Association between Interleukin-15 and Obesity: Interleukin-15 as a Potential Regulator of Fat Mass

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Objective: IL-15 decreases lipid deposition in preadipocytes and decreases the mass of white adipose tissue in rats, indicating that IL-15 may take part in regulating this tissue. IL-15 is expressed in human skeletal muscle and skeletal muscle may be a source of plasma IL-15 and in this way regulate adipose tissue mass.

Design: The relation between skeletal muscle IL-15 mRNA expression, plasma IL-15, and adipose tissue mass was studied in 199 humans divided into four groups on the basis of obesity and type 2 diabetes. Furthermore, using a DNA electrotransfer model, we assessed the effect of IL-15 overexpression in skeletal muscle of mice.

Results: In humans, multiple regression analysis showed a negative association between plasma IL-15 and total fat mass ($P < 0.05$), trunk fat mass ($P < 0.01$), and percent fat mass ($P < 0.05$), independent of type 2 diabetes. Negative associations were also found between muscle IL-15 mRNA and obesity parameters. IL-15 overexpression in skeletal muscle of mice reduced trunk fat mass but not sc fat mass.

Conclusions: Our results indicate that IL-15 may be a regulator of trunk fat mass. (***J Clin Endocrinol Metab* 93: 4486–4493, 2008**)

IL-15 is expressed in human skeletal muscle (1) and has been identified as an anabolic factor in muscle growth (2, 3). Interestingly, IL-15 exerts its effects on fully differentiated myotubes, independently of IGF-I (4). IL-15 stimulates protein synthesis and inhibits protein degradation in cultured skeletal myotubes (4). However, the main mechanism involved in the anabolic effects of IL-15 seems to rely on a decrease in the proteolytic rate (5).

In addition to its anabolic effects on skeletal muscle *in vitro* and *in vivo*, IL-15 may also take part in reducing adipose tissue mass. IL-15 administration to adult rats for 7 d results in a decrease in white adipose tissue mass (6). In leptin-deficient obese (ob/ob) mice, IL-15 induces a reduction in white adipose tissue and in lipoprotein lipase activity with no effect on food intake. The same effects are observed in lean Zucker rats. In contrast, leptin receptor-deficient Zucker rats do not respond to IL-15 administration. The tissue response to IL-15 is related to the amount of IL-15/IL-15 receptor complex expression, suggesting a direct action of IL-15 on adipose tissue (7).

Whereas IL-15 is expressed in muscle (1), little or no IL-15 mRNA has been detected in either the undifferentiated preadipocytes or the differentiated adipocytes (8). Administration of IL-15 inhibits lipid accumulation in differentiating 3T3-L1 preadipocyte cultures and stimulates secretion of adiponectin from 3T3-L1 adipocytes (8), supporting the hypothesis that IL-15 functions in a muscle-to-fat endocrine axis, which plays a role in regulating fat-lean body composition. The effect on adipose tissue mass and the apparent ability to stimulate adiponectin se-

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Abbreviations: BMI, Body mass index; CRP, C-reactive protein; DXA, dual-energy x-ray absorptiometry; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HbA1c, glycosylated hemoglobin; HOMA2-IR, homeostasis model assessment version 2 for insulin resistance; HRP, horseradish peroxidase; NGT, normal glucose tolerance; TAG, triacylglycerides; TC, tibialis cranialis.

cretion could further make IL-15 an important cytokine with regard to insulin sensitivity.

Accordingly, we hypothesized that muscle IL-15 mRNA and plasma IL-15 concentration would be associated with body composition. We included individuals with a wide range of body weight, with and without type 2 diabetes and demonstrated that high levels of plasma IL-15 were inversely associated with fat mass, independently of type 2 diabetes. These findings were supported by experiments in mice, in which IL-15 overexpression in muscles was found to be associated with reduced trunk fat mass.

Subjects and Methods

Human study

Subjects

Using a cross-sectional, case-control design, the participants in this study were divided into four distinct groups according to their body mass index (BMI) (<30 or ≥ 30 kg/m^2) and according to diagnosis of type 2 diabetes [normal glucose tolerance (NGT) or type 2 diabetes]. Subjects (n = 233) were recruited by advertising in a local newspaper, and information of diagnosis of type 2 diabetes was based on information from each subject respectively. To verify correct diagnosis, the World Health Organization diagnostic criteria for diabetes were used. Participants were carefully screened to isolate metabolic conditions, other than type 2 diabetes, known to influence body composition and the immune system. Exclusion criteria were treatment with insulin, recent or ongoing infection, history of malignant disease, or treatment with antiinflammatory drugs. Subject characteristics are given in Table 1. Participants with impaired glucose tolerance or participants who did not fulfill the criteria for any of the four main groups were excluded from the analysis (n = 34). Participants were given both oral and written information about the experimental procedures before giving their written informed consent. The study was approved by the Ethical Committee of Copenhagen and Frederiksberg communities, Denmark (KF 01-141/04) and performed according to the Declaration of Helsinki.

Protocol

Participants reported in the laboratory between 0800 and 1000 h after an overnight fast. Subjects did not take any medication for 24 h preceding the examination, and type 2 diabetics did not take oral antidiabetic medication for 1 wk preceding the examination day. A general health examination was performed. Blood samples were drawn from an antecubital vein. On the same day, one muscle biopsy was obtained, an oral glucose tolerance test and a fitness test were performed, and subjects were scanned on a dual-energy x-ray absorptiometry (DXA) whole-body scanner.

Oral glucose tolerance test

Blood samples were drawn before and 1 and 2 h after drinking 500 ml of water containing 75 g of dissolved glucose. The World Health Organization diagnostic criteria were applied.

Fitness test

Cardiorespiratory fitness was measured by the Åstrand-Rhyming indirect test of maximal oxygen uptake (9).

Body composition

Fat and fat-free tissue masses for the whole body, trunk and extremities were measured using DXA scanning (Lunar Prodigy Advance; GE Medical Systems Lunar, Milwaukee, WI). DXA scanning does not distinguish between sc and intraabdominal fat located in the trunk region. Software (Prodigy, enCORE 2004, version 8.8, GE Lunar Corp., Madison, WI) was used to estimate regional and total fat and fat-free tissue.

TABLE 1. General characteristics of the study population

	NGT					Type 2 diabetes					
	Nonobese		Obese			Nonobese		Obese			
n (male/female)	62	(42/20)	41	(28/13)		50	(38/12)	46	(34/12)		
Age (yr)	56	(53–59)	48	(45–52)	[b]	58	(55–61)	58	(55–61)		
Smoker (%)	17	(27%)	10	(24%)		11	(22%)	9	(20%)		
Fitness (VO_2/kg/FFM)	49.2	(45.8–52.9)	39.7	(36.4–43.2)	[b]	39.1	(35.9–42.7)	34.7	(31.7–37.9)		[a]
Body composition											
BMI (kg/m^2)	25.7	(24.8–26.6)	36.7	(35.6–37.8)	[b]	26.6	(25.6–27.6)	35.5	(34.4–36.5)	[b]	
Total fat mass (kg)	20.5	(18.1–22.9)	44.3	(41.4–47.2)	[b]	22.9	(20.8–25.0)	37.5	(35.3–39.7)	[b]	
Trunk fat mass (kg)	11.6	(10.4–12.9)	24.8	(23.4–26.3)	[b]	13.9	(12.8–15.1)	22.1	(20.9–23.3)	[b]	
Limb fat mass (kg)	8.2	(6.9–9.6)	18.5	(16.9–20.2)	[b]	8.3	(7.1–9.5)	14.5	(13.2–15.7)	[b]	
Total fat-free mass (kg)	52.4	(49.7–55.0)	67.3	(64.1–70.5)	[b]	54.3	(51.1–57.4)	65.4	(62.0–68.7)	[b]	
Metabolic regulation											
p-HDL (mM)	1.7	(1.5–1.8)	1.3	(1.2–1.4)	[b]	1.3	(1.2–1.4)	1.3	(1.2–1.4)		
p-LDL (mM)	3.6	(3.4–3.9)	3.3	(3.0–3.6)		2.9	(2.6–3.2)	3.0	(2.7–3.3)		[a]
p-TAG (mM)	1.0	(0.9–1.2)	1.4	(1.2–1.7)	[b]	1.6	(1.3–2.0)	1.5	(1.2–1.9)		
p-Glucose$_{0\ h}$ (mM)	5.1	(5.0–5.2)	5.2	(5.1–5.4)		9.1	(8.2–10.1)	9.0	(8.0–10.1)		[a]
p-Insulin$_{0\ h}$ (mM)	34.8	(29.8–40.6)	68.6	(56.9–82.6)	[b]	52.6	(43.3–64.0)	101	(82.5–124.0)	[b]	[a]
HOMA2-IR	0.66	(0.56–0.76)	1.28	(1.07–1.54)	[b]	1.22	(1.0–1.50)	2.27	(1.83–2.81)	[b]	[a]
HbA1c	5.5	(5.4–5.6)	5.6	(5.5–5.6)		7.3	(6.9–7.8)	6.9	(6.5–7.3)		[a]
Inflammation											
p-CRP (mg/liter)	1.6	(1.3–1.9)	3.9	(3.1–5.0)	[b]	2.3	(1.8–3.0)	3.7	(2.9–4.8)	[b]	

General characteristics of the study population were divided into four groups on the basis of definition of obesity and diagnosis of type 2 diabetes: NGT/nonobese, NGT/obese, type 2 diabetes/nonobese, and type 2 diabetes/obese. Data are presented as numbers for categorical variables and means with 95% confidence interval for continuous variables. VO_2/kg/FFM, Maximal oxygen consumption relative to fat-free body mass; p-HDL, plasma high-density lipoprotein; p-LDL, plasma low-density lipoprotein; p-TAG, plasma TAG; p-glucose, fasting plasma glucose; p-insulin, fasting plasma insulin; p-CRP, plasma CRP.

[a] Difference between glycaemia groups (NGT *vs.* type 2 diabetes).

[b] Difference between obesity groups within each glycaemia group.

Blood sampling

Plasma was obtained by drawing blood from an antecubital vein into glass tubes containing EDTA. The tubes were immediately spun at 3500 × *g* for 15 min at 4 C, and the plasma was isolated and stored at −20 C until further analysis. Plasma levels of cholesterol content of lipoprotein fractions and triacylglycerides (TAG), C-reactive protein (CRP), glucose, insulin, and glycosylated hemoglobin (HbA1c) were measured using routine methods. Based on the fasting plasma concentrations of glucose and insulin, the level of insulin resistance was calculated using the homeostasis model assessment version 2 (HOMA2-IR) from 1996, because the original homeostasis model assessment (10) tends to overestimate insulin resistance (11).

Plasma concentrations of IL-15 were measured using a commercial kit (Quantikine; R&D Systems, Minneapolis, MN). To optimize the IL-15 assay, 100 μl of sample were used instead of 50 μl, for both subject samples and the standard curve. The range of the standard curve was 0.46–72.1 pg/ml, in which a double-logarithmic scale showed a linear relationship with $r^2 = 0.999$. All samples were within the range of the standard curve. The detection limit was calculated to be 0.23 pg/ml. The intra- and interassay coefficients of variation were validated within our work and were 2.7 and 7.7%, respectively.

Muscle biopsies

Muscle biopsies were obtained from the vastus lateralis of musculi quadriceps femoris using the percutaneous needle method with suction (12). Muscle tissue was immediately placed on an ice-cold glass plate. If present, superficial blood was quickly removed and the biopsy was frozen in liquid nitrogen and stored at −80 C until further analysis.

Muscle IL-15 mRNA

Total RNA was isolated from 20–25 mg of skeletal muscle tissue with TriZol (Life Technology, Foster City, CA) as described by the manufacturer. RNA was re-suspended in 15 μl diethyl pyrocarconate-treated H_2O. The concentration of the isolated RNA and the ratio of absorbance at 260:280 nm (A_{260}/A_{280} ratio) were measured with the FLUOstar Optima absorbance reader (BMG Labtechnologies, Offenburg, Germany). The threshold inclusion values for the RNA samples were greater than 1.9 for the A_{260}/A_{280} ratio.

Random hexamer primed reverse transcription reactions were performed from 2 μg of total RNA in a 100 μl setup using Taqman reverse transcription reagents following the manufacturer's instructions (Applied Biosystems, Foster City, CA). The reactions were run in a PerkinElmer GeneAmp PCR system 9700 (Applied Biosystems) with the following conditions: 25 C for 10 min, 48 C for 30 min, and 95 C for 5 min. Real-time PCR was performed on an ABI PRISM 7900HT sequence detector (Applied Biosystems). Each assay included a cDNA standard curve of five serial dilution points (ranging from 1 to 0.01), a no-template control, a no-reverse transcriptase control, and 150 ng [35 ng for 18S rRNA, β-actin, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH)] of each sample cDNA and was run in accordance to the manufacturer's instructions. The PCR amplification was performed in triplicates in a total reaction volume of 10 μl. The primers and probes used for 18S, β-actin, GAPDH, and IL-15 (Hs00542562_m1) were predeveloped TaqMan assays (Applied Biosystems). The amplification mixtures were amplified according to standard conditions (50 C for 2 min, 95 C for 10 min followed by 50 cycles of 95 C for 15 sec, 60 C for 1 min). The relative expression of IL-15 was determined after normalization to the endogenous control, β-actin.

Muscle IL-15 protein

Muscle tissue was freeze dried and dissected free of visual blood, fat, and connective tissues. Muscle lysate was then prepared by mixing the muscle tissue with a modified radioimmunoprecipitation assay cell lysis buffer [50 mM Tris-HCL (pH 7.4), 150 mM NaCl, 1 mM EGTA, 1 mM EDTA, 0,25% Na deoxycholate, 1% Triton X-100] containing 1 μg/ml Pepstatin A, 1 mM sodium-orthovanadate, 1 mM sodium-fluoride, and a complete protease inhibitor cocktail (Roche, Basel, Switzerland) followed by homogenization in precooled racks using a Tissuelyzer (QIAGEN, Valencia, CA) for 1 min at 30 Hz followed by 15 min incubation on ice. The homogenization and incubation on ice were repeated two or three times depending on the degree of homogenization of the tissue. Homogenates were then rotated end over end for 1 h at 4 C and centrifuged at 16,000 × *g* at 4 C for 1 h. The supernatant protein concentrations were determined using the Bio-Rad DC kit (Bio-Rad, Hercules, CA), using BSA as standard. All determinations were done in triplicates.

Twenty-five micrograms of muscle protein lysate per lane were boiled in Laemmli buffer, separated on 12% Bis-Tris gels (Invitrogen, Taastrup, Denmark), and transferred to polyvinyl difluoride membranes (Hybond-P; GE Healthcare, Little Chalfont, UK). Membranes were then blocked for 1 h at room temperature in blocking buffer (Tris-buffered saline with 0.1% Tween 20 and 5% Top-Block; Sigma-Aldrich, St. Louis, MO), washed three times for 5 min in wash buffer (Tris-buffered saline with 0.1% Tween 20), and cut in pieces to determine both IL-15 and β-actin in the same samples. Subsequently the membranes were incubated overnight at 4 C in blocking buffer containing a primary antibody against either human IL-15 (AF315; R&D Systems, Minneapolis, MN) at a final concentration of 0.2 μg/ml or against actin (A3853; Sigma) at a final concentration of 0.3 μg/ml. The membranes were then washed three times in wash buffer and incubated for 1 h at room temperature with rabbit-antigoat horseradish peroxidase (HRP; P0449) or rabbit-antimouse HRP (P0260) (Dako, Glostrup, Denmark) secondary antibody at a 1:2000 dilutions in blocking buffer, followed by three times 5 min washing in wash buffer. After detection using Supersignal West Femto (Pierce, Rockford, IL; IL-15) or enhanced chemiluminescence (GE Healthcare; actin) and quantification using a CCD image sensor (Chemi-Doc XRS; Bio-Rad) and software (Quantity One; Bio-Rad), the IL-15 protein content was expressed as arbitrary units relative to actin protein content. The IL-15 species measured using Western blot were the approximately 19-kDa cell associated form (13, 14), whereas we were unable to detect the 15-kDa mature form of IL-15.

Animal study

Animals

All animal experiments were conducted in accordance with the recommendations of the European Convention for the Protection of Vertebrate Animals used for Experimentation and after permission from the Danish Animal Experiments Inspectorate. Mice experiments were performed at Copenhagen University, Herlev Hospital, on 8- to 10-wk-old female C57BL/C mice from Taconic (Tornbjerggaard, Denmark). The mice were maintained in a thermo-stated environment under a 12-h light, 12-h dark cycle with free access to food and drinking water. Each group consisted of eight mice, and the mice were fed either a standard diet (Altromin pellets; Spezialfutter-Werke, Germany) or a high-fat diet containing 60 kilocal% fat (D12492; Research Diets, Bomholtgaard, Denmark). Drinking water was weighed daily, whereas fodder was weighed once a week. After 14 wk the mice were killed by quick cervical dislocation, and intact organs were dissected out and quickly frozen on dry ice and absolute alcohol. Trunk fat defines intraabdominal adipose deposits and includes ependymal, perirenal, and visceral fat.

Plasmid constructs and *in vivo* DNA electrotransfer

Plasmids encoding the regulatory elements of the doxycycline-inducible Tet-On system (pTet-On, encoding the transactivator, and pTetS, encoding a silencer) were obtained from CLONTECH (Palo Alto, CA). pBI-IL15, encoding murine IL-15 under the control of an Tet-On dependent promoter, was cloned by Geneart (Regensburg, Germany). All DNA preparations were performed using Qiafilter Plasmid Maxiprep kits (QIAGEN, Hilden, Germany), and the concentration and quality of the plasmid preparations were controlled by spectrophotometry and gel electrophoresis.

The mice were anesthetized 15 min before electrotransfer by injection of Hypnorm (0.4 ml/kg; Janssen Saunderton, Buckinghamshire, UK) and Dormicum (2 mg/kg; Roche, Basel, Switzerland). DNA electrotransfer was performed as previously described (15). Shortly, 20 μl plasmid solution or saline (control animals) were injected im along the fibers into the tibialis cranialis (TC) muscle using a 29G insulin syringe. Plate electrodes with 4 mm gap were fitted around the hind legs. Good contact between electrode and skin was ensured by hair removal and use of electrode gel. The electric field was applied using the Cliniporator (IGEA, Carpi, Italy), applying a combination of a high voltage pulse [1000 V/cm (applied voltage = 400 V), 100 μsec] followed by a low-voltage pulse [100 V/cm (applied voltage = 40V), 400 ms]. Both IL-15-transfected and control mice received drinking water containing 0.2 mg/ml doxycycline (doxycycline hyclate; Sigma-Aldrich, Brondby, Denmark) for induction of gene expression (16).

IL-15 measurement

Plasma concentrations of murine IL-15 were determined using mouse IL-15 BioPlex bead assay (Bio-Rad, Sundbyberg, Sverige, Sweden). The assay was performed according to the manufacturer's guidelines using 12 μl of sample per measurement. The range of the standard curve was 1–32,000 pg/ml, in which a double-logarithmic scale showed a linear relationship ($r^2 = 0.999$). All samples were within the range of the standard curve.

Fifty micrograms of muscle protein lysate per lane were boiled in Laemmli buffer, separated on 10% Bis-Tris gels (Invitrogen), and transferred to polyvinyl difluoride membranes (Hybond-P, GE Healthcare). Membranes were then blocked for 1 h at room temperature in blocking buffer (Tris-buffered saline with 0.1% Tween 20 and 5% BSA; Sigma-Aldrich) and washed three times for 5 min in wash buffer (Tris-buffered saline with 0.1% Tween 20). Subsequently the membranes were incubated overnight at 4 C in blocking buffer containing a primary antibody against mouse IL-15 (sc-7889; Santa Cruz Biotechnology, Inc., Santa Cruz, CA) at a 1:200 dilution. The membranes were then washed three times in wash buffer and incubated for 1 h at room temperature with antirabbit HRP (P0448) (Dako) secondary antibody at a 1:5000 dilution in blocking buffer, followed by washing three times for 5 min in wash buffer. IL-15 was detected using Supersignal West Femto (Pierce) and quantified using a CCD image sensor (ChemiDoc XRS; Bio-Rad) and software (Quantity One; Bio-Rad). The IL-15 band measured was the 15 kDa mature form of IL-15.

Statistics

Plasma levels of TAG, plasma glucose, plasma insulin, insulin resistance was calculated using the HOMA2-IR, HbA1c, CRP, and plasma IL-15 and measures of fitness and muscle IL-15 mRNA were log10 transformed to approximate normal distribution. For these parameters results are presented as geometric means with 95% confidence interval. Plasma IL-15 and muscle IL-15 mRNA are presented as geometric mean ± SEM. All other parameters results are presented as means with 95% confidence interval. Differences between glycemia and obesity groups were tested in a two-way ANOVA (PROC GLM with the LSMEANS statement). Multiple regression analysis (PROC REG) was performed to identify whether the level of plasma IL-15 (explanatory variable) could explain the variation in parameters of obesity and type 2 diabetes (dependent variable). Normality of the residuals was assessed graphically. A *t* test was performed to test differences between wild-type mice and IL-15-transfected mice. $P < 0.05$ was considered significant. All analyses was performed with SAS 9.1 (SAS Institute, Cary, NC).

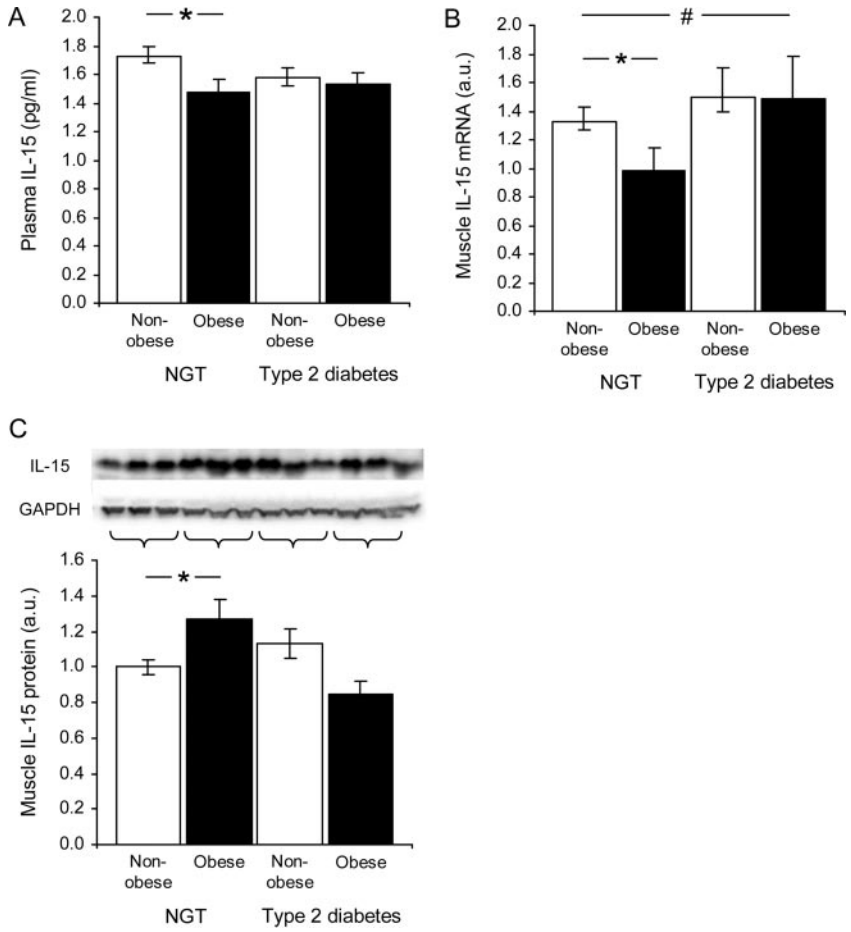

FIG. 1. Measurement of IL-15 in the four groups; NGT/nonobese, NGT/obese, type 2 diabetes/nonobese, and type 2 diabetes/obese. A, Level of plasma IL-15 (n = 199). B, Expression level of muscle IL-15 mRNA (n = 177). C, Western blot measurement of muscle IL-15 protein content. The photo shows Western blot from three different subjects from the four main groups. The four bars represent the sum of nine subjects. GAPDH is used as protein of reference for the protein levels. #, Significant difference between glycemia groups (NGT *vs.* type 2 diabetes); *, significant difference between obesity groups within each glycemia group.

Results

Subject characteristics

Characteristics of the four main groups are shown in Table 1. Figure 1A illustrates the level of plasma IL-15 in the four groups. Plasma IL-15 concentrations were decreased in NGT/obese subjects compared with NGT/nonobese ($P < 0.01$). There was no difference in plasma IL-15 concentration between obesity groups of type 2 diabetics ($P = 0.65$) and no difference between glycemia groups (NGT *vs.* type 2 diabetics) ($P = 0.42$). There were no gender differences in plasma IL-15 (data not shown). From Fig. 1B we found that muscle IL-15 mRNA expression was lower in the NGT/obese group compared with NGT/nonobese ($P < 0.05$). No difference was found between obesity subgroups in type 2 diabetics ($P = 0.42$), but muscle IL-15 mRNA expression

TABLE 2. Univariate and multivariate regression analyses with plasma IL-15 expression as predictors for parameters of obesity

	Plasma IL-15						
	NGT and type 2 diabetes						
	Univariate				Multivariate		
Covariate	RC	CI	R^2	*P* value	RC	CI	*P* value
BMI	−8.0	(−14.6 to −1.3)	0.03	<0.05	−5.8	(−11.8 to 0.2)	ns
Total FM	−22.5	(−36.7 to −8.3)	0.05	<0.01	−15.4	(−28.1 to −2.7)	<0.05
Trunk FM	−12.4	(−20.1 to −4.7)	0.05	<0.01	−9.7	(−16.8 to −2.7)	<0.01
Limb FM	−9.7	(−16.7 to −2.7)	0.04	<0.01	−5.4	(−11.4 to 0.6)	ns
Percent FM	−15.1	(−24.5 to −5.7)	0.05	<0.01	−9.7	(−17.3 to −2.1)	<0.05
Total FFM	−2.1	(−15.9 to 11.8)		ns	−3.2	(−13.4 to 7.1)	ns

IL-15 measures were log10 transformed, and hence, a 1-U change signifies a 10-fold increase. Multivariate analyses were adjusted for age (year), sex (male/female), fitness [log(VO_2/kg/fat free mass)], and smoking (current smoker/not smoker). FFM, Fat free mass; FM, fat mass; RC, regression coefficient, CI, 95% confidence interval; ns, not significant.

was higher in type 2 diabetics compared with NGT ($P < 0.05$). No gender differences were found (data not shown).

IL-15 and obesity

Univariate and multivariate regression analysis with IL-15 as the explanatory variable and parameters of obesity as the dependent variables are shown in Tables 2 and 3. Plasma IL-15 was negatively associated with BMI, total, trunk, limb, and percent fat mass in the univariate models, but only significantly with regard to total fat mass ($P < 0.05$), trunk fat mass ($P < 0.01$), and percent fat mass ($P < 0.05$) in multiple regression analysis adjusting for age, sex, fitness level, and smoking. Further adjustment for CRP, TAG, and HbA1c did not change the results. Plasma IL-15 did not show any association with total fat-free mass. The association between plasma IL-15 and trunk fat mass is presented in Fig. 2.

Because muscle IL-15 mRNA differed between glycemia groups, analysis was done for NGT and type 2 diabetic participants separately. In NGT individuals, muscle IL-15 mRNA showed negative associations with BMI, total, trunk, limb, and percent fat mass and was further negatively associated with fat-free mass. In multiple regression analysis the negative associations were still significant for BMI ($P < 0.001$), total fat mass ($P < 0.001$), trunk fat mass ($P < 0.01$), limb fat mass ($P < 0.001$), percent fat mass ($P < 0.05$), and total fat-free mass ($P < 0.01$). No association between body composition measurements and muscle IL-15 mRNA was found in type 2 diabetics.

Age, sex, and fitness level showed a separate negative association with body fat mass. There was no interaction between plasma IL-15 and these confounders, whereas muscle IL-15 mRNA levels showed interaction with age. Although the same negative relation was found in both young and old, it was significant only in the young subjects.

No correlation was found between muscle IL-15 mRNA levels and plasma IL-15.

IL-15 protein in human skeletal muscle

Western blot for IL-15 in skeletal muscle biopsies was performed from 36 representative individuals, nine from each of the four main groups; hence, the subjects were matched for age, sex, and BMI. Muscle IL-15 protein expression was higher in NGT/obese compared with NGT/nonobese ($P < 0.05$), but no difference between obesity groups was found in the type 2 diabetics and no difference was found between glycemia groups (Fig. 1C).

IL-15 overexpression in murine muscle

Control and IL-15-transfected mice did not differ with regard to food intake, total weight, skeletal muscle mass, spleen, or sc

TABLE 3. Univariate and multivariate regression analyses with muscle IL-15 mRNA expression as predictors for parameters of obesity

	Muscle IL-15 mRNA												
	NGT							Type 2 diabetes					
	Univariate				Multivariate			Univariate				Multivariate		
Covariate	RC	CI	R^2	*P* value	RC	CI	*P* value	RC	CI	R^2	*P* value	RC	CI	*P* value
BMI	−10.5	−15.6 to −5.3	0.15	<0.001	−8.3	−12.7 to −3.9	<0.001	−1.6	−5.6 to 2.5		ns	−2.5	−6.8 to 1.8	ns
Total FM	−23.2	−35.0 to −11.3	0.15	<0.001	−18.9	−28.6 to −9.1	<0.001	−3.8	−11.8 to 4.2		ns	−2.8	−10.8 to 5.2	ns
Trunk FM	−11.4	−17.8 to −5.0	0.12	<0.001	−8.7	−14.2 to −3.2	<0.01	−1.3	−5.8 to 3.2		ns	−1.3	−6.0 to 3.3	ns
Limb FM	−11.5	−17.2 to −5.7	0.15	<0.001	−9.9	−14.4 to −5.3	<0.001	−2.4	−6.2 to 1.4		ns	−1.4	−5.0 to 2.2	ns
Percent FM	−8.8	−16.7 to −1.0	0.05	<0.05	−7.8	−13.9 to −1.7	<0.05	−2.4	−8.3 to 3.6		ns	−0.6	−5.6 to 4.3	ns
Total FFM	−16.9	−27.4 to −6.5	0.10	<0.05	−10.9	−17.7 to −4.1	<0.01	−0.2	−9.9 to −9.4		ns	−2.2	−10.2 to 5.8	ns

IL-15 measures were log10 transformed, and hence, a 1-U change signifies a 10-fold increase. Multivariate analyses were adjusted for age (year), sex (male/female), fitness [log(VO_2/kg/fat free mass)], and smoking (current smoker/not smoker). FM, Fat mass; RC, regression coefficient; CI, 95% confidence interval; ns, not significant.

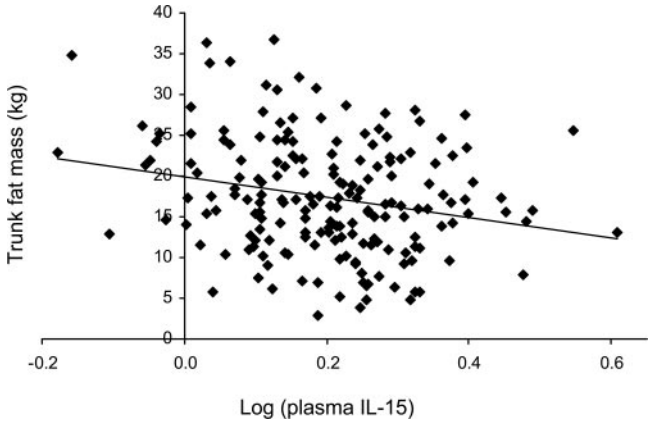

FIG. 2. Scatterplot showing logarithmic-transformed plasma IL-15 against trunk fat mass (kilograms). Regression coefficient, −12.4, R^2, 0.05.

fat tissue (Table 4). In both the normal-fed group and the high-fat-fed group, the IL-15-transfected mice had reduced trunk fat mass compared with control mice ($P < 0.01$ and $P < 0.05$, respectively). No differences were found between serum IL-15 levels in either the normal-fed or the high-fat-fed group. There was a difference in muscle IL-15 protein between IL-15-transfected and control mice in the normal-fed groups ($P < 0.05$), but no difference was found in the muscle IL-15 protein levels in the high-fat-fed groups. In the high-fat-fed IL-15-transfected group, the weight of the heart and liver was reduced compared with controls, whereas no difference in heart and liver weight was observed in the normal-fed groups. In the normal-fed group, the IL-15 transfected mice showed a lower concentration of triglycerides than the control group. The same difference was not present in the high-fat-fed group. There were no differences between control and IL-15 transfected groups with regard to total cholesterol, glucose, or insulin levels.

Discussion

The present study demonstrated a negative association between plasma IL-15 concentration and body fat mass in humans, independent of the diagnosis of type 2 diabetes. Furthermore we demonstrated a decrease in trunk fat mass in mice overexpressing muscle IL-15.

Previous *in vitro* and *in vivo* studies have demonstrated an effect of IL-15 on fat metabolism (6–8), and it has been hypothesized (17) that production of IL-15 takes place in muscle and that a release of IL-15 from this tissue is able to regulate fat mass (17, 18).

Multivariate analysis showed an interesting negative association between plasma IL-15 and total, trunk, and percent fat mass. Not surprisingly, age, sex, and fitness level showed an independent effect on the parameters of obesity. However, no interaction was found between plasma IL-15 and these confounders, and thus, the negative association between plasma IL-15 and fat mass cannot be explained by the effect of age, sex, or fitness level. This may suggest that IL-15 plays a specific role in regulating fat mass.

For type 2 diabetics we did not find any association between muscle IL-15 mRNA and body fat mass, but muscle IL-15 mRNA levels in the NGT group were negatively associated with all obesity markers. In contrast to plasma IL-15, muscle IL-15 mRNA was highly associated with BMI and limb fat mass and also with fat-free mass. These different findings may explain why

TABLE 4. Mice characteristics

	Normal food			High-fat food		
	Control	**IL-15**	***P* value**	**Control**	**IL-15**	***P* value**
Body composition						
Food intake (g/mouse/d)	4.3	4.4	ns	3.0	2.8	ns
Body weight (g)						
Initial	20 ± 0.4	20 ± 0.3	ns	21 ± 0.3	20 ± 0.2	ns
Final	23 ± 0.4	22 ± 0.5	ns	34 ± 1.6	31 ± 1.4	ns
Skeletal muscle (mg)						
Left TC	44 ± 2.1	46 ± 2.8	ns	40 ± 2.5	45 ± 2.8	ns
Right TC	37 ± 2.5	34 ± 2.5	ns	40 ± 2.5	45 ± 3.5	ns
Heart (mg)	104 ± 2.5	109 ± 2.8	ns	131 ± 2.8	116 ± 2.5	<0.01
Liver (mg)	962 ± 35	962 ± 25	ns	1141 ± 27	993 ± 51	<0.05
Spleen (mg)	105 ± 4.2	103 ± 7	ns	109 ± 3.9	109 ± 4.2	ns
Trunk WAT (mg)	500 ± 54	303 ± 34	<0.01	2928 ± 404	1798 ± 310	<0.05
Subcutaneous WAT (mg)	100 ± 8	81 ± 9	ns	520 ± 93	404 ± 73	ns
IL-15						
Serum IL-15 (pg/liter)	12.1 ± 0.6	11.6 ± 1.2	ns	23.6 ± 5.9	19.5 ± 3.3	ns
Muscle IL-15 protein (a.u.)	892 ± 132	2214 ± 400	<0.05	2284 ± 518	3102 ± 759	ns
Metabolic regulation						
Total cholesterol (mM)	1.97 ± 0.17	1.87 ± 0.25	ns	3.76 ± 0.25	3.92 ± 0.7	ns
Triglycerides (mM)	0.85 ± 0.13	0.61 ± 0.01	<0.05	1.03 ± 0.19	0.89 ± 0.24	ns
Glucose (mM)	10.3 ± 1.9	10.8 ± 1.1	ns	10.6 ± 2.2	11.6 ± 0.8	ns
Insulin (ng/ml)	0.45 ± 0.12	0.48 ± 0.09	ns	1.3 ± 0.39	0.99 ± 0.41	ns

Data are presented as means ± SEM. WAT, White adipose tissue; ns, not significant. The P values show the difference between control mice and IL-15 transfected mice. a.u., Arbitrary unit.

we did not find muscle IL-15 mRNA levels to correlate with plasma IL-15, suggesting that measurement of muscle IL-15 mRNA might provide independent information.

In healthy subjects, obesity was associated with IL-15. IL-15 mRNA expression, and circulating IL-15 levels were decreased in obese subjects. However, muscle IL-15 protein was increased in the obese subjects. IL-15 production in muscle cells is not well understood, but regulation downstream of transcription could explain the discrepancy. In type 2 diabetics, obesity did not show any association with IL-15 in plasma or muscle, suggesting that the impact of obesity on the insulin-resistant skeletal muscle has been abolished. The lack of association between muscle IL-15 content and systemic concentrations of IL-15 might suggest that skeletal muscle is not the major source of origin with regard to IL-15. The apparent lack of correlation between plasma IL-15 and fat-free tissue mass supports this hypothesis. Besides skeletal muscle, IL-15 mRNA has been determined in lung, liver, kidney, and heart (1). Members of the monocyte/macrophage lineage also produce IL-15 mRNA and protein (19, 20), which is why we need to consider other possible tissues contributing to circulating IL-15 levels.

In the mouse model, IL-15-transfected mice displayed decreased amount of trunk fat mass compared with control mice. No difference was observed in sc fat mass. This is interesting because plasma IL-15 in humans was negatively associated with trunk fat mass and not limb fat mass, suggesting that the main effect of IL-15 could be regulation of trunk/visceral fat and to a lesser extent sc fat. In muscle we found an increase in muscle IL-15 protein in the normal-fed IL-15 transfected muscle, when comparing with the normal-fed control group. There was no difference between the high-fat-fed groups. However, we were not able to show an increase in circulating IL-15 in the IL-15-transfected groups. We postulate that the difference in visceral fat mass between the IL-15 transfected and nontransfected groups are a consequence of the IL-15 transfection. However, our study is limited by the fact that we are not able to show any differences in serum IL-15 levels. It is possible that a difference could have been seen at other time points, but the study design did not leave the possibility of illuminating this. Further studies are needed to identify IL-15 as a protein secreted from muscle tissue.

A previous study by Carbo *et al.* (6) showed that injections of IL-15 decreased plasma levels of triglycerides in adult rats. However, conflicting results have been reported (7). In the human subjects, we did not find any association between plasma IL-15 and plasma levels of triglycerides, high-density lipoprotein, or low-density lipoprotein (data not shown).

Finally, Busquets *et al.* (21) showed that IL-15 stimulation of C2C12 muscle cell cultures increased glucose uptake and increased GLUT4 mRNA expression, suggesting that IL-15 could regulate peripheral glucose metabolism. From our human data, we were not able to show any association between plasma IL-15 and parameters of type 2 diabetes (data not shown), which is why the present study does not support these previous findings (21). It seems more likely that a possible effect of IL-15 on glucose metabolism would be indirect through the effect on fat mass.

In conclusion, the present study demonstrated a negative association between plasma IL-15 and body fat mass independent of the diagnosis of type 2 diabetes. Additionally, IL-15 overexpression in mice skeletal muscle resulted in reduced trunk fat mass, suggesting, that IL-15 might be involved in the regulation of fat mass.

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