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Effects of interleukin-15 (IL-15) on adipose tissue mass in rodent obesity models: evidence for direct IL-15 action on adipose tissue

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Abstract

Interleukin-15 (IL-15) is a proinflammatory cytokine with multifunctional effects outside the immune system. Previous studies have indicated that treatment of normal rats with IL-15 reduces white adipose tissue (WAT) mass, but it was unclear if these effects were direct or indirect. In the present study, the effects of IL-15 on WAT mass and lipid metabolism were studied in two genetic models of obesity: the leptin receptor-negative *falfa* Zucker rat and the leptin-deficient *ob*/*ob* mouse. Lean Zucker rats, lean (+/+), and obese mice (*ob*/*ob*) responded to IL-15 with reductions in WAT mass and lipoprotein lipase activity (LPL), with no decreases in food intake. In contrast, *falfa* Zucker rats did not respond to IL-15 administration by any of the above measures of fat mass or lipid metabolism. In addition, ribonuclease protection assays (RPAs) were used to demonstrate that all three subunits (γc, β and α) of the IL-15 receptor complex are expressed by rat and mouse WAT, suggesting that the effects of IL-15 on adipose tissue metabolism could be direct. Additionally, the *falfa* rats expressed 84% lower levels of the γc signaling receptor subunit than lean Zucker rats, suggesting this decrease may play a role in the lack of adipose tissue response to IL-15 in the *falfa* genotype and lending further support for a direct action of IL-15 on adipose tissue.

Keywords: Interleukin-15; White adipose tissue; Zucker rat; *ob*/*ob* mouse; Obesity

1. Introduction

Interleukin-15 (IL-15) is a multifunctional cytokine originally described as a T-cell stimulatory factor with actions similar to IL-2 [1]. IL-15 has since been found to have numerous activities outside the immune system, including muscle fiber anabolism [2–4]. IL-15 mRNA is also more widely expressed outside the immune system than IL-2, with high expression in placenta, skeletal muscle, kidney, lung and heart [1]. The tripartite receptor for IL-15 consists of a γc signaling subunit common to many cytokine receptors, a β subunit (IL2Rβ) utilized by IL-2 and IL-15, and a nonsignaling IL-15 specific α subunit (IL15Rα) believed to confer IL-15-specific binding [5]. The IL15Rα is also broadly expressed in many tissues [6], suggesting the various effects of IL-15 are direct.

In previous publications [4,7], we showed that administration of IL-15 to normal rats caused significant reductions in white adipose tissue (WAT) mass via both a decreased rate of tissue lipogenesis and a reduction in lipoprotein lipase (LPL) activity, without concomitant reductions in food intake. It was unclear if these effects on adipose tissue metabolism were due to direct or indirect actions of IL-15 on WAT. In the present study, we examined the effects of IL-15 administration on adipose tissue

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mass and lipid metabolism in two genetic models of obesity, the leptin-receptor negative *fa/fa* Zucker rat [8] and the leptin-deficient *ob/ob* mouse [9]. We also examined the expression of mRNAs for the γc, α, and β IL-15 receptor subunits in adipose tissue from each genotype. Adipose tissue IL-15 response among the four lean/obese rodent genotypes correlated with the levels of γc receptor subunit expression, suggesting IL-15 action on adipose tissue may be direct.

2. Material and methods

2.1. Animals and cytokine administration

Male lean (+/?) and obese (*fa/fa*) Zucker rats weighing an average of 170 g and 310 g respectively, C57BL/6 (+/+) mice weighing an average of 25 g, and *ob/ob* mice weighing an average of 43 g, were fed ad libitum on a chow diet (Panlab, Barcelona, Spain) consisting (by weight) of 54% carbohydrate, 17% protein, and 5% fat (the residue was non-digestible material). Animals had free access to drinking water and were maintained at an ambient temperature of 22 ± 2°C with a 12:12-h light/dark cycle (lights on from 08:00 20:00 h). Animals in each genotype were divided in two groups: treated and controls. The former received a daily s.c. dose of IL-15 (100 mg kg^{-1} body weight dissolved in physiological saline solution) for 7 days, the latter a corresponding volume of vehicle. Food intake was measured daily. IL-15 treatment did not result in any differences in either the well-being or the spontaneous activity of the animals. At the end of the treatment, animals were weighed and anesthetized. Blood was collected from the abdominal aorta into heparinized tubes and centrifuged (3500 × *g*, 10 min, 4°C) to obtain plasma. Dorsal WAT was rapidly excised, weighed, and frozen in liquid nitrogen. As previously reported [4], neither the controls nor the IL-15-treated animals developed overt diabetes at the time of sacrifice.

2.2. LPL activity

WAT LPL activity was measured by a modification of the technique of Nilsson-Ehle and Eckman [10]. Tissue samples were homogenized and used in an assay system containing [^{3}H]triolein as substrate; [^{3}H]fatty acids released after a 30-min incubation period were extracted and determined by the method of Nilsson-Ehle and Schotz [11].

2.3. Plasma triacylglycerols levels

Plasma triacylglycerols (TAG) were measured by the method of Eggstein and Kreutz [12]. In brief, plasma samples were saponified with alcoholic 3 N KOH and glycerol measured fluorimetrically.

2.4. RNA isolation and RNAse protection assays

Total RNA from WAT was extracted using the acid guanidinium isothiocyanate/phenol/chloroform method [13]. RNAse protection assays (RPAs) were used to detect expression of IL-15 receptor subunits, using the mCR-3 BD Riboquant Multi-Probe Template Set (BD PharMingen, SanDiego, CA, USA) and the BD PharMingen transcription kit. Hybridized RNA was fractionated by electrophoresis and gels were exposed to Hyperfilm (Amersham) and quantified by densitometry (Phoretix 2.51, Phoretix International). A GADPH probe was used for mRNA normalization.

3. Results

3.1. Effects of IL-15 on WAT mass

As reported previously [4,7], IL-15 treatment did not

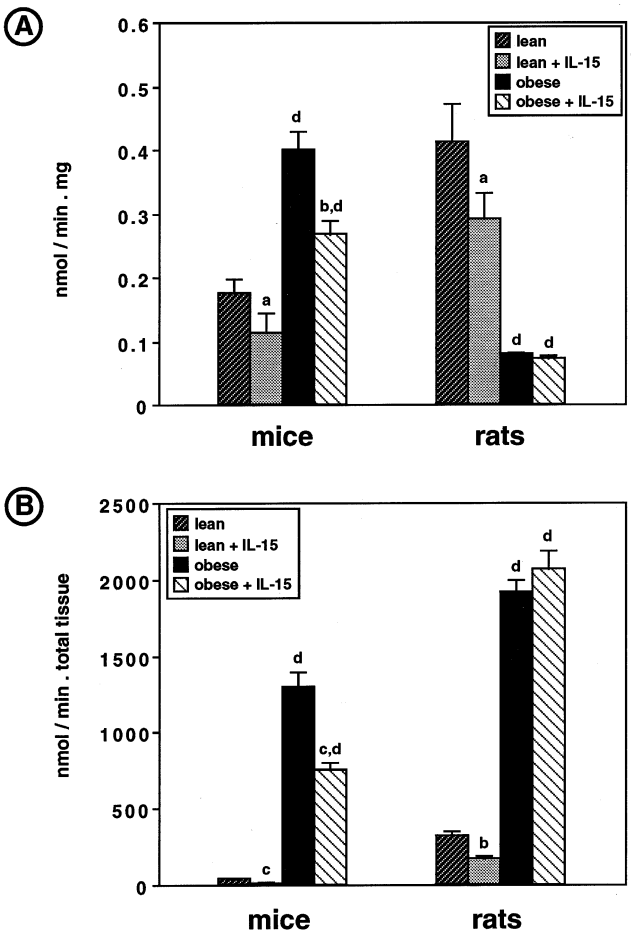

Fig. 1. White adipose tissue lipoprotein lipase activity. Lipoprotein lipase activity (LPL) is expressed as nmol of fatty acid released/min per mg of tissue (A) or per total tissue (B). Results shown are means ± S.E.M. for five animals in each group. Statistical significance of the differences (Student's *t*-test): [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$ (treated vs. control); [d] $P < 0.001$ (obese vs. lean).

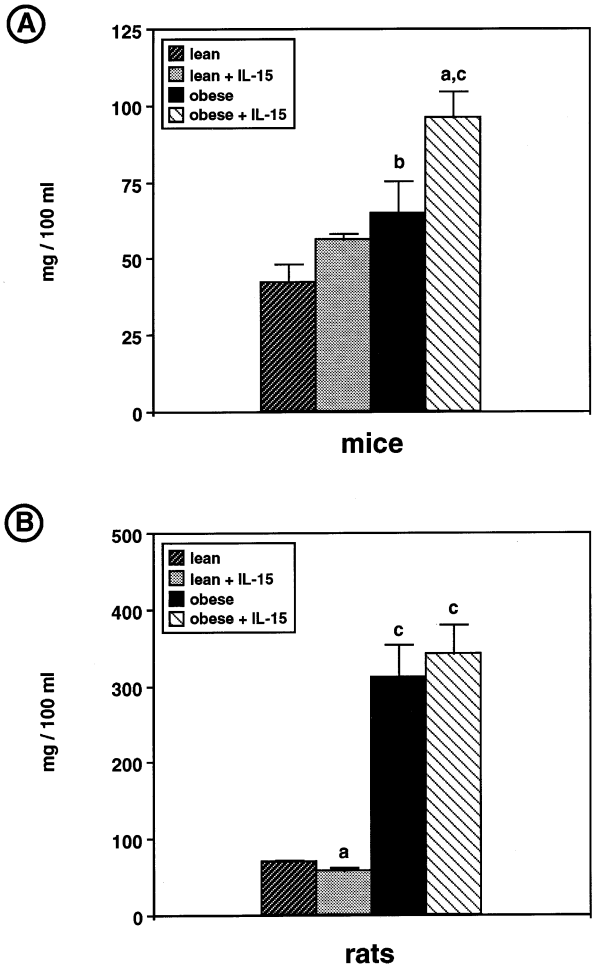

Fig. 2. Triacylglycerols in plasma. Data are expressed as mg per 100 ml of plasma. Results shown are mean ± S.E.M. for five animals in each group. (A) Mice; (B) rats. Statistical significance of the differences (Student's *t*-test): $^{a}P<0.05$ (treated vs. control); $^{b}P<0.05$, $^{c}P<0.001$ (obese vs. lean).

affect either food or water intake in any of the groups studied (not shown). In lean Zucker rats, IL-15 treatment resulted in a 26% decrease ($P<0.001$) in dorsal WAT (Table 1). However, IL-15 treatment did not result in changes in WAT mass in obese (*falfa*) rats (Table 1). Conversely, both lean and *ob/ob* mice responded to IL-15 with 44% ($P<0.05$) and 14% ($P<0.01$) decreases, respectively, in dorsal WAT (Table 1).

3.2. Effects of IL-15 on LPL activity

IL-15 treatment significantly decreased LPL activity in both lean and obese mice (Fig. 1). In lean mice, total LPL activity decreased 68% in response to IL-15 administration (Fig. 1B) and decreased 43% when expressed as LPL activity per mg WAT (Fig. 1A). Likewise, LPL activity in obese mice decreased 42% (per total tissue) and 32% (per mg WAT). IL-15 administration also significantly decreased LPL activity in lean Zucker rats (Fig. 1) by 48% (per total tissue) and 29% (per mg WAT). IL-15 admin-

Table 1
Effects of IL-15 administration on white adipose tissue mass

Experimental group	Treatment	Dorsal WAT weight (mg/100 g ibw)
Rats		
fa/?	None	766 ± 29
fa/?	IL-15	567 ± 38**
falfa	None	1133 ± 33
falfa	IL-15	1200 ± 69
Mice		
+/+	None	210 ± 39
+/+	IL-15	118 ± 16*
ob/ob	None	3260 ± 100
ob/ob	IL-15	2800 ± 90**

Adipose tissue weights. WAT, white adipose tissue; ibw, initial body weight. Data are mean ± S.E.M. for five animals in each group. Statistical significance of the differences (Student's *t*-test): $^{*}P<0.05$, $^{**}P<0.01$.

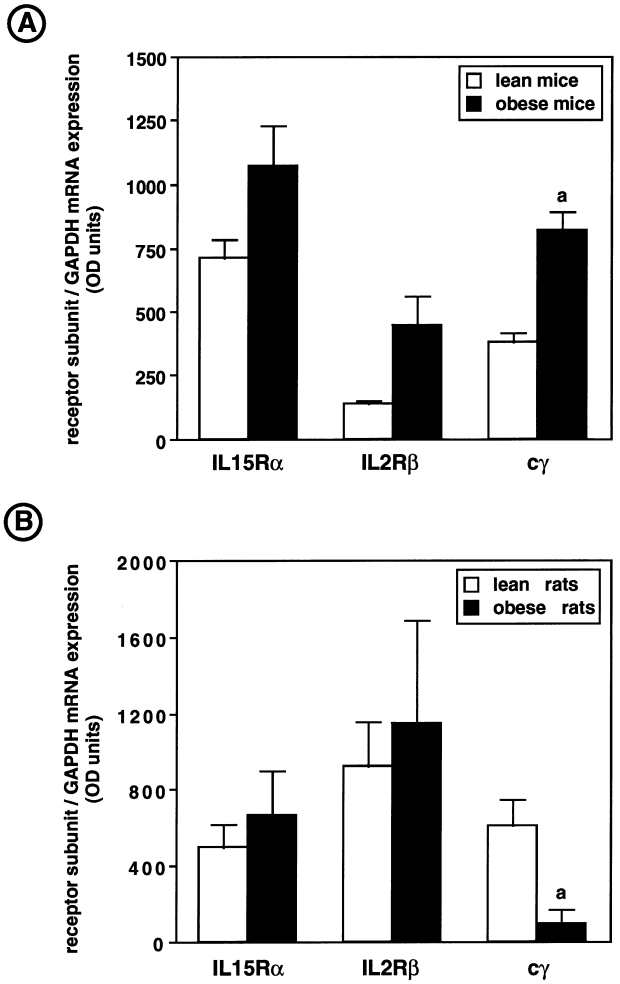

Fig. 3. IL-15 receptor subunit expression in WAT of lean and obese rodents. Expression of IL15Rγ, IL2Rβ, and γc subunits was assessed using ribonuclease protection assays (RPAs) and is expressed as arbitrary densitometry units; signals were normalized to GADPH mRNA expression. (A) Mice; (B) rats. Statistical significance of the differences (Student's *t*-test): $^{a}P<0.05$ (obese vs. lean).

istration to obese Zucker rats had no significant effect on LPL activity, expressed either per total tissue or per mg WAT (Fig. 1). These findings are similar to the effects of IL-15 on WAT mass reported above. By both measures, lean mice, obese mice, and lean rats, but not obese rats, responded to IL-15 administration.

3.3. Effects of IL-15 on circulating TAG

Plasma TAG levels were assessed as a measure of lipolysis. Levels of circulating TAG were not significantly different in IL-15-treated and control (no treatment) lean mice (Fig. 2A). However, IL-15 administration caused a 49% increase in plasma TAG levels in *ob/ob* mice (Fig. 2A). In lean Zucker rats, the circulating TAG levels were decreased 17% in response to IL-15 (Fig. 2B). No changes in TAG were observed in the obese Zucker rats.

3.4. Expression of IL-15 receptor subunit mRNAs in rodent WAT

Expression of mRNAs for the three IL-15 receptor subunits in lean and obese mice and rat adipose tissue was assessed using RPAs (Fig. 3). Levels of IL15Rα and IL2Rβ mRNA expression did not differ significantly between lean and obese Zucker rats (Fig. 3B). However, expression of the γc subunit was decreased 84% in obese rats compared to controls ($P < 0.05$). In obese mice, expression levels of all three receptor subunits were somewhat elevated compared to those in lean mice (Fig. 3A).

4. Discussion

Previous studies have indicated that treatment of normal rats with IL-15 reduces WAT mass, accompanied by profound changes in WAT metabolism [4,7]. In the present study, the effects of IL-15 on adipose tissue mass and lipid metabolism were studied in two genetic models of obesity, the leptin receptor-negative *fa/fa* Zucker rat and the leptin-deficient *ob/ob* mouse. Both models have been extensively used for studies on obesity and exhibit features generally observed in obese humans, such as decreased lean body mass, reduced thermogenic capacity, hyperglycemia, glucose intolerance, and hypertriglyceridemia [14].

We have reported that normal Wistar rats treated with IL-15 exhibited a 33% decrease in WAT mass without changes in food intake [4,7]. This decrease was accompanied by reductions in LPL activity and lipogenic rate in WAT, changes that can account for the observed reduction in adipose tissue mass. In contrast to adipose tissue, IL-15 produced no changes in skeletal muscle mass in normal animals [4]. The observations of decreased WAT mass with no changes in skeletal muscle mass or food intake suggested that IL-15 may be of use in treatment of obesity. Indeed, the results presented here suggest that alterations in IL-15 receptors could be responsible for some types of obesity.

The present study showed that both lean (+/+) and obese mice (*ob/ob*), as well as lean Zucker rats, responded to IL-15 with reductions in WAT mass and decreased LPL activity, with no decreases in food intake. In contrast, *fa/fa* Zucker rats did not respond to IL-15 administration by either of these measures. RPAs demonstrated that all three subunits (γc, IL2Rβ and IL15Rα) of the IL-15 receptor complex are expressed by rat and mouse WAT, suggesting that the effects of IL-15 on adipose tissue metabolism could be direct. Moreover, the non-responding *fa/fa* rats expressed 85% lower levels of mRNA for the γc signaling receptor subunit than lean Zucker rats. While the γc chain of the receptor is not necessary for IL-15 binding, it is indispensable for signal transduction [15]. Diminished expression of this subunit in *fa/fa* WAT could be a possible explanation for the lack of IL-15 effects on this tissue. Although not direct proof of a direct effect of IL-15 on WAT, the correlation of decreased γc mRNA expression levels and the lack of response by *fa/fa* rats to IL-15 is suggestive of a direct action of this cytokine on adipose tissue.

Other cytokines have been implicated in WAT metabolism. Tumor necrosis factor-α (TNF-α) is overexpressed in WAT during genetic obesity and in type II diabetes [16]. In addition, TNF-α decreases LPL activity in cultured 3T3-L1 adipocytes [17] and organotypic WAT cultures [18]. The cytokine is also able to induce apoptosis in WAT [19].

Although the mechanism by which IL-15 decreases fat mass in mice and lean rats is unknown, several mechanisms can be ruled out by our observations. First, IL-15 treatment did not alter food intake in any of the groups studied. Leptin, a hormone produced by WAT [9], is involved in energy metabolism and body weight regulation. Leptin works as a circulating factor that sends a satiety signal to the hypothalamus [20]. Although previous studies [7] have shown that IL-15 can decrease circulating leptin levels in rats, a role for leptin in mediating the effects of IL-15 on WAT can be ruled out by our study, since *ob/ob* mice are leptin-negative [9], but nevertheless responded to IL-15 by decreasing WAT mass.

The decreased LPL activity induced by IL-15 is likely to be a major contributor to the reduction in fat mass. LPL activity was decreased by IL-15 in all genotypes studied except *fa/fa* rats, which also did not exhibit decreased WAT mass in response to IL-15. LPL hydrolyzes plasma triacylglycerols (TAGs) and directs uptake of the resulting long-chain fatty acids into WAT. These changes in LPL activity (which in some of the groups were also associated with increases in TAGs) did not result in an induction on insulin resistance in lean animals.

Through its capacity to reduce WAT storage, IL-15 may

be a candidate molecule for the treatment of some forms of obesity. Since both the binding and signaling IL-15 receptor subunits are expressed in WAT, our study indicates that IL-15 action might be direct. Furthermore, modulation of receptor subunit expression levels in WAT could in turn modulate IL-15 responses by that tissue.

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References

[1] K. Grabstein, J. Eisenman, K. Shanebeck, C. Rauch, S. Srinivasan, V. Fung, C. Beers, J. Richardson, M.A. Schoenborn, M. Ahdieh, L. Johnson, M.R. Alderson, J.D. Watson, D.M. Anderson, J.G. Giri, Cloning of a T cell growth factor that interacts with the b chain of the interleukin-2 receptor, Science 264 (1994) 965–968.

[2] L.S. Quinn, K.L. Haugk, K.H. Grabstein, Interleukin-15: a novel anabolic cytokine for skeletal muscle, Endocrinology 136 (1995) 3669–3672.

[3] L.S. Quinn, K.L. Haugk, S.E. Damon, Interleukin-15 stimulates C2 skeletal myoblast differentiation, Biochem. Biophys. Res. Commun. 239 (1996) 6–10.

[4] N. Carbó, J. López-Soriano, P. Costelli, S. Busquets, B. Alvarez, F.M. Baccino, L.S. Quinn, F.J. López-Soriano, J.M. Argilés, Interleukin-15 antagonizes muscle protein waste in tumour-bearing rats, Br. J. Cancer 83 (2000) 526–531.

[5] J.G. Giri, S. Kumaki, M. Ahdieh, D.J. Friend, A. Loomis, K. Shanebeck, R. DuBose, D. Cosman, L.S. Park, D. Anderson, Identification of a novel IL-15 binding protein that is structurally related to the alpha chain of the IL-2 receptor, EMBO J. 14 (1995) 3654–3663.

[6] T. Waldmann, Y. Tagaya, R. Bamford, Interleukin-2, interleukin-15, and their receptors, Int. Rev. Immunol. 16 (1998) 205–226.

[7] N. Carbó, J. López-Soriano, P. Costelli, B. Alvarez, S. Busquets, F.M. Baccino, L.S. Quinn, F.J. López-Soriano, J.M. Argilés, Interleukin-15 mediates reciprocal regulation of adipose tissue and muscle mass: a potential role in body weight control, Biochim. Biophys. Acta 1526 (2001) 17–24.

[8] S.C. Chua, W.K. Chung, X.S. Wu-Peng, Y. Zhang, S.M. Lui, L. Tartaglia, R.L. Leibel, Phenotypes of mouse diabetes and rat fatty due to mutation in the Ob (leptin) receptor, Science 271 (1996) 994–996.

[9] Y. Zhang, R. Proenca, M. Maffei, M. Barone, L. Leopold, J. Friedman, Positional cloning of the mouse obese gene and its human homologue, Nature 372 (1994) 425–432.

[10] P. Nilsson-Ehle, R. Ekman, Rapid, simple and specific assays for lipoprotein lipase and hepatic lipase, Artery 3 (1977) 197–209.

[11] P. Nilsson-Ehle, M.C. Schotz, A stable, radioactive substrate emulsion for assay of lipoprotein lipase, J. Lipid Res. 17 (1976) 536–541.

[12] M. Eggstein, F.H. Kreutz, A new method for the determination of neutral fats in blood serum and tissues, Klin. Wochensch. 44 (1966) 262–267.

[13] N. Chomczynski, N. Sacchi, Single-step method of RNA isolation by acid guanidinium-thiocyanate-phenol-chloroform extraction, Anal. Biochem. 162 (1991) 156–159.

[14] P.R. Johnson, M.R.C. Greenwood, B.A. Horwitz, J.S. Stern, Animal models of obesity: genetic aspects, Annu. Rev. Nutr. 11 (1991) 325–353.

[15] J.G. Giri, M. Ahdieh, K.S. Einsenman, K. Grabstein, S. Kumaki, A. Namen, L.S. Park, D. Cosman, D. Anderson, Utilization of the β and γ chains of the IL-2 receptor by the novel cytokine IL-15, EMBO J. 13 (1994) 2822–2830.

[16] G.S. Hotamisligil, P. Arner, J.S. Caro, R.L. Atkinson, B.M. Spiegelman, Increased adipose tissue expression of tumor necrosis factor-α in human obesity and insulin resistance, J. Clin. Invest. 95 (1995) 2409–2415.

[17] S.R. Price, T. Olivecrona, P.H. Pekala, Regulation of lipoprotein synthesis by recombinant tumor necrosis factor: the primary regulatory role of the hormone in 3T3-L1 adipocytes, Arch. Biochem. Biophys. 251 (1986) 738–746.

[18] P. Cornelius, S. Enerback, G. Bjursell, T. Olivecrona, P.H. Pekala, Regulation of the lipoprotein lipase mRNA content in 3T3-L1 cells by tumor necrosis factor, Biochem. J. 249 (1988) 765–769.

[19] J.B. Prins, C.U. Niesler, C.M. Winterford, N.A. Bright, K. Siddle, S. O'Rahilly, N.I. Walker, D.P. Cameron, Tumor necrosis factor-alpha induces apoptosis of human adipose cells, Diabetes 46 (1997) 1939–1944.

[20] M.A. Pelleymounter, M.J. Cullen, M.B. Baker, R. Hecht, D. Winters, T. Boone, F. Collins, Effects of the obese gene product on bodyweight regulation in ob/ob mice, Science 269 (1995) 546–549.