

# Adipose Tissue Infiltration in Skeletal Muscle of Healthy Elderly Men: Relationships With Body Composition, Insulin Resistance, and Inflammation at the Systemic and Tissue Level

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**Background.** Association between inflammatory markers and intermuscular adipose tissue (IMAT) has been reported. We hypothesized that subclinical inflammation of adipose tissue surrounding and infiltrating muscle could be related to the metabolic and functional abnormalities of the “aging muscle.”

**Methods.** In 20 healthy elderly men undergoing elective vertebral surgery, IMAT within erector spinae was evaluated by magnetic resonance imaging and body composition by dual-energy x-ray absorptiometry. Fasting glucose, insulin, high-sensitive C-reactive protein (hs-CRP), leptin, adiponectin, and interleukin 6 (IL-6) were measured, and insulin resistance was estimated by homeostasis model assessment (HOMA) index. In subcutaneous adipose tissue (SAT) biopsies near the erector spinae, quantification of gene expression was performed.

**Results.** IMAT showed a significant association with body mass index and total and regional body fat, even after adjustment for age. Insulin, HOMA, and leptin were significantly correlated with IMAT, whereas hs-CRP presented an association of borderline significance. IL-6 expression in SAT was significantly associated with IMAT; IL-6 messenger RNA (mRNA) was negatively associated with adiponectin and peroxisome proliferator-activated receptor gamma expression. In multivariate regression analysis, 68% of IMAT variance was explained by fat mass and age, independent of waist circumference, leptin, HOMA, and IL-6 mRNA.

**Conclusion.** IMAT was primarily related to age and total body adiposity; subclinical inflammation in fat significantly contributes to IMAT.

**Key Words:** IMAT—Body composition—Insulin resistance—Inflammation—Aging.

**A**DIPPOSE tissue deposition in skeletal muscle, defined as intermuscular adipose tissue (IMAT) within the fascia, has been recently emerged as the object of great interest in geriatric and metabolic research.

IMAT has been proposed as an independent risk factor for insulin resistance and diabetes (1). The degree of fat infiltration into skeletal muscle, liver, and other organs is highly correlated with insulin sensitivity (2) and has been considered as an early participant in the development of insulin resistance and diabetes (3), reversed by weight loss (4).

Muscle fat infiltration has been observed to be related to age and adiposity among different race groups (5–8) and to be associated not only with metabolic abnormalities (1–4,9) but also with poorer strength and scores on performance tests (5,10), as well as with incident mobility disability (11).

In the Health ABC Study population, thigh intermuscular fat was significantly associated with higher concentrations of inflammatory markers (12). Insulin resistance and type 2

diabetes, obesity, and the age-related loss of muscle mass and quality, termed sarcopenia, have all been linked to a chronic subclinical inflammation state (13).

Thus, it is tempting to speculate that a chronic subclinical inflammation of adipose tissue surrounding and infiltrating muscle could be related to the metabolic and functional abnormalities of the “aging muscle.”

To test this hypothesis, we studied, as a source of tissue inflammation, subcutaneous adipose tissue (SAT) near the muscle, evaluated by magnetic resonance imaging (MRI) for IMAT. We examined the relationships between IMAT, systemic and tissue inflammation, and insulin resistance in a small sample of healthy elderly men.

## METHODS

A total of 20 men, aged between 58 and 80 years with body mass index (BMI) ranging from 24.5 to 44 kg/m<sup>2</sup>, were studied.

All participants were selected among those undergoing elective surgery for medullar lumbar stenosis in the Department of Neurosurgery of our hospital.

All individuals had been weight stable over the previous 6 months, in general good health, and had no history of type 2 diabetes. Patients receiving steroid and/or immunosuppressive medications in the previous 6 months and using anti-inflammatory medications more than once a week were excluded.

All participants gave their informed consent, and the experimental protocol was approved by the Ethical Committee of our hospital.

#### *Anthropometry and Body Composition Measurements*

BMI was computed as weight divided by stature squared (kilograms per square meter); waist circumference was obtained as previously described (4).

Body composition was studied using dual-energy x-ray absorptiometry (Hologic QDR 4500; Waltham, MA) fan beam with software version 8.21. Total body fat mass (FM) was expressed in kilograms and as percentage of body weight (FM%). Lean total body mass (LFM) was the difference between fat-free mass and bone mineral mass and expressed in kilograms. Appendicular skeletal muscle mass was calculated as the sum of arms and legs lean soft tissue masses. As index of sarcopenia, appendicular lean mass to squared height ratio (ASMMI) was used (14).

The coefficient of variation for double determinations in 10 elderly participants was 1% for FM and 1.3% for LFM.

#### *Study of Muscle Fat Infiltration With MRI*

Participants were positioned supine on a MRI scanner, and transverse sections were obtained at L3–L4 disk level using a 1.0-T Siemens Armony Expert (Siemens AG, Erlangen, Germany). A high-resolution T1-weighted sequence was obtained for every participant. Erector spinae measurements on the right and left side (mean values) were analyzed (15) using Sliceomatic image software (version 4.2; Tomovision, Montreal, Canada).

IMAT was defined as adipose tissue area visible between muscle groups and beneath the muscle fascia (6). Muscle area (MA) was the difference between total area (TA) and IMAT; the IMAT/MA ratio was also calculated. Intraclass correlation coefficients for IMAT, TA, and MA were all higher than 0.97.

#### *Subcutaneous Fat Biopsies*

SAT biopsies were taken at the beginning of vertebral surgery in the site of surgical incision (L3–L4) in a standardized manner. Adipose tissue was immediately frozen for gene expression analysis with real-time polymerase chain reaction (PCR).

Total RNA was extracted with Qiazol (Qiagen, Germantown, MA) and chloroform, and the aqueous phase was purified using an RNeasy Mini or Micro Kit (Qiagen). Fifteen nanograms of total RNA for each gene was reverse transcribed into complementary DNA in 20  $\mu$ L reactions with the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA). Aliquots of the reverse transcriptase were PCR amplified with QuantiTect SYBR Green PCR Kit (Qiagen) and with QuantiTect Primer Assays (Qiagen) for each gene in the iCycler Thermocycler (Bio-Rad). Gene expression was normalized against 18S rRNA for each sample and performed in triplicate (Bio-Rad iQ5 software).

#### *Biochemical Analyses*

Venous blood samples were obtained after overnight fasting. Plasma glucose was measured using a glucose analyzer and plasma immunoreactive insulin by double antibody radioimmunoassay using a commercial kit. Insulin resistance was estimated with the homeostasis model assessment (HOMA) method. Serum leptin, adiponectin, and interleukin 6 (IL-6) were measured using specific enzyme-linked immunosorbent assay kits. High-sensitive C-reactive protein (hs-CRP) was measured with the immunoturbidimetric method.

#### *Statistical Analysis*

Log transformation was performed before analysis for not normal variables. Comparison of the main characteristics of the study sample, stratified for BMI (25–30  $\text{kg}/\text{m}^2$  and  $>30 \text{ kg}/\text{m}^2$ ), was performed by analysis of variance. Pearson and partial correlations were used to test associations between variables. Backward regression analysis was used to test the effects of independent variables on IMAT. Analyses referring to MRI measurements or gene expression in adipose tissue were available only for 18 participants.

The level for statistical significance was  $p < .05$ . All statistical analyses were performed using the SPSS 16.0 for Windows.

## **RESULTS**

Waist circumference, FM and FM% (all  $p < .001$ ), LFM and ASMMI (all  $p < .05$ ), and IMAT ( $p < .05$ ) were significantly higher in obese than in overweight participants. Obese participants had significantly higher levels of hs-CRP, insulin, and HOMA (all  $p < .05$ ) as well as of serum leptin ( $p < .001$ ). In the whole study sample, age was negatively related to MA, IMAT, and IMAT/MA (all  $p < .05$ ). BMI, weight, waist circumference, FM, and FM% presented significant correlations with IMAT (all  $p < .01$ ) and IMAT/MA (all  $p < .05$ ). ASMMI did not correlate with IMAT nor with IMAT/MA. After adjustment for age, associations between FM, waist circumference and, respectively, IMAT ( $p < .01$ ), and IMAT/MA ( $p < .05$ ) were still significant.

Table 1 shows correlations between metabolic variables, indices of inflammation, anthropometry, body composition, and muscle fat infiltration. Hs-CRP was related to BMI, total body fat, and waist circumference. Insulin and HOMA were significantly associated with BMI, weight, FM, FM%, waist circumference, and IMAT. Serum leptin was positively associated with all indices of adiposity and IMAT, whereas no significant association between circulating adiponectin or IL-6 and IMAT was found.

Hs-CRP presented a significant association with leptin ( $r = .641, p < .01$ ) and adiponectin ( $r = -.508, p < .05$ ). Insulin and HOMA were significantly and positively related to serum leptin ( $r = .746, p < .001$  and  $r = .713, p < .001$ , respectively).

Gene expression of IL-6 in SAT near the erector spinae presented a significant negative association with adiponectin gene expression and a borderline negative association with peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ) (Table 2). Adiponectin and PPAR $\gamma$  gene expressions were highly related to each other, and both were significantly correlated to vascular endothelial growth factor expression in SAT (Table 2). Insulin receptor substrate-1 (IRS-1) showed a significant positive association with PPAR $\gamma$  and a trend toward a positive association also with adiponectin; glucose transporter 4 (GLUT-4) and IRS-1 gene expressions in SAT were positively related to each other (Table 2).

Among the genes analyzed, only IL-6 expression in SAT was significantly associated with IMAT ( $r = .504, p < .05$ ) and IMAT/MA ( $r = .484, p < .05$ ).

We found a negative correlation between GLUT-4 and IRS-1 expressions in adipose tissue and insulin ( $r = -.496, p < .05$  and  $r = -.518, p < .05$ , respectively) or HOMA ( $r = -.582, p < .01$  and  $r = -.542, p < .05$ , respectively). Moreover, a significant negative relation between hs-CRP

levels and PPAR $\gamma$  gene expression in SAT was observed ( $r = -.523, p < .05$ ).

In Table 3 are presented different models of backward regression analysis where IMAT was the dependent variable and age, waist circumference, FM, serum leptin, HOMA, and IL-6 gene expression in SAT the independent variables. About 68% of total variance of IMAT was explained by FM and age, independently of other factors.

**DISCUSSION**

Our data show, in a group of healthy overweight and obese elderly men, that IMAT increases with age, adiposity, and subclinical local inflammation, as evaluated by IL-6 gene expression in SAT near the erector spinae that was positively related to the degree of adipose tissue infiltration in skeletal muscle.

Our study confirms previous findings of a significant and strong relation between age, total body adiposity, and IMAT measured by midhigh CT (5,16), whole-body MRI (6,8), or <sup>1</sup>H nuclear magnetic resonance spectroscopy of the calf (9) and expands them also to a group of muscles previously unstudied.

The mechanisms by which an increase in total body adiposity may lead to an increase in adipose tissue infiltrating muscle are still not understood (2).

Fat cell derived peptides have been recently proposed to explain the link between increasing adiposity, insulin resistance, and IMAT (17). We observed a strong association between serum leptin and IMAT. High serum level of leptin, as observed in obese participants, has been suggested as surrogate marker of leptin resistance (18,19). Thus, leptin resistance may lead to lower fatty acid oxidation in muscle and to ectopic fat deposition and insulin resistance (18). In turn, this downregulation of lipid oxidation may lead to an

Table 1. Correlations Between Metabolic Variables, Indices of Inflammation, Anthropometry, Body Composition, and Muscle Fat Infiltration Measured by MRI (n = 20 men)

Variables <sup>†</sup>	hs-CRP <sup>‡</sup>	Glucose	Insulin	HOMA	Leptin <sup>‡</sup>	Adiponectin <sup>‡</sup>	IL-6 <sup>‡</sup>
Age	0.332	-0.081	-0.046	-0.003	0.255	0.173	0.101
BMI	0.438*	-0.230	0.604**	0.482*	0.770***	-0.125	0.272
Weight	0.302	-0.334	0.594**	0.466*	0.722***	-0.069	0.214
Waist circumference	0.446*	-0.156	0.434*	0.380	0.737***	-0.054	0.208
FM	0.560*	-0.125	0.674**	0.612**	0.864***	-0.167	0.226
FM%	0.703**	0.124	0.554**	0.583**	0.794***	-0.224	0.162
LFM	0.058	-0.407	0.462*	0.314	0.507*	0.006	0.170
ASMMI	0.023	-0.452*	0.422	0.237	0.456*	-0.114	0.242
MA	0.081	-0.194	0.133	0.017	0.010	-0.259	0.159
IMAT	0.404	-0.214	0.554**	0.499*	0.764***	0.012	0.106
IMAT/MA	0.257	-0.076	0.371	0.378	0.593*	0.116	-0.015

Notes: ASMMI = appendicular lean mass to squared height ratio (measured by DXA); BMI = body mass index; DXA = dual-energy x-ray absorptiometry; FM = fat mass (measured by DXA); FM% = fat mass percentage (measured by DXA); HOMA = homeostasis model assessment of insulin resistance; hs-CRP = high-sensitive C-reactive protein; IL-6 = interleukin 6; IMAT = intermuscular adipose tissue area (measured by MRI in 18 men); LFM = lean total body mass (measured by DXA); MA = muscle area (measured by MRI in 18 men); MRI = magnetic resonance imaging.

\* $p < .05$ ; \*\* $p < .01$ ; \*\*\* $p < .001$ .

<sup>†</sup>r values are shown.

<sup>‡</sup>Log transformation before analysis.

Table 2. Reciprocal Correlations Between Subcutaneous Fat Gene Expression of Leptin, Adiponectin, IL-6, PPAR $\gamma$ , VEGF, GLUT-4, and IRS-1 ( $n = 18$  men)

	Leptin	Adiponectin	IL-6	PPAR $\gamma$	VEGF	GLUT-4	IRS-1
Leptin	1 <sup>†</sup>						
Adiponectin	-0.019	1					
IL-6	0.314	-0.466*	1				
PPAR $\gamma$	-0.150	0.848***	-0.442	1			
VEGF	-0.183	0.611**	-0.251	0.704**	1		
GLUT-4	0.033	0.219	-0.238	0.197	0.279	1	
IRS-1	0.018	0.419	-0.396	0.479*	0.033	0.606*	1

Notes: GLUT-4 = glucose transporter 4; IL-6 = interleukin 6; IRS-1 = insulin receptor substrate-1; PPAR $\gamma$  = peroxisome proliferator-activated receptor gamma; VEGF = vascular endothelial growth factor.

\* $p < .05$ ; \*\* $p < .01$ ; \*\*\* $p < .001$ .

<sup>†</sup>  $r$  values are shown; all variables were log transformed before analysis.

accumulation of lipid metabolites interfering with insulin signaling (2).

Subacute low-grade inflammation has been associated with obesity, insulin resistance, and type 2 diabetes. In this study, elderly participants with higher levels of hs-CRP tended to have greater IMAT, at least partially in agreement with a previous report describing association between C-reactive protein and thigh intermuscular fat (12).

However, the mechanisms linking inflammation and intermuscular fat are not yet clear. It is known that inflammation could be dependent on the secretory profile of many adipocyte-derived factors, acting both at a local and at a

systemic level. Interestingly, we observed a significant positive relation between IL-6 expression in SAT near the erector spinae and IMAT, but not between circulating IL-6 and IMAT, partially in contrast with recent observations (12), but in agreement with other studies (20,21). Because it is thought that much of the effect of cytokines occurs in a paracrine or autocrine fashion, circulating levels may not be accurate reflections of the in vivo activity of cytokines at the tissue level. The existence of a relation between IMAT and IL-6 expression in SAT near the erector spinae seems thus to suggest a prevalent role of tissue inflammation than of systemic inflammation.

In this study, we observed a negative relation between IL-6, adiponectin, and PPAR $\gamma$  gene expression in SAT. Furthermore, hs-CRP was negatively related to PPAR $\gamma$ , a transcription factor involved in fatty acid oxidation (17). It is suggestive to hypothesize that systemic inflammation may impair whole-body fat oxidation, leading to ectopic fat accumulation by decreasing PPAR $\gamma$  gene expression with downregulation of adiponectin and upregulation of IL-6 production in adipose tissue.

Finally, in SAT biopsies, we found a positive association between PPAR $\gamma$  and IRS-1, a marker of tissue insulin resistance. Moreover, GLUT-4 and IRS-1 expressions in SAT were strongly related to each other and to systemic insulin resistance. These data may suggest a possible link between inflammation, IMAT, and insulin resistance at the tissue and systemic level.

Some limitations of the present study should be acknowledged. First, the small sample size confers to study low statistical power, and the lack of women and of physical activity does not allow generalization of the present findings. Second, the design of the study allows us only to describe associations and not cause-effect relationships. Third, the single slice measure of IMAT obtained at L3-L4 level could not entirely account for whole-body IMAT (22).

Wider population studies are needed to better explain the mechanisms involved in the interplay between muscle fat infiltration, inflammation, and insulin resistance and to support the hypothesis that inflammation and insulin resistance in fat represent the secondary mechanisms by which

Table 3. Backward Regression Analysis Where IMAT Was the Dependent Variable and Age, Waist Circumference, FM, Serum Leptin, HOMA, and Subcutaneous Fat IL-6 Expression Were the Independent Variables ( $n = 18$  men)

Variables Entered in the Models	Total $R^2$	$\beta$ -Coefficient	Significance
Waist circumference	0.584	-0.301	0.457
FM		0.634	0.244
Leptin		0.212	0.634
HOMA		-0.054	0.865
IL-6 (SAT)		0.176	0.563
Age		0.387	0.098
Waist circumference	0.629	-0.269	0.422
FM		0.632	0.218
Leptin		0.163	0.607
IL-6 (SAT)		0.154	0.550
Age		0.398	0.062
Waist circumference	0.656	-0.292	0.361
FM		0.807	0.035
IL-6 (SAT)		0.109	0.639
Age		0.423	0.035
Waist circumference	0.680	-0.289	0.348
FM		0.883	0.010
Age		0.418	0.030
FM	0.681	0.655	0.001
Age		0.370	0.039

Note: FM = fat mass (measured by dual-energy x-ray absorptiometry); HOMA = homeostasis model assessment of insulin resistance; IL-6 = interleukin-6; IMAT = intermuscular adipose tissue (measured by magnetic resonance imaging in 18 men).

increase in FM could lead to ectopic fat deposition in muscle.

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