Adipocyte 1:2, 112–115; April/May/June 2012; © 2012 Landes Bioscience

Role of cardiotrophin-1 in obesity and insulin resistance

María J. Moreno-Aliaga,[1] M. Asunción Romero-Lozano,[2] David Castaño,[2] Jesús Prieto[2] and Matilde Bustos[2,*]

[1]Department of Nutrition, Food Sciences, Physiology and Toxicology; University of Navarra; Pamplona, Spain; [2]Division of Hepatology and Gene Therapy; Center for Applied Medical Research (CIMA); University of Navarra; Pamplona, Spain

Keywords: gp130 cytokines, IL-6 family of cytokines, cardiotrophin-1, adipocytokines, obesity, insulin resistance

Abbreviations: CT-1, cardiotrophin-1; IL, interleukin; LIF, leukemia inhibitory factor; CNTF, ciliary neurotrophic factor; OSM, oncostatin M; CLC, cardiotrophin like cytokine; NP, neuropoietin; STAT, signal transducer and activator of transcription; EE, energy expenditure; rCT-1, recombinant CT-1; WAT, white adipose tissue; UCP1, uncoupling protein 1; Dio2, deiodinase iodothyronine type II; TNF-α, tumor necrosis factor-α; PAI-1, plasminogen activator inhibitor-1; ROS, reactive oxygen species; SOCS, suppressor of cytokine signaling; IRS, insulin receptor substrate; GLP-1, glucagon-like-peptide-1; ITT, insulin tolerance test; FFA, free fatty acids; cAMP, cyclic adenosine monophosphate; STZ, streptozotocin

Submitted: 01/09/12

Revised: 02/11/12

Accepted: 02/13/12

http://dx.doi.org/10.4161/adip.19696

*Correspondence to: Matilde Bustos; Email: mbustos@unav.es

Commentary to: Moreno-Aliaga MJ, Pérez-Echarri N, Marcos-Gómez B, Larequi E, Gil-Bea FJ, Viollet B, et al. Cardiotrophin-1 is a key regulator of glucose and lipid metabolism. Cell Metab 2011; 14:242–53; PMID:21803294; http://dx.doi.org/10.1016/j.cmet.2011.05.013

Cardiotrophin-1 (CT-1) is a member of the gp130 family of cytokines. In a recent study we examined the metabolic features of *ct-1* null mice and the effects on body composition, glucose and lipid metabolism of acute and chronic administration of recombinant CT-1. Our data revealed that CT-1 is a key regulator of energy metabolism with potential applications in the treatment of obesity and the metabolic syndrome. This commentary discusses the significance of these findings in the context of other key studies in the field of obesity and insulin resistance.

Obesity is considered to be a worldwide epidemic disease, the prevalence of which has dramatically increased among children, adolescents and adults during the last decades. It has been, in fact, recognized as one of the major global health problems. This health hazard is linked to increased risk of several types of common diseases including cardiovascular dysfunction, insulin resistance and type 2 diabetes mellitus, hypertension, dyslipidemia, non-alcoholic fatty liver and also various types of cancer.[1] Progress in understanding the regulation of energy metabolism and the pathogenesis of obesity is of paramount importance for the design of novel therapeutic options.

The interleukin (IL)-6 family of cytokines includes IL-6, IL-11, IL-27, leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF), cardiotrophin-1 (CT-1), oncostatin M (OSM), cardiotrophin like cytokine (CLC) and neuropoietin (NP). These cytokines are commonly referred to as gp130 ligands/cytokines because all members of this family utilize glycoprotein 130 as a common signal transducer within their signaling receptor complex. Although there is some cross-talk among the gp130 cytokines, the complex signal transduction cascade is not common to all family members. IL-6 and IL-11 first bind to the IL-6 receptor α and IL-11 receptor α respectively, and then the complex associates with a gp130 homodimer β (gp130Rβ) complex for signaling. CNTF, CT-1, CLC and NP after binding to their specific α receptors induce formation of a heterodimer of the signal-transducing gp130Rβ and LIF receptor β (LIFRβ).[2] However, NP has also been reported to activate signal transducer and activator of transcription (STAT)3 in adipocytes independently of LIFRβ.[3] OSM interacts with gp130/OSM receptor (OSMRβ) complex and has also being reported to use gp130/LIFRβ.[2] In recent years, the IL-6 family of cytokines have been proposed as potential therapies in obesity and insulin resistance, based on their ability to modulate metabolic functions in adipocytes and myocytes and because of the efficacy of some of these cytokines in promoting weight loss and insulin sensitivity.[4] In particular, it has been reported that CNTF decreases fat mass and improves glucose tolerance in humans and rodents acting both centrally and peripherally.[5,6] However, disappointing results were obtained in clinical trials using a human recombinant variant of CNTF (Axokine®).[7] On the other hand, there has been considerable debate regarding the effects of IL-6 on insulin sensitivity[8,9] but the pro-inflammatory properties of this cytokine limits its clinical use.

CT-1 is a 21.5 kDa protein that was named by its ability to induce myocyte hypertrophy,[10] and it is expressed in and released from the heart in response to

stress.[11] CT-1 is also expressed in many other tissues such as skeletal muscle, liver, brain, lungs and kidneys with a broad spectrum of biological activities, including potent cytoprotective properties.[12] A recent publication by our group revealed that CT-1 is a master regulator of energy metabolism.[13] Mice lacking CT-1 exhibited reduced energy expenditure (EE) and developed adult-onset obesity, hypercholesterolemia and type 2 diabetes, closely mimicking the human metabolic syndrome. Given the fact, that CT-1 modulated fat and glucose metabolism we proposed that this cytokine might be a potential therapy for obesity and insulin resistance. Indeed, we observed that recombinant CT-1 (rCT-1) was able to correct obesity and associated diabetes in animal models of genetic (*ob/ob* mice) and acquired obesity (high fat diet-fed mice). These effects were related to an increase in EE and decreased food intake. The study also demonstrated a potent effect of rCT-1 on white adipose tissue (WAT), leading to a reduction of fat stores together with a dramatic remodeling of WAT, characterized by shrinkage of adipocytes and upregulation of genes involved in lipolysis, fatty acid oxidation, mitochondrial biogenesis and genes typifying brown-fat phenotype. Analogous metabolic remodeling of adipocytes has been described for CNTF. However, there are some important differences between CNTF and CT-1. In particular, rCT-1 is able to directly upregulate *UCP1* (uncoupling protein 1) and *Dio2* (deiodinase iodothyronine type II) in cultured adipocytes, while CNTF has no comparable effect on these genes. Moreover, while rCT-1 enhances lipolysis in cultured adipocytes (unpublished observations), CNTF fails to do this. Our findings showed that CT-1 can activate fat mobilization and fat utilization in WAT, which might explain the adipose-tissue lowering effect of this cytokine.

WAT is no longer considered a passive reservoir for storing lipids, but rather an important organ influencing energy metabolism, insulin sensitivity and inflammation by the secretion of a number of signaling hormones (referred to generically as adipokines) including leptin, adiponectin, resistin, visfatin, tumor necrosis factor (TNF)-α and plasminogen activator inhibitor-1 (PAI-1). Our data have revealed that CT-1 is a cytoadipokine that critically influences WAT metabolism.

CT-1 mRNA expression levels are higher in skeletal muscle, heart and liver compared with WAT.[13] CT-1 lacks signal peptide, but it has been shown that CT-1 can be released to extracellular milieu by different stimuli such as hypoxia or reactive oxygen species (ROS).[14] In fact, CT-1 is circulating in plasma and its concentration is elevated in arterial hypertension and coronary artery disease.[11,12] Recently, increased serum CT-1 levels have been found in obesity,[15,16] possibly as result of augmented secretion by a diversity of organs altered in this disease. The metabolic, cytoprotective and hypotensor properties described for CT-1 suggest that the reported overproduction of CT-1 observed in subjects with these pathologies could be a protective mechanism to counteract the emergence of obesity-related disorders such as type 2 diabetes or cardiovascular dysfunctions. In *ob/ob* mice CT-1 mRNA abundance was significantly increased in muscle, but not in WAT, as compared with wild-type animals (unpublished observations). Accordingly, it is tempting to speculate that the high circulating levels of CT-1 described in obesity derives more from skeletal muscle than from WAT. It has been shown that muscles have the capacity to secrete several myokines which act in a hormone-like fashion and exert specific endocrine effects on distant organs or locally via paracrine mechanisms.[17] Our data showed that rCT-1 markedly stimulates fatty acid oxidation and influences glucose homeostasis by acting on skeletal muscle. Indeed, acute rCT-1 administration activates AKT in this tissue and enhances insulin-induced AKT activation. Interestingly, in vitro incubation of L6E9 myocytes with rCT-1 increases insulin-mediated AKT phosphorylation and glucose uptake. As the skeletal muscle is the largest organ in human body, the decreased plasma glucose observed after systemic administration of rCT-1 could be explained by the specific effects of rCT-1 in muscle. Importantly, chronic rCT-1 treatment increased insulin-induced AKT phosphorylation in muscle of wild-type mice and when given chronically for 10 d to *ob/ob* mice reduced significantly the levels of glucose and insulin together with an improvement in ITT compared with both saline and pair-fed groups.[13] It has been reported that CT-1 may cause insulin resistance in adipocytes. As other members of IL-6 family of cytokines, CT-1 induces the expression of suppressor of cytokine signaling (SOCS)3,[18] a molecule that binds to the insulin receptor inhibiting insulin receptor substrate (IRS)-1 phosphorylation and downstream insulin signaling. In addition, SOCS3 has been shown to inhibit insulin signaling by targeting IRS-1 and -2 for proteasomal degradation.[19] As SOCS3 is upregulated in WAT from different models of obese mice,[20] it has been proposed that this molecule might promote insulin resistance in WAT. However, the study of the role of SOCS3 in WAT by creating fat-specific SOCS3 transgenic mice showed that, although overexpression of this molecule in adipocytes inhibited local insulin action, it improved systemic glucose metabolism in animals subjected to a high-fat diet. It was found that SOCS3 transgenics showed increased serum levels of adiponectin and decreased WAT mass indicating that the reduction of adipocyte size and the increase of insulin-sensitizing adipokines exert a dominant effect on systemic insulin sensitivity, overcoming local adipocyte insulin resistance.[21] In fact, adipose tissue is responsible for only 10% of whole-body glucose uptake, while muscle accounts for 80–90%.[22] Therefore, insulin resistance in WAT may not necessarily cause systemic insulin resistance. Furthermore, a previous report has shown that CNTF is effective on insulin sensitivity of skeletal muscle by bypassing the effects of SOCS3 induction by high-fat diet.[23] Although we did not test the effects of SOCS3 overexpression on CT-1-induced signaling in WAT, it is important to mention that neither acute nor chronic rCT-1 treatment inhibited insulin-induced AKT phosphorylation in WAT from obese mice and cultured adipocytes,[13] suggesting that CT-1 effects are not impaired by SOCS3.

Taken into account these effects and the fact that CT-1 mRNA expression increased in skeletal muscle in circumstances of obesity and insulin resistance,

the overall interpretation could be that increased CT-1 mRNA expression in skeletal muscle could represent a protective mechanism to counteract fat accumulation and to facilitate glucose uptake by the muscle acting in an autocrine or paracrine manner. In this context, it has been shown that mRNA levels of IL-6, a prototypic myokine, increase after 30 min of exercise, sensitizing myocytes to insulin action,[24] which underscores the fact that proteins from skeletal muscle influence local muscle biology. Myokines could also induce metabolic effects in distant organs such as adipose tissue, liver and brain. Recently, it has been shown that IL-6 secreted by skeletal muscle or WAT mediates crosstalk between insulin-sensitive tissues, intestines and pancreatic islets through glucagon-like-peptide-1 (GLP-1).[25]

CT-1 mRNA expression increased markedly when mice were subjected to 48 h fast and decreased rapidly upon refeeding. Those changes were observed in a variety of organs including skeletal muscle, WAT and liver, indicating that CT-1 is regulated by nutritional status.[13] During fasting, circulating glucagon and glucocorticoids levels are elevated as well as free fatty acids (FFA), all those factors serve as mediators of the adaptive response to starvation by inducing changes in gene expression to deal with the new metabolic conditions. Because CT-1 mRNA is induced during fasting, further investigations will be needed in order to determine the role of cAMP (the mediator of glucagon action), glucocorticoids or FFA in the regulation of CT-1. It has been reported that glucose upregulates CT-1 mRNA levels in cultured murine adipocytes and that serum CT-1 levels are significantly higher in subjects with hyperglycemia.[15] Our data would argue against the mentioned hypothesis, since glucose levels tend to decrease in fasting when CT-1 is upregulated; however, more investigations are required to better understand the nutritional regulation of CT-1. As CT-1 is induced by fasting, it appears to play a role in the biological response to starvation by enhancing hydrolysis of triglycerides and providing FFA to other tissues to be oxidized in situations of energy depletion. In this regard, as mentioned before, we found that rCT-1 enhances the expression of lipolytic genes in WAT, promotes lipolysis in adipocytes and boosts FFA oxidation in muscle.[13]

Future Perspectives

Our data point to CT-1 as a promising therapy for obesity and type 2 diabetes. This is not only because CT-1 activates fat utilization and improves insulin sensitivity acting on WAT and muscle, but also because it possesses anorexigenic properties. Another hallmark of our study was the finding that the administration of rCT-1 caused a significant fall in plasma glucose in mice with streptozotocin (STZ)-induced insulin deficiency, demonstrating an insulin-independent effect of CT-1 on glucose homeostasis, and suggesting a potential application in type 1 diabetes by helping to obtain a better glycemic control and reducing the requirements of insulin. Further research is needed to determine the actions of CT-1 in pancreatic islet including if the cytokine would be able to preserve the function of insulin-producing β cells. Liver is also a key organ for glucose and lipid metabolism. Previous studies of our group have evidenced the hepatoprotective properties of CT-1.[14,26-28] Research should be also focused in the metabolic effects of CT-1 on hepatic glucose and lipid metabolism, including the regulation of lipogenesis, gluconeogenesis as well as hepatic insulin sensitivity and glucose production, which play important roles in the development of hyperglycemia and hyperlipidemia of diabetes. Another issue to be solved is the physiological role of CT-1 in the regulation of food intake and EE. We clearly demonstrated the anorexigenic properties of pharmacological doses of rCT-1, but we also observed that *ct-1* null mice represent a model of hypophagic obesity. We hypothesized that the hypophagia of these animals may be a compensatory mechanism to slow down excessive weight gain as result of low EE.[13] Nevertheless, the underlying biochemical/molecular mechanisms by which CT-1 regulates food intake and basal metabolic rate remain to be elucidated. Moreover, given the potential of CT-1 to influence energy homeostasis, it is important to determine whether variability in the *ct-1* gene is associated with a higher risk of developing obesity in humans. Taking into account the beneficial effects described for CT-1 in obesity and insulin resistance in rodents, we may hypothesize that the reported overproduction of CT-1 in obese subjects with metabolic syndrome[15,16] could be a protective mechanism opposing the emergence of obesity-related disorders. More importantly, the efficacy of safe and well-tolerated doses of CT-1 in the treatment of human obesity and metabolic syndrome remains to be demonstrated in clinical trials.

References

1. Prieto-Hontoria PL, Pérez-Matute P, Fernández-Galilea M, Bustos M, Martínez JA, Moreno-Aliaga MJ. Role of obesity-associated dysfunctional adipose tissue in cancer: a molecular nutrition approach. Biochim Biophys Acta 2011; 1807:664-78; PMID:21111705; http://dx.doi.org/10.1016/j.bbabio.2010.11.004
2. White UA, Stewart WC, Stephens JM. Gp130 cytokines exert differential patterns of crosstalk in adipocytes both in vitro and in vivo. Obesity (Silver Spring) 2011; 19:903-10; PMID:21164505; http://dx.doi.org/10.1038/oby.2010.293
3. White UA, Stephens JM. Neuropoietin activates STAT3 independent of LIFR activation in adipocytes. Biochem Biophys Res Commun 2010; 395:48-50; PMID:20353755; http://dx.doi.org/10.1016/j.bbrc.2010.03.132
4. Febbraio MA. gp130 receptor ligands as potential therapeutic targets for obesity. J Clin Invest 2007; 117:841-9; PMID:17404609; http://dx.doi.org/10.1172/JCI30453
5. Watt MJ, Dzamko N, Thomas WG, Rose-John S, Ernst M, Carling D, et al. CNTF reverses obesity-induced insulin resistance by activating skeletal muscle AMPK. Nat Med 2006; 12:541-8; PMID:16604088; http://dx.doi.org/10.1038/nm1383
6. Steinberg GR, Watt MJ, Fam BC, Proietto J, Andrikopoulos S, Allen AM, et al. Ciliary neurotrophic factor suppresses hypothalamic AMP-kinase signaling in leptin-resistant obese mice. Endocrinology 2006; 147:3906-14; PMID:16675525; http://dx.doi.org/10.1210/en.2005-1587
7. Ettinger MP, Littlejohn TW, Schwartz SL, Weiss SR, McIlwain HH, Heymsfield SB, et al. Recombinant variant of ciliary neurotrophic factor for weight loss in obese adults: a randomized, dose-ranging study. JAMA 2003; 289:1826-32; PMID:12684362; http://dx.doi.org/10.1001/jama.289.14.1826
8. Pedersen BK, Febbraio MA. Point: Interleukin-6 does have a beneficial role in insulin sensitivity and glucose homeostasis. J Appl Physiol 2007; 102:814-6; PMID:17068210; http://dx.doi.org/10.1152/japplphysiol.01208.2006

9. Mooney RA. Counterpoint: Interleukin-6 does not have a beneficial role in insulin sensitivity and glucose homeostasis. J Appl Physiol 2007; 102:816-8, discussion 818-9; PMID:17284655; http://dx.doi.org/10.1152/japplphysiol.01208a.2006
10. Pennica D, King KL, Shaw KJ, Luis E, Rullamas J, Luoh SM, et al. Expression cloning of cardiotrophin 1, a cytokine that induces cardiac myocyte hypertrophy. Proc Natl Acad Sci U S A 1995; 92:1142-6; PMID:7862649; http://dx.doi.org/10.1073/pnas.92.4.1142
11. Pemberton CJ, Raudsepp SD, Yandle TG, Cameron VA, Richards AM. Plasma cardiotrophin-1 is elevated in human hypertension and stimulated by ventricular stretch. Cardiovasc Res 2005; 68:109-17; PMID:15978561; http://dx.doi.org/10.1016/j.cardiores.2005.05.014
12. Calabrò P, Limongelli G, Riegler L, Maddaloni V, Palmieri R, Golia E, et al. Novel insights into the role of cardiotrophin-1 in cardiovascular diseases. J Mol Cell Cardiol 2009; 46:142-8; PMID:19059413; http://dx.doi.org/10.1016/j.yjmcc.2008.11.002
13. Moreno-Aliaga MJ, Pérez-Echarri N, Marcos-Gómez B, Larequi E, Gil-Bea FJ, Viollet B, et al. Cardiotrophin-1 is a key regulator of glucose and lipid metabolism. Cell Metab 2011; 14:242-53; PMID:21803294; http://dx.doi.org/10.1016/j.cmet.2011.05.013
14. Iñiguez M, Berasain C, Martinez-Ansó E, Bustos M, Fortes P, Pennica D, et al. Cardiotrophin-1 defends the liver against ischemia-reperfusion injury and mediates the protective effect of ischemic preconditioning. J Exp Med 2006; 203:2809-15; PMID:17178916; http://dx.doi.org/10.1084/jem.20061421
15. Natal C, Fortuño MA, Restituto P, Bazán A, Colina I, Díez J, et al. Cardiotrophin-1 is expressed in adipose tissue and upregulated in the metabolic syndrome. Am J Physiol Endocrinol Metab 2008; 294:E52-60; PMID:17940213; http://dx.doi.org/10.1152/ajpendo.00506.2007
16. Malavazos AE, Ermetici F, Morricone L, Delnevo A, Coman C, Ambrosi B, et al. Association of increased plasma cardiotrophin-1 with left ventricular mass indexes in normotensive morbid obesity. Hypertension 2008; 51:e8-9, author reply e10; PMID:18180396; http://dx.doi.org/10.1161/HYPERTENSIONAHA.107.105346
17. Pedersen BK, Febbraio MA. Muscle as an endocrine organ: focus on muscle-derived interleukin-6. Physiol Rev 2008; 88:1379-406; PMID:18923185; http://dx.doi.org/10.1152/physrev.90100.2007
18. Zvonic S, Hogan JC, Arbour-Reily P, Mynatt RL, Stephens JM. Effects of cardiotrophin on adipocytes. J Biol Chem 2004; 279:47572-9; PMID:15339920; http://dx.doi.org/10.1074/jbc.M403998200
19. Rui L, Yuan M, Frantz D, Shoelson S, White MF. SOCS-1 and SOCS-3 block insulin signaling by ubiquitin-mediated degradation of IRS1 and IRS2. J Biol Chem 2002; 277:42394-8; PMID:12228220; http://dx.doi.org/10.1074/jbc.C200444200
20. Emanuelli B, Peraldi P, Filloux C, Chavey C, Freidinger K, Hilton DJ, et al. SOCS-3 inhibits insulin signaling and is up-regulated in response to tumor necrosis factor-alpha in the adipose tissue of obese mice. J Biol Chem 2001; 276:47944-9; PMID:11604392
21. Shi H, Cave B, Inouye K, Bjørbaek C, Flier JS. Overexpression of suppressor of cytokine signaling 3 in adipose tissue causes local but not systemic insulin resistance. Diabetes 2006; 55:699-707; PMID:16505233; http://dx.doi.org/10.2337/diabetes.55.03.06.db05-0841
22. Kahn BB, Flier JS. Obesity and insulin resistance. J Clin Invest 2000; 106:473-81; PMID:10953022; http://dx.doi.org/10.1172/JCI10842
23. Watt MJ, Dzamko N, Thomas WG, Rose-John S, Ernst M, Carling D, et al. CNTF reverses obesity-induced insulin resistance by activating skeletal muscle AMPK. Nat Med 2006; 12:541-8; PMID:16604088; http://dx.doi.org/10.1038/nm1383
24. Wojtaszewski JF, Hansen BF, Gade J, Kiens B, Markuns JF, Goodyear LJ, et al. Insulin signaling and insulin sensitivity after exercise in human skeletal muscle. Diabetes 2000; 49:325-31; PMID:10868952; http://dx.doi.org/10.2337/diabetes.49.3.325
25. Ellingsgaard H, Hauselmann I, Schuler B, Habib AM, Baggio LL, Meier DT, et al. Interleukin-6 enhances insulin secretion by increasing glucagon-like peptide-1 secretion from L cells and alpha cells. Nat Med 2011; 17:1481-9; PMID:22037645; http://dx.doi.org/10.1038/nm.2513
26. Bustos M, Beraza N, Lasarte JJ, Baixeras E, Alzuguren P, Bordet T, et al. Protection against liver damage by cardiotrophin-1: a hepatocyte survival factor up-regulated in the regenerating liver in rats. Gastroenterology 2003; 125:192-201; PMID:12851883; http://dx.doi.org/10.1016/S0016-5085(03)00698-X
27. Beraza N, Marqués JM, Martínez-Ansó E, Iñiguez M, Prieto J, Bustos M. Interplay among cardiotrophin-1, prostaglandins, and vascular endothelial growth factor in rat liver regeneration. Hepatology 2005; 41:460-9; PMID:15723445; http://dx.doi.org/10.1002/hep.20590
28. Marquès JM, Belza I, Holtmann B, Pennica D, Prieto J, Bustos M. Cardiotrophin-1 is an essential factor in the natural defense of the liver against apoptosis. Hepatology 2007; 45:639-48; PMID:17326158; http://dx.doi.org/10.1002/hep.21508