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Original article

Tumor growth factor expression in obesity and changes in expression with weight loss: another cause of increased virulence and incidence of cancer in obesity

Daniel Cottam, M.D.[a,*], Barry Fisher, M.D.[b], Amy Ziemba, M.D.[c], James Atkinson, M.D.[b], Brian Grace, P.A.C.[b], David C. Ward, Ph.D.[c], Giuseppe Pizzorno, Ph.D.[c]

[a]*Surgical Weight Loss Center of Utah, Salt Lake City, Utah*
[b]*Surgical Weight Control Center of Nevada, Las Vegas, Nevada*
[c]*Nevada Cancer Institute, Las Vegas, Nevada*

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Abstract

Background: Obesity is associated with increased tumerogenesis. Previously, we demonstrated that inflammation in obesity caused cancer fighting cells to display greater surface receptor levels, predisposing them to early cell death. We measured the inflammatory tumor growth factor levels to determine whether inflammation in obesity increases expression of these factors, potentially predisposing these patients to greater rates of neoplasia.

Methods: A total of 24 patients undergoing weight loss surgery had samples collected preoperatively and at 6 and 12 months after surgery. The growth factors analyzed included tumor necrosis factor (TNF)-α, granulocyte-macrophage colony-stimulating factor, interferon-γ, interleukin (IL)-1b, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, vascular endothelial growth factor, hepatocyte growth factor, TNF-receptor I (TNF-RI), TNF-RII, death receptor 5, leptin, and adiponectin. Control samples were obtained from 10 healthy, normal weight volunteers.

Results: The tumor growth factors TNF-α, TNF-RI, TNF-RII, vascular endothelial growth factor, hepatocyte growth factor, interferon-γ, IL-2, IL-5, and IL-6 all decreased significantly ($P < .05$) compared with the preoperative values. The IL-4, IL-8, leptin, death receptor 5, adiponectin, and granulocyte-macrophage colony-stimulating factor levels did not change significantly over time. The IL-1b and IL-10 levels were less than the detection limit at all points. When obese patient serum was compared with healthy volunteer pooled serum, we found that the leptin, death receptor 5, hepatocyte growth factor, vascular endothelial growth factor, TNF-RI, TNF-RII, TNF-α, IFN-γ, granulocyte-macrophage colony-stimulating factor, IL-4, IL-5, IL-6, and IL-8 levels were all 2–37 times greater than the levels in the controls at baseline. The concentrations of these same growth factors had decreased levels only 1–3.5 times greater than those of the controls at 12 months postoperatively.

Conclusion: Many inflammatory tumor growth factors are present in greater concentrations in obese individuals. This could explain the greater prevalence of neoplasia in the morbidly obese population. (Surg Obes Relat Dis 2010;6:538–541.)

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*Correspondence: Daniel Cottam, M.D., Surgical Weight Control Center of Nevada, 24th South 11th East, Suite 304, Salt Lake City, UT 84102.

E-mail: drdanielcottam@yahoo.com

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Morbid obesity is a worldwide epidemic that most people have associated with diabetes, hypertension, heart disease, and metabolic syndrome as causes of death. However, obesity also markedly increases the overall cancer death rate by a factor of 1.5. The most common cancers afflicting the morbidly obese are esophageal, postmenopausal breast, kidney, stomach, pancreatic, gallbladder, uterine, cervical, colon, rectal, hepatic, and ovarian cancer [1–6]. These sobering statistics led us to investigate the possible causes of this issue. Specifically, we believed there must be an environment that promotes tumor growth. We selected a broad range of tumor growth factors for the tumors that afflict the morbidly obese and studied their expression levels both before and after surgery.

Methods

The Western institutional review board approved the present study (identifier 20052059). All patients provided written informed consent before beginning the study. All samples were drawn and processed at the Nevada Cancer Institute. All follow-up examinations were done in the office of the Surgical Weight Control Center of Nevada by physicians of the same practice. The present trial was not registered with the National Institutes of Health website because the study had been completed before this had become a requirement for publication.

Serum and plasma isolation

Serum and plasma samples were archived from each patient for analysis of the cytokines. The samples were collected before and 6 and 12 months after surgery. Blood sample of 6 mL were collected in both BD Vacutainer purple-capped plasma tubes containing ethylenediaminetetraacetic acid (EDTA) and BD Vacutainer red-capped serum tubes (BD, Franklin Lakes, NJ). The samples were immediately mixed by inverting the containers several times and placed upright for 20 minutes before centrifugation at 1000 rpm (400*g*) for 15 minutes at room temperature with the centrifuge break turned off. The samples were isolated within 30 minutes of venipuncture and visually inspected for hemolysis. The serum and plasma samples were transferred into single-freeze aliquots for storage at −80°C until analysis. The samples were evaluated within 4 months of processing.

Luminex analysis

The human cytokine 10-plex panel was purchased from Invitrogen's Biosource division (Carlsbad, CA). For the remainder of the analytes, a custom 7-plex panel was developed by Biosource, and all quality control was optimized by the manufacturer. The 7-plex panel included the following analytes: vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), tumor necrosis factor (TNF)-receptor I (RI), TNF-RII, death receptor 5 (DR5), leptin, and adiponectin. The samples were thawed on ice, and the samples clarified by centrifuging at 14,000 rpm for 10 minutes. The plasma samples were used as input for the 10-plex panel, and the serum samples were used for the custom 7-plex panel according to the manufacturer's recommendation. Analytes for the standard curves were provided by Biosource and were reconstituted fresh for each experiment. The samples were analyzed in duplicate on 2 separate days using a 1:2 sample diluent in accordance with the manufacturer's instructions.

Regression analysis was performed using Bio-Plex Manager, version 4.0, software with 5-parameter logistics (Bio-Rad Laboratories, Hercules, CA).

Results

The patients' weight characteristics are listed in Table 1. No significant differences were seen between the band and bypass patients in preoperative weight. The median age for both groups was 40 years. When preoperative analyses were performed using Pearson correlation coefficients, no correlation was seen between the body mass index (BMI) or weight and the tumor growth factor levels. These nonlinear values were also present in weight loss because no correlation was seen between the amount of weight lost, change in BMI, and levels of tumor growth factors. These facts held true regardless of the surgery type.

The tumor growth factors TNF-α, TNF-RI, TNF-RII, VEGF, HGF, IFN-γ, IL-2, IL-5, and IL-6 all decreased significantly ($P < .05$) compared with the preoperative values (Table 2). The IL-4, IL-8, leptin, DR5, adiponectin, and granulocyte-macrophage colony-stimulating factor (GM-CSF) levels did not change significantly over time.

When the obese patients' serum was compared with the healthy volunteer pooled serum, we found that the leptin, DR5, HGF, VEGF, TNF-RI, TNF-RII, TNF-α, IFN-γ, GM-CSF, IL-4, IL-5, IL-6, and IL-8 levels were all 2–37 times greater than those of the controls at baseline (Table 3). The concentrations of these same growth factors had decreased to levels only 1–3.5 times greater than those of the controls at 12 months (Table 3).

From the 10-plex panel, the IL-1b and IL-10 levels were less than the detection limit of the multiplex assay; therefore, these data were not evaluated.

Table 1
Weight loss of study participants

Variable	Patients (n)	Mean ± SD	Median	Minimum	Maximum	*P* value
Weight						
Preoperative	23	291.7 ± 38.9	289.0	233.0	373.0	
6-mo Postoperative	19	228.7 ± 32.9	228.4	164.1	290.1	
Difference	19	−61.0 ± 31.8	−59.4	−130.0	−17.2	<.001
12-mo Postoperative	13	211.4 ± 39.9	225.4	127.3	274.0	
Difference	13	−70.6 ± 32.9	−66.3	−130.7	−30.6	<.001
BMI						
Preoperative	23	45.8 ± 5.8	45.0	37.8	62.2	
6-mo Postoperative	19	35.8 ± 5.1	35.9	28.0	44.9	
Difference	19	−9.1 ± 4.9	−8.7	−17.0	−.2	<.001
12-mo Postoperative	13	33.1 ± 5.7	32.2	21.9	42.9	
Difference	13	−11.3 ± 5.7	−9.3	−22.4	−4.7	<.001

BMI = body mass index.
Paired *t* test.

Discussion

Most previously published reports have focused on cancer epidemiology. That morbidly obese patients have greater rates of cancer is no longer in question [1–8]. Additionally, new evidence from Canada and the United States have highlighted the decrease in cancer incidence after surgically induced weight loss [1–4]. Christou et al. [9,10] have shown that cancer deaths begin to decrease within 6 months after surgery, far shorter than the cancer generation times.

Our previous studies highlighted the cellular immune system and showed how cancer fighting cells were at a greater risk of dying of excess expression of apoptosis-inducing surface receptors [11–14]. This finding correlated with the cancers that afflict the morbidly obese, showing an increased expression of apoptotic-inducing ligands.

Our next hypothesis was that obesity fosters an environment that supports tumorigenesis and increased virulence. This theory was reached when we investigated chronic inflammation in morbidly obese patients and realized that many of the tumor growth factors were also components of the chronic inflammatory cascade [14].

The results from the present pilot study suggest that the chronic inflammation present in obesity provides a hormonal milieu that might allow tumors, once formed, to flourish. However, we were surprised that weight and BMI did not correlate directly with tumor growth factor expression. The decrease in tumor growth hormonal expression also did not correlate with weight loss or with any surgical procedure for any of the tumor growth factors measured (see the Results section). Age plays a role in cancer expression; however, because both surgical groups had the same

Table 2
Growth factor changes over time (Kruskal-Wallis one-way analysis of variance on ranks, Dunn's method when folded over norms)

Growth factor	Preoperative median	6-mo median	12-mo median	*P* value
Leptin	2.50	1.60	1.70	.057
DR5	1.10	1.30	1.20	.516
Adiponectin	1.1	1.2	1.1	.989
HGF	2.5	1.9	1.9	<.001
VEGF	1.610	1.40	1.1	.002
TNF-RI	1.910	1.4	1.2	<.001
TNF-RII	1.75	1.1	.90	.001
TNF-α	6.256	3.935	1.922	.026
IFN-γ	7.952	3.784	1.526	.001
GM-CSF	2.376	2.049	1.602	.254
IL-4	2.343	1.785	1.615	.437
IL-5	15.815	6.261	1.111	<.001
IL-6	3.481	2.333	1.309	.024
IL-8	1.723	1.606	1.771	.928
IL-2	3.012	.889	2.117	.001

DR5 = death receptor 5; HGF = hepatocyte growth factor; VEGF = vascular endothelial growth factor; TNF = tumor necrosis factor; IFN = interferon; GM-CSF = granulocyte-macrophage colony-stimulating factor; IL = interleukin.

Table 3
Obese tumor growth factor multiples of healthy volunteers' cancer-free pooled serum concentrations*

Growth factor	Preoperative level	6-mo Level	12-mo Level
Leptin	3.85	.3	.025
DR5	1.91	1.51	1.43
Adiponectin	−.007	−.003	−.009
HGF	4.6	1.93	1.75
VEGF	2.0	1.47	1.13
TNF-RI	2.47	1.54	1.22
TNF-RII	2.36	1.31	1.02
TNF-α	16.1	9.9	3.5
IFN-γ	9.6	5.2	2.1
GM-CSF	3.1	2.5	1.6
IL-4	4.5	3.0	2.1
IL-5	37.5	16.6	1.36
IL-6	5.6	2.6	2.2
IL-8	2.5	1.9	1.6

Abbreviations as in Table 2.
* All numeric values represent multiples of healthy volunteer's serum values, except for leptin's mean preoperative concentration, which was 3.85 times greater than the controlled serum value expressed in nanograms per milliliter.

mean age, it was impossible to determine what role age might have played in this cohort [15]. Co-morbid conditions also varied widely; therefore, it was impossible to focus on any single co-morbid condition as causing or resulting in increased tumor growth factor expression. The relationships have been difficult to establish between many co-morbid conditions and the hormonal milieu. One example would be the seemingly obvious associations such as the homostasis model assessment–insulin resistance, ghrelin, hunger, and obesity [16–18]. Outcomes such as glucose and ghrelin made us focus on weight and surgery types as factors. The weight dichotomous results suggested, but in no way have proved, that becoming "morbidly obese" is not necessary for the increased risk of tumorigenesis but that just being obese or overweight is necessary. If the hormonal environment is important to the greater rates of malignancy, the effects of weight loss that decrease cancer risk might not accrue in a linear fashion or as a result of a specific procedure. With a small sample size such as in the present study, we would need large differences between the procedures to show any significance. These results have been supported by large epidemiologic studies that showed that increased cancer risk begins at a BMI of 28 kg/m^2 and not at a BMI of 35 or 40 kg/m^2 [3].

This was a pilot study and thus had the methodologic flaws related to the small sample size and possible variance within samples. An example would be adiponectin, which generally increases with weight loss, but showed no change in our sample. Because we used healthy, normal weight, cancer-free volunteers for our pooled serum comparisons, we could not preclude errors from our control group with such a small sample set. We also chose 2 different surgical procedures. We did this specifically to determine whether large differences were present between the 2 groups. Small differences would not have been captured in this sample size.

Conclusion

The results from the present study have strongly suggested that morbidly obese patients have elevated tumor growth hormones and that weight loss by either band or bypass can drastically reduce these hormonal levels. Whether these changes or those seen with apoptotic receptors are real or causally related to the decrease in cancer rates after surgery needs additional study. The results from the present study suggest which hormones might be appropriate for additional consideration.

Disclosures

The authors have no commercial associations that might be a conflict of interest in relation to this article.

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