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Diet-Induced Obesity Accelerates Acute Lymphoblastic Leukemia Progression in Two Murine Models

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Abstract

Obesity is associated with an increased incidence of many cancers, including leukemia, although it is unknown whether leukemia incidence is increased directly by obesity or rather by associated genetic, lifestyle, health, or socioeconomic factors. We developed animal models of obesity and leukemia to test whether obesity could directly accelerate acute lymphoblastic leukemia (ALL) using *BCR/ABL* transgenic and AKR/J mice weaned onto a high-fat diet. Mice were observed until development of progressive ALL. Although obese and control *BCR/ABL* mice had similar median survival, older obese mice had accelerated ALL onset, implying a time-dependent effect of obesity on ALL. Obese AKR mice developed ALL significantly earlier than controls. The effect of obesity was not explained by WBC count, thymus/spleen weight, or ALL phenotype. However, obese AKR mice had higher leptin, insulin, and interleukin-6 levels than controls, and these obesity-related hormones all have potential roles in leukemia pathogenesis. In conclusion, obesity directly accelerates presentation of ALL, likely by increasing the risk of an early event in leukemogenesis. This is the first study to show that obesity can directly accelerate the progression of ALL. Thus, the observed associations between obesity and leukemia incidence are likely to be directly related to biological effects of obesity. *Cancer Prev Res; 3(10); 1259–64. ©2010 AACR.*

Introduction

Obesity is associated with increased incidence and mortality from several types of cancer, including leukemia (1, 2). The association between acute lymphoblastic leukemia (ALL) risk and caloric intake (3) and obesity (4, 5) is evident across many populations, although why this occurs is unknown. Because obesity is associated with numerous physiologic effects and potential confounding lifestyle, socioeconomic, genetic, and health factors, it is difficult to investigate this association in human studies. Also, there are currently no obese mouse models that develop ALL. Given the high prevalence of obesity, it is critical to determine whether obesity directly increases cancer incidence or is merely a marker of some other associated exposure.

The present study was designed to test whether diet-induced obesity would directly increase the rate of progression of ALL in two distinct mouse models.

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Materials and Methods

Mouse model

We used a high-fat diet (60% calories from fat, Research Diets; ref. 6) to induce obesity in two mouse models of ALL. Male *BCR/ABL* mice on a C57Bl background, which develop pro-B ALL (7), were randomized at weaning to a high-fat diet (*n* = 40) or standard laboratory chow (*n* = 41, 13.5% calories from fat, Labdiet). Male AKR/J are a strain of inbred mice which develop T-cell ALL starting at 5 to 6 months of age due to recombinant retroviruses that target thymocytes (8). These mice were randomized to high-fat diet (*n* = 12) or control diet (*n* = 12, 10% calories from fat, Research Diets) at 5 weeks of age. Although it was not possible to define the precise onset of ALL in these experiments, mice were removed from the study when they developed signs of progressive ALL [palpable mass (>1 cm), hind limb paralysis, moribund state, weight loss, or respiratory distress]. ALL was verified by necropsy on all mice.

In additional experiments, fat-fed and control male AKR mice (*n* = 12 per group) were sacrificed at various time points before ALL onset. To test whether obesity alters T-cell maturation, thymocytes were isolated, lymphocytes were selected by forward and side scatter on a BD FACScan, and gated populations were analyzed for CD4/CD8 and TCR expression (TCR-αβ and TCR-γδ, BD Pharmingen).

All animal experiments were approved by the Childrens Hospital Los Angeles Institutional Animal Care and Use
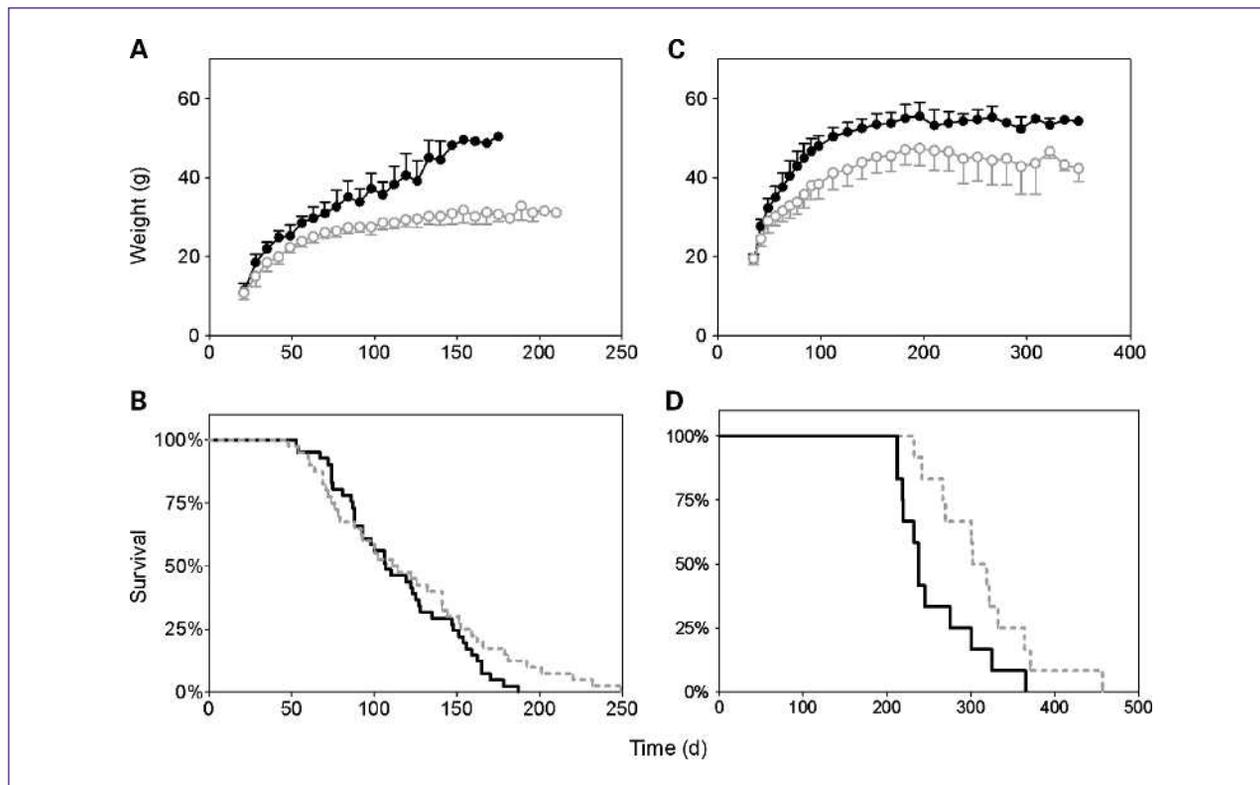
Fig. 1. Weight and survival of two mouse models of leukemia in obesity. A, weights of mice transgenic for human *BCR/ABL* randomized to a high-fat diet (60% of calories from fat; closed circles) or lab chow (13.5% calories from fat; open circles). B, survival curves generated from Kaplan-Meier life tables of obese (solid line) versus control (dashed line) *BCR/ABL* mice. Weights (C) and survival (D) curves of AKR/J mice randomized to high-fat diet (60% of calories from fat) versus control (10% of calories from fat) diet. Weights were significantly higher in fat-fed mice in both models at all time points after weaning ($P < 0.05$), and survival was significantly shorter (see Materials and Methods; $P < 0.05$, log-rank test).

Committee and were done in accordance with the USPHS Policy on Humane Care and Use of Laboratory Animals.

Assays

Plasma samples from the AKR mice, which were sacrificed at various time points before ALL onset, were analyzed for adiponectin concentration using an ELISA (Millipore). Leptin, interleukin-6 (IL-6), and insulin were measured using a Milliplex mouse adipokine kit (Millipore) in the Beckman Center for Immune Monitoring at the University of Southern California Norris Cancer Center and analyzed with the Bio-Plex Suspension Array System (BIO-RAD).

Calculations

Body weights were compared using two-sided *t* tests. Weight loss just before ALL onset was excluded from the weight curves. Kaplan-Meier survival curves were generated on Prism 5.00 (GraphPad Software, Inc.) and compared by log-rank test. Due to the small number of AKR mice included in the survival analysis, *P* values for the difference in survival were confirmed using a permutation distribution of the log-rank test with 2,000 replicates. For the *BCR/ABL* mice, the survival curves diverged at later

time points, implying that obesity might have a late or cumulative effect to accelerate ALL. Thus, these data were reanalyzed (*post hoc*) by Cox regression using the day before the first death occurred as time 0. Test of Schoenfeld residuals showed that the proportional hazard assumption was violated, so the interaction between obesity and time was evaluated and the hazard ratio of death of obese mice compared with controls was calculated at various time points. Organ weights and adipokines were compared between groups using ANOVA. When diet effects were significant by ANOVA, groups were compared at each time point by *t* test. Multivariate regression analysis (9) was used to examine whether there was any difference in thymocyte expression patterns between diet groups. All *P* values are two sided. Analyses were done using Stata 9.2 (StataCorp) and Prism 5.00.

Results

Both *BCR/ABL* and AKR mice were significantly heavier than controls after 1 week on the high-fat diet ($P < 0.001$; Fig. 1A and C). The *BCR/ABL* mice began presenting with signs of ALL at ~7 weeks of age. Whereas fat-fed *BCR/ABL* mice had a similar median survival to the

controls (107 versus 113 days; Fig. 1B, $P = 0.2$, log-rank test), in *post hoc* analysis, the hazard ratio of ALL significantly varied over time by diet group ($P = 0.047$). At 60 days of age, the risk of ALL in obese mice was 0.61 times (95% confidence interval, 0.24-1.5) that of controls, whereas at 150 days, the risk was twice as high (95% confidence interval, 1.1-3.7) as controls. This was reflected by the decreased maximum life span in obese mice compared with control mice ($175 ± 10$ versus $226 ± 26$ days for the longest living 10% of each group, $P < 0.005$). Thus, obesity increased the risk of ALL in older *BCR/ABL* transgenic mice.

AKR mice began presenting with ALL at ~7 months of age. Cause of death could not be confirmed in one mouse in each diet group; otherwise, all mice presented with labored breathing and/or tumor. Obese AKR mice developed ALL earlier than control mice, resulting in a 2.5-month lower median survival (237 versus 310 days; Fig. 1D, $P = 0.035$, log-rank test; $P = 0.039$, permutation analysis). Thymus weights from AKR mice before ALL were increased by both obesity and age ($P = 0.005$ and 0.003, respectively, ANOVA, $n = 3$ per group per time point; Fig. 2A), but there was no interaction between these variables ($P = 0.59$). A similar pattern was observed with spleen weight (diet, $P < 0.001$; age, $P = 0.026$; interaction, $P = 0.15$; Fig. 2B). When expressed as a percentage of body weight, there was no effect of diet on thymus size and only a borderline significant effect of high-fat diet to increase spleen size ($P = 0.066$, data not shown). Also, age and diet did not seem to alter the thymocyte expression patterns of CD4/CD8 (Fig. 2C) or the TCR subtype (α/β versus γ/δ,

data not shown; $P = 0.26$). In both obese and control AKR mice sacrificed at the time of progressive ALL, the thymus samples showed a predominant outgrowth of cells with similar expression (most frequently $CD4^+/CD8^+$ or $CD4^-/CD8^-$; data not shown), and this also did not differ between diet groups.

To investigate potential mediators of the obesity-leukemia link in this study, we measured various obesity-related hormones in plasma from the AKR mice sacrificed at various time points. High-fat diet was associated with increased (nonfasting) levels of insulin ($P < 0.001$), leptin ($P = 0.004$), and IL-6 ($P < 0.001$, ANOVA; Fig. 3). Leptin also increased over time ($P = 0.041$). There was no significant effect of diet or age on plasma adiponectin level.

Discussion

The present results are the first to our knowledge to show that obesity can directly accelerate ALL progression. Obesity increased the risk of ALL in older, but not younger, *BCR/ABL* mice, whereas it had an even more significant effect on the AKR mice, which did not develop ALL until after 7 months of age. This is consistent with a time-dependent and/or cumulative effect of obesity, such as that observed for other exposure-related cancers, such as lung carcinoma (smoking) and breast cancer (estrogen). This is also consistent with other comorbidities of obesity, which are related to a cumulative exposure, such as heart disease, diabetes, and arthritis. Additionally, a certain threshold of obesity may need to be reached before it

Fig. 2. Wet weights of thymuses (A) and spleens (B) from obese (solid) and control (open circles) AKR/J mice. C, CD4/8 expression of lymphocytes isolated from thymuses of AKR/J mice. Left columns are from obese mice, and right columns are from controls. Black, $CD4^+/CD8^+$; hatched, $CD4^+/CD8^-$; white, $CD4^-/CD8^+$; gray, $CD4^+/CD8^+$.
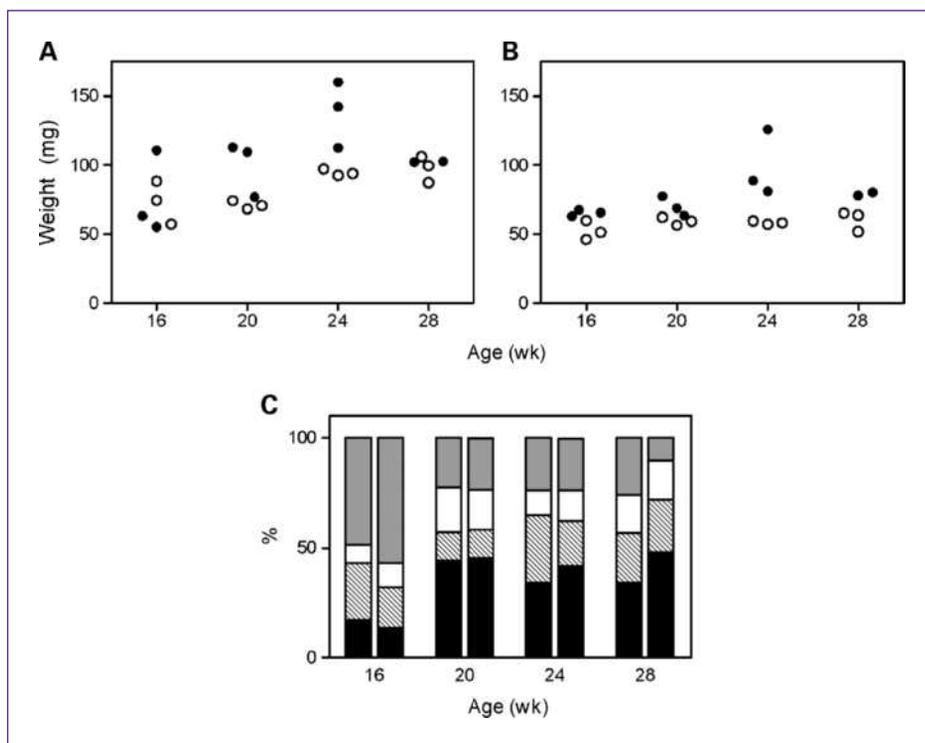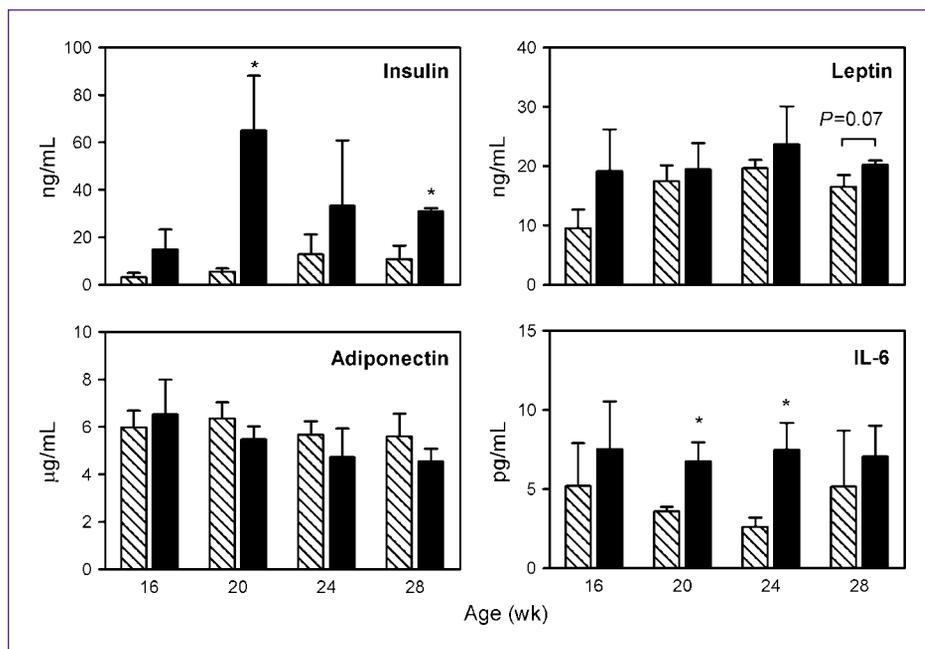
Fig. 3. Plasma levels of insulin, leptin, adiponectin, and IL-6 from AKR/J mice sacrificed at various ages on low (hatched bars) or high-fat diet (solid bars). *, $P < 0.05$, compared with control mice. See text for ANOVA results.

begins to exert an effect on ALL progression. This is suggested by human cancer incidence and mortality data. Reeves et al. found that a modest degree of overweight (body mass index, 25-27.4 kg/m^2) was not associated with increased leukemia or overall cancer incidence (2). However, leukemia and overall cancer incidence were increased in women with more substantial obesity. Calle et al. reported similar effects of obesity on leukemia and cancer mortality in men, although they did not detect an effect of obesity on leukemia mortality in women (1). Finally, it is possible that ALL, which develops in young mice, may be intrinsically more aggressive and thus may be less influenced by environmental or physiologic modulators of the disease such as obesity. In any case, it is plausible that the observed effects of obesity might be relevant in adolescents and adults, who may have been obese for years or decades.

In this study, we cannot differentiate between the effects of a high-fat diet and of obesity *per se* on ALL progression. However, it is interesting to note that a subset (11 of 41) of the *BCR/ABL* mice were "obesity resistant," with weights within 2 SD of the control group (10). These mice had a median survival of 135 days compared with 99 days in the remaining "obesity-prone" mice ($P = 0.10$, in *post hoc* analysis). Removal of the "obesity-resistant" mice from the survival analysis does not change the results ($P = 0.12$, log-rank control versus "obesity-prone" mice). However, the tendency for the "obesity-resistant" mice to have prolonged survival, despite being on the high-fat diet, does imply that obesity itself is responsible for the accelerated appearance of leukemia. Further studies would be needed to formally test this hypothesis.

Although ALL presented earlier in both obese mouse models, we did not identify any factors that predicted this.

WBC counts were similar between groups of *BCR/ABL* mice at 2 and 3 months of age (data not shown). Although thymus weights were increased by obesity in 4- to 7-month-old AKR mice, we identified no difference in TCR subtype or thymocyte expression pattern or TCR subtype. Interestingly, diet-induced obesity has recently been shown to increase adipocytes in the thymus, associated with decreased thymocyte counts and increased thymocyte apoptosis (11). Thus, obesity does not seem to increase risk of T-cell ALL simply by increasing leukocyte or thymocyte number or rate of turnover. Together, these results imply that obesity acts by increasing the risk of an early event in leukemogenesis rather than accelerating ALL proliferation once it is started.

As has been shown in other mouse models of obesity, we found that obese AKR/J mice had higher plasma levels of insulin, leptin, and IL-6. Insulin is a potent growth factor and has been shown to increase ALL cell proliferation *in vivo* (12). Interestingly, the common pre–B-cell ALL translocation, $t(1;19)$, may enhance insulin receptor signaling, implicating this pathway as a potential contributor to ALL pathogenesis (13). Leptin has been shown to stimulate hematopoietic progenitors and multiple leukemia cell types (14, 15), although a direct effect on ALL has not been shown to our knowledge. However, ALL blasts can express leptin receptors, and so it is possible that this hormone could contribute to ALL proliferation and/or survival (16). IL-6 has been implicated in the pathogenesis of several types of cancer and may play a role in bone metastasis and cancer-stromal cell interactions (17). As IL-6 is increased in obesity, it has been investigated as a potential link between obesity and several types of cancer (18), IL-6 induces B-cell proliferation (19), enhances differentiation

(20), and so could, in theory, alter ALL progression. Thus, these and other obesity-related hormones (e.g., insulin-like growth factor-I) could potentially be responsible for all or part of the accelerated ALL presentation in the present study. Further mechanistic work is necessary to tease apart these possibilities.

These results complement a previous study by Shields et al., which showed that caloric restriction causes an increased life span in AKR mice (21). In that study, a ~25%–30% lower body weight in the restricted mice caused a 50% longer median life span. In our study, a ~15% higher body weight in the obese mice was associated with a 25% shorter life span. Thus, body weight seems to have a continuous, bidirectional effect on life span in this model.

One weakness in the present study is that the decision to remove mice from the experiment was made by an unblinded investigator and was therefore subject to bias. This potential bias is minimal in the P190 mouse model, as these mice develop signs of ALL, which are fairly obvious (swollen lymph nodes or tumor, hind limb paralysis, hunched posture, acute weight loss, and/or labored breathing). These symptoms progress fairly rapidly to moribund status and death if the mouse is not removed from the study. Furthermore, other causes of death are rare in the background C57Bl/6 strain of this age range (22). The AKR/J mice were significantly older when they were removed from the study. Thymoma and/or T-cell ALL was confirmed by necropsy, fluorescence-activated cell sorting analysis, and/or presentation with respiratory distress in all but one mouse per diet group. Exclusion of these two mice from the data analysis did not change the survival curve comparison. Thus, the effect of obesity

to decrease survival in these two mouse models most likely reflects accelerated ALL.

The present study shows that obesity directly accelerates the development of both B cell– and T cell–derived ALL in mouse models. This supports the epidemiologic data showing an increased incidence of leukemia in obese adults (4, 5) and argues that these observations are indeed due to obesity *per se* and not to confounding genetic, socioeconomic, and/or lifestyle factors. This also adds ALL to the list of cancers that are accelerated by obesity in mice, including colon (23) and breast cancer (10). These results are particularly relevant in light of the evidence that obesity can directly impair ALL and acute myelogenous leukemia treatment (24–26). It is important that future research be aimed at elucidating the mechanisms linking cancer and obesity, so that the burdens from both of these diseases can be reduced.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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