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LETTER TO THE EDITOR

# Balance of adiponectin and leptin modulates breast cancer cell growth

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**Dear Editor,**

There is lack of information concerning how the ratio of adiponectin to leptin in serum affects breast cancer (Brca) proliferation. It is possible that the link between obesity and increased risk for aggressive Brca is in part due to the milieu of cytokines synthesized and released by the adipose tissue [1]. Adiponectin (also known as adipocyte complement-related protein of 30 kDa (Acrp30)) and leptin are two specific cytokines secreted by the adipose tissue. Acrp30 serum levels decrease with increasing fatness while leptin levels increase. Functionally, they appear to oppose each other's actions. Acrp30 can block proliferation of Brca cells [2]. *In vitro* assays have shown that a number of different Brca cell lines express one or both of the Acrp30 receptors and show reduced growth and/or increased apoptosis in response to Acrp30 [3]. Leptin has been implicated as a growth-promoting factor for Brca [1]. Animal studies also support a role for leptin in mammary tumor development as evidenced by the fact that mice deficient in leptin, $Lep^{ob}Lep^{ob}$ [4], or with non-functioning leptin receptors, $Lepr^{db}Lepr^{db}$ [5], did not develop transgene-induced mammary tumors. It is possible that the levels of adiponectin and leptin receptors, as well as the balance of serum adiponectin and leptin, are critical factors in mammary tumorigenesis.

We investigated the effects of Acrp30 and a naturally truncated form known as globular Acrp30 [6] (gAcrp30) in the presence or absence of leptin. There are two different Acrp30 receptors, designated as AdipoR1 and AdipoR2 [7]. Full-length Acrp30 binds with highest affinity to AdipoR2, and gAcrp30 binds with highest affinity to AdipoR1 [7]. The serum levels of gAcrp30 in women with Brca have not yet been fully investigated, nor has the role of gAcrp30 in Brca development. We performed proliferation assays with the MDA-MB-231 (MDA-wt) ERα negative Brca cell line and the MDA-ERα7 cell line which we developed by stably integrating the ERα gene such that it now exhibits increased growth *in vivo* in the presence of exogenous estradiol [8]. Leptin is present in the serum of almost all humans in the range of 5-50 ng/ml [9]. However, in obese individuals levels in excess of 100 ng/ml are common. The addition of leptin (50 ng/ml) to these two cell lines caused a slight increase in proliferation (Figure 1A) for both the MDA-wt and MDA-ERα7 cells after 48 h. Acrp30 is measured in human serum in the range of 2-20 μg/ml and is negatively correlated with body weight, BMI, body fat and serum leptin in women [10]. When these two cell lines were treated with Acrp30 and gAcrp30 there was a reduction in proliferation of the MDA-wt and MDA-ERα7 cells as compared to leptin-treated cells. To investigate how changes in the ratio of leptin and Acrp30 or gAcrp30 affect cell growth, we performed proliferation assays using ratios of Acrp30 to leptin that simulate the physiological balance found with increasing body weight, i.e., by decreasing adiponectin and increasing leptin. A high ratio of Acrp30 to leptin caused a reduction in proliferation (Figure 1B), while a low ratio of Acrp30 to leptin (2.5/66.4) did not cause a reduction in cell proliferation of either cell line (Figure 1B). In Figure 1C the cells were treated with different ratios of gAcrp30 and leptin. The ratios of Acrp30 and gAcrp30 to leptin are in μg/ng, as is the custom when performing serum measurements, but gAcrp30 is actually smaller than Acrp30. Hence, in order to be sure that any increased activity of gAcrp30 was not due to an increase in the actual molar ratios, we utilized less gAcrp30 on a μg/ng scale. We found that both the MDA-ERα7 and MDA-wt cells had reductions in cell proliferation at the

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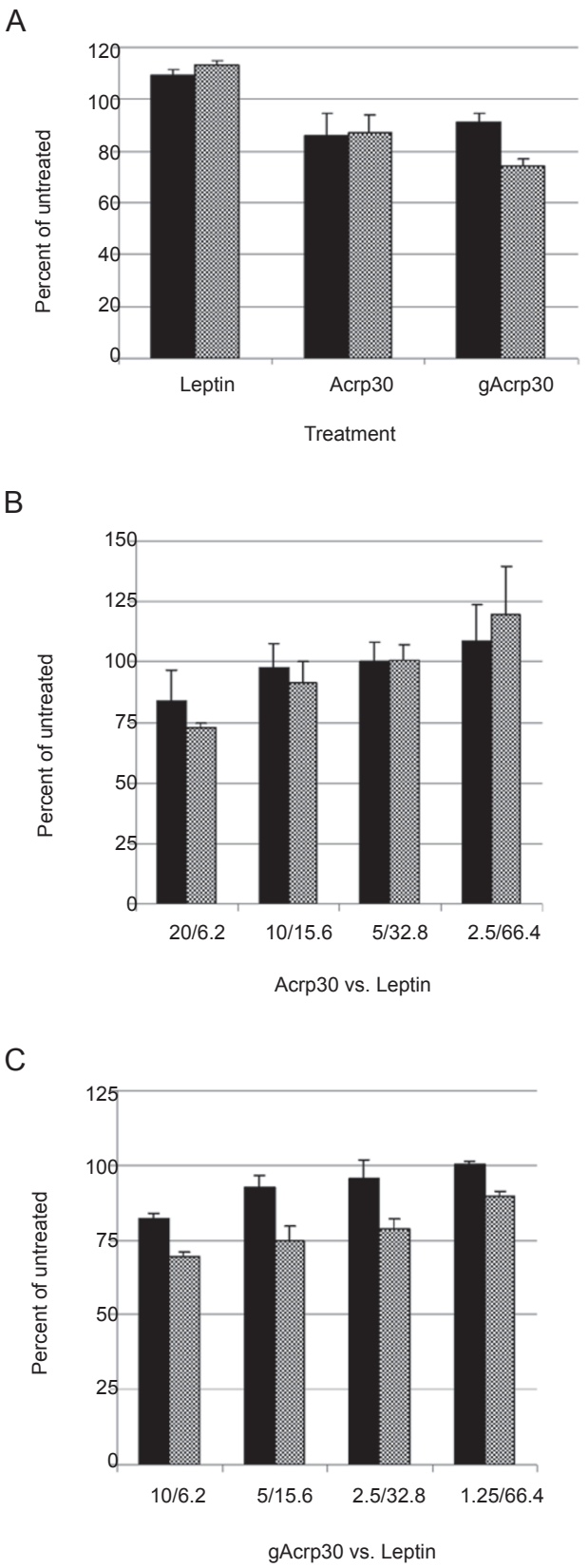


**Figure 1** Proliferation of breast cancer cell lines and expression of Acrp30 and leptin receptors in tumor tissue. **(A)** Two-way ANOVA, *P*=0.0025 for differences among the leptin, Acrp30 and gAcrp30 treatments. Cells were treated with leptin (50 ng/ml), Acrp30 (20 μg/ml) or gAcrp30 (10 μg/ml) for 48 h. The *y*-axis represents percent untreated and *x*-axis different treatments. The MDA-wt cell line is denoted by solid bars and the MDA-ERα7 cell line by checkered bars. Standard error of the mean is shown above each bar. Each bar represents 3-8 experiments using triplicate wells in each experiment. **(B)** Two-way ANOVA, *P*=0.0473 for differences among the treatments. **(C)** Two-way ANOVA, *P*=0.0006 for differences among the treatments and *P*<0.0001 for differences overall between the cell lines. Concentrations are shown along the *x*-axis of (B) and (C), with leptin to the right of the slash. The *y*-axis is shown as percent, where 100% represents the amount for proliferation of each individual cell line in serum-free media after 48 h. All proliferation assays were performed using 10 μl of CCK-8 reagent from the Cell Counting Kit-8 as per the manufacturer's instructions (Dojindo Laboratories, Japan). In this assay a formazan dye is generated by the activity of dehydrogenases in cells that is directly proportional to the number of living cells. The plates were then incubated for 1.5-3 h depending on the cell line in a $CO_2$ incubator, after which the plates were read on an ELISA reader at 450 nm.

highest ratios of gAcrp30 to leptin. Very interestingly, we also found that cell proliferation of the ERα-positive MDA-ERα7 cells was lower than that of MDA-wt cells which are ERα negative.

Here we have moved a step beyond assessing individual adipokines by determining the effects of a changing ratio between Acrp30 and leptin on human Brca cells. The actions of gAcrp30 have received very little attention in relation to Brca growth. We found that gAcrp30 is capable of decreasing cell proliferation of the MDA-ERα7 cell line (Figure 1A). Furthermore, when gAcrp30 and leptin were combined at ratios that simulate the serum levels of non-obese individuals there was a reduction in Brca cell proliferation and the MDA-ERα7 cells were more sensitive as compared to ER-negative MDA-wt cells (Figure 1C). This illustrates that the ratio of gAcrp30 to leptin as well as the ERα status of the cells can be determinants in Brca growth. Nutritional interventions that affect mammary tumor development will now be undertaken to investigate specific links between Brca and the ratio of Acrp30/gAcrp30 to leptin *in vivo*.

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*Disclosures*

The authors indicate no potential conflicts of interest.

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