isolated fat cells. J Biol Chem 1964;239:375-80.
[29] Kather H, Schroder F, Simon B. Microdetermination of glycerol using bacterial NADH-linked luciferase. Clin Chim Acta 1982;120: 295-300.
[30] Taskinen MR, Nikkila M, Huttunen JK, et al. A micromethod for assay of lipoprotein lipase activity in needle biopsy samples of human adipose tissue and skeletal muscle. Clin Chim Acta 1980;104:107-17.
[31] Anonymous. Executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001;285:2486-97.
[32] Lamarche B, Rashid S, Lewis GF. HDL metabolism in hypertriglyceridemic states: an overview. Clin Chim Acta 1999;286:145-61.
[33] Garg A. Gender differences in the prevalence of metabolic complications in familial partial lipodystrophy (Dunnigan variety). J Clin Endocrinol Metab 2000;85:1776-82.
[34] Robbins DC, Horton ES, Tulp O, et al. Familial partial lipodystrophy: complications of obesity in the non-obese? Metabolism 1982;31: 445-52.
[35] Simha V, Garg A. Lipodystrophy: lessons in lipid and energy metabolism. CurrOpinLipidol 2006;17:162-9.
[36] Cutler DA, Sullivan T, Marcus-Samuels B, et al. Characterization of adiposity and metabolism in Lmna-deficient mice. Biochem Biophys Res Commun 2002;291:522-7.
[37] Sullivan T, Escalante-Alcalde D, Bhatt H, et al. Loss of A-type lamin expression compromises nuclear envelope integrity leading to muscular dystrophy. J Cell Biol 1999;147:913-20.
[38] Lewis GF, Carpentier A, Adeli K, et al. Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes. Endocr Rev 2002;23:201-29.
[39] Gray SL, Vidal-Puig AJ. Adipose tissue expandability in the maintenance of metabolic homeostasis. Nutr Rev 2007;65:S7-12.
[40] Medina-Gomez G, Gray S, Vidal-Puig A. Adipogenesis and lipotoxicity: role of peroxisome proliferator–activated receptor gamma (PPARgamma) and PPARgamma coactivator–1 (PGC1). Public Health Nutr 2007;10:1132-7.
[41] Kim JK, Fillmore JJ, Chen Y, et al. Tissue-specific overexpression of lipoprotein lipase causes tissue-specific insulin resistance. Proc Natl Acad Sci U S A 2001;98:7522-7.