Reduced body fat and increased hepatic lipid synthesis in mice bearing interleukin-6-secreting tumor

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Metzger, S., T. Hassin, V. Barash, O. Pappo, and T. Chajek-Shaul. Reduced body fat and increased hepatic lipid synthesis in mice bearing interleukin-6-secreting tumor. Am J Physiol Endocrinol Metab 281: E957–E965, 2001.—Chronic secretion of interleukin-6 (IL-6) in mice causes metabolic alteration in the liver, leading to increased synthesis of hepatic cholesterol and fatty acids (FA). Mice were injected with allogeneic tumor cells transduced with the murine IL-6 gene. During the 3 wk after tumor inoculation, elevated serum IL-6 levels were associated with increased spleen and liver weight. Histological examination of sections from the liver showed increased hepatocyte proliferation, resulting in liver enlargement. Body composition analysis revealed that IL-6 caused a significant loss in fat tissue without affecting lean body mass and water content. Hepatic de novo synthesis of FA and cholesterol, as measured by 3H2O incorporation, was three to five times as high in mice secreting IL-6 (IL-6 mice) as in pair-fed mice bearing nonsecreting tumors. This increase in FA and cholesterol synthesis is sufficient to maintain hepatic triglyceride secretion at levels comparable with those of pair-fed mice bearing nonsecreting tumors and, presumably, is the main source of cholesterol and FA-phospholipids necessary for hepatocyte proliferation.

hepatocyte proliferation; adipose tissue; fatty acids; cholesterol; triglyceride; glucose-6-phosphate dehydrogenase

INTERLEUKIN-6 (IL-6) is a cytokine mediating the inflammatory response in the liver through the induction of STAT3 (21), as well as a growth factor of many cells, including hematopoietic precursors, B and T cells, endothelial cells, neuronal cells, keratinocytes, and osteoclasts (11, 17, 37). IL-6 has also been reported to enhance mouse hepatocyte growth in primary culture (20).

Several IL-6 transgenic models have been described (5, 7, 16, 24). The chronic elevation of serum IL-6 in these mice is associated with hematologic disorders, severe renal failure, and alterations in liver morphology and gene expression. The phenotypic presentation is dependent on multiple variables, including the specific tissue expressing the IL-6 gene, the mouse species’ genetic background, the duration of exposure, and serum IL-6 levels. Liver regeneration after partial hepatectomy, combined hepatic ischemia and hepatectomy, or carbon tetrachloride treatment was impaired in IL-6-deficient mice (5). IL-6 injection into IL-6-deficient mice corrected the defective liver regeneration in these mice.

We previously described a mouse model of chronic elevated secretion of IL-6 achieved by injecting tumor cells transduced with the murine IL-6 (mIL-6) gene (26). Development of the tumor was associated with elevated concentrations of IL-6 in the serum. Mice bearing IL-6-secreting tumors (IL-6 mice) became anorectic and lost body weight comparable to body weight loss of Neo pair-fed (NPF) mice. Unlike NPF mice, IL-6 mice became hypoglycemic, probably through the effect of IL-6 on appetite and the major effect on carbohydrate metabolism in the liver. Liver glycogen content was depleted, and activity and gene expression of the gluconeogenic enzyme glucose-6-phosphatase were inhibited.

The aim of this study was to extend the use of this model to determine the effect of chronic secretion of IL-6 on lipid metabolism and body composition. Acute administration of IL-6 to rodents (12, 29) results in elevation of serum triglyceride (TG), cholesterol, and free fatty acid (FFA) concentrations and an increased hepatic TG secretion rate. A variety of cytokines also inhibit lipoprotein lipase (LPL) activity in adipose tissue (9, 12); however, LPL activity in heart muscle is increased (4). Other studies demonstrate that the increase in serum TG levels after acute administration of tumor necrosis factor-α, interleukin-1, IL-6, and interferon-α is mainly due to an increase in the secretion of TG by the liver. The increase in very-low-density lipoprotein (VLDL)-TG secretion is derived from the induction of hepatic de novo synthesis of fatty acids (FA) and cholesterol, reesterification of adipose tissue-derived FA, and the uptake of partially lipolyzed VLDL remnants (4, 8), but not from inhibition of TG clearance.

We report here that IL-6 mice develop enlarged spleen and liver. Histological sections from the liver showed hepatocyte proliferation. IL-6 mice lost fat tissue, with no effect on lean body mass. Hepatic de novo

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synthesis of FA and cholesterol was higher in IL-6 than in NPF mice. Serum cholesterol levels of IL-6 and NPF mice were similar. A slight increase in serum TG was observed compared with NPF mice or pair-fed mice without tumor, with no change in TG secretion rate. Serum FFA levels are lower than in NPF mice and even lower than in control fed mice. Enhanced hepatic de novo synthesis of FA and cholesterol was only sufficient to maintain hepatic TG secretion at the level of NPF mice and also might be the source of FA-phospholipids and cholesterol for hepatocyte proliferation.

METHODS

Retroviral vector and transduction. The tumor cell line MCA 102 is a methylcholanthrene-induced fibrosarcoma derived from female C57BL/6 mice. The N2-IL6 retrovirus (generously provided by Dr. David Bodine, Clinical Hematology Branch, National Heart, Lung, and Blood Institute) expressed the mIL-6 gene, driven by the Moloney murine leukemia virus LTR promoter, and the neomycin resistance gene, driven by the simian virus 40 promoter (2). Tumor cells (10⁶) were incubated with retrovirus at a multiplicity of infection of 4:1 and polybrene (6 μg/ml Sigma). The cells were washed after 24 h and placed in medium containing G418 (GIBCO Laboratories) after 72 h. Tumor cells growing in 0.4 mg/ml G418 were screened for mIL-6 production by enzyme-linked immunosorbent assay (Endogen). The high-producer clone 4JK-mIL6 (1,000 ng IL-6/10⁶ cells·ml⁻¹) was used in this study.

Mice and tumor creation. Male C57BL/6 mice weighed 20–25 g and were maintained in a 12:12-h light-dark cycle. Five × 10⁶ MCA 102 NeoR IL-6 (IL-6) or MCA 102 NeoR (Neo) cells were injected subcutaneously into the flank area. Tumor size was measured at different time points after tumor inoculation, and IL-6-secreting tumors were twice as large as those removed from mice bearing nonsecreting tumors. To compensate for differences in tumor size, we doubled the dose of MCA 102 Neo R cells (10⁷ cells) injected into the NPF mice. NPF mice were pair fed in accordance with the food intake of the IL-6 mice on the previous day. IL-6 and NPF mice were killed at different times after tumor inoculation. NPF mice were killed 1 day after IL-6 mice to achieve the same food intake after pair feeding. Body weight, tumor weight, food consumption, and body composition were measured. Blood was drawn from the retroorbital plexus for analytic procedures.

Analytic procedure. Blood glucose levels were determined as described elsewhere (3). Serum TG, total cholesterol, and high-density-lipoprotein cholesterol (HDL-C) were determined by enzymatic methods according to the LRC method (22) using a Vitatron autoanalyzer (Vital Scientific). Concentration of FFA in the serum was determined as described previously (18).

Determination of LPL and glucose-6-phosphate dehydrogenase activity. LPL activity was measured in muscle, heart, and epididymal fat tissues as described previously (3, 4). The activity of glucose-6-phosphate dehydrogenase (G6PDH) was measured as described elsewhere (32).

TG secretion. Anesthetized mice were injected intravenously with Triton WR-1339 (15% wt/vol, 500 mg/kg body wt) to block TG removal from the circulation (1). Animals were bled from the retroorbital plexus at 0, 45, and 90 min after Triton WR-1339 injection, and serum TG levels were measured.

Body composition analysis. After removal of internal organs and tumors, animals were dehydrated in an oven at 90°C for 4–7 days until a constant mass was achieved, and water content was calculated. The dried carcass was homogenized, and lipids were extracted from 1-g aliquots with 1:1 chloroform-methanol. The extracted aliquots and the chloroform-methanol supernatants were dried and weighed and represented the lean body mass and the fat mass, respectively.

Incorporation of [3H]2O into liver cholesterol and FA. Animals were injected intraperitoneally with 10 μCi of [3H]2O and deprived of water. At 60 min after injection, the mice were killed and blood was withdrawn from the retroorbital plexus. The liver, small intestine, and adipose tissue were subjected to alkaline hydrolysis using 7.1 ethanol-5 M KOH at 70°C for 12 h. [14C]cholesterol was added to the hydrolysate as a standard reference.

The nonsaponified lipids were extracted with petroleum ether and ether and analyzed on TLC plates with 1:5 ethyl acetate-ether and analyzed on TLC plates with 1:5 ethyl acetate-

RESULTS

Effect of IL-6 on body composition. IL-6 mice exhibited an increase in tumor size, elevated serum IL-6 levels, and reduced food intake from day 12 after tumor inoculation, as we previously described (26). Injection of 5 × 10⁶ tumor cells to mice resulted in a twofold increase in weight of IL-6-secreting tumors compared with nonsecreting tumors. To create NPF tumors comparable in size with IL-6-secreting tumors, we injected twice the number of MCA 102 Neo R cells (10⁷ cells). Tumors were weighed at different times after inoculation of cells (Table 1). After tumor removal, the loss in body weight was similar in IL-6 and NPF mice and the eviscerated body weight was comparable in the two experimental groups (Table 1).

Eviscerated carcasses underwent body composition analysis. On day 6 after tumor inoculation, there were no differences in percentages of fat, lean body mass, and water content among NPF, IL-6 mice, and fed mice without tumor (Fig. 1). Fat content in mice fed ad libitum without tumor was 13.4 ± 0.2% (2.1 ± 0.2 g). Fat content was reduced to 9.5 ± 0.7% (1.4 ± 0.1 g) due to pair feeding according to food intake of IL-6 mice during the 18-day period (Fig. 1A). The percentage of fat and fat content in NPF mice dropped from 11.8 ± 0.86% (1.66 ± 0.19 g) on day 6 to 9.6 ± 0.96% (1.4 ± 0.16 g) on day 14 and 6.1 ± 0.6% (0.8 ± 0.09 g) on day...
IL-6 EFFECT ON BODY COMPOSITION AND LIPID METABOLISM

Table 1. Effect of IL-6 secretion on body and tumor weight

<table>
<thead>
<tr>
<th>Day</th>
<th>Treatment</th>
<th>Initial Body Weight, g</th>
<th>Final Body Weight, g</th>
<th>Tumor Weight, g</th>
<th>Final Weight Without Tumor, g</th>
<th>Eviscerated Body Weight, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Fed</td>
<td>20.3 ± 1.0</td>
<td>20.3 ± 1.1</td>
<td>0.4 ± 0.04</td>
<td>15.6 ± 0.6</td>
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</tr>
<tr>
<td>19</td>
<td>IL-6</td>
<td>21.5 ± 0.5</td>
<td>19.2 ± 0.3</td>
<td>0.3 ± 0.03</td>
<td>14.1 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>IL-6</td>
<td>20.2 ± 0.3</td>
<td>19.2 ± 0.3</td>
<td>0.3 ± 0.03</td>
<td>14.1 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>NPF</td>
<td>21.1 ± 0.6</td>
<td>20.2 ± 0.5</td>
<td>3.6 ± 0.3</td>
<td>19.5 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>IL-6</td>
<td>19.8 ± 0.4</td>
<td>23.1 ± 0.6</td>
<td>2.9 ± 0.5</td>
<td>20.4 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>IL-6</td>
<td>19.3 ± 0.4</td>
<td>23.2 ± 0.9</td>
<td>5.0 ± 0.4</td>
<td>19.5 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>NPF</td>
<td>20.3 ± 1.0</td>
<td>23.9 ± 0.9</td>
<td>4.4 ± 0.5</td>
<td>18.2 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>NPF</td>
<td>20.2 ± 1.0</td>
<td>23.2 ± 0.5</td>
<td>4.4 ± 0.5</td>
<td>18.2 ± 0.5</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE of 8–10 mice. Mice were killed at indicated days after tumor inoculation and analyzed for body, tumor, and eviscerated weight. Groups represent fed ad libitum (Fed) and pair-fed mice without tumor (PF), pair-fed mice bearing nonsecreting tumors (NPF mice), and mice with interleukin-6-(IL-6)-secreting tumors (IL-6 mice). PF and NPF mice were pair fed in accordance to food intake of IL-6 mice on the previous day.

19. On day 19, the percentage of fat and fat content were significantly lower in NPF mice than in pair-fed mice without tumor, probably because of tumor burden. The percentage of fat in IL-6 mice on day 6 was 12.3 ± 1% (1.65 ± 0.14 g), similar to NPF mice and control mice without tumors. On day 13 the percentage dropped to 6.9 ± 0.35% (0.96 ± 0.06 g), and on day 18 it was 3.45 ± 0.35% (0.42 ± 0.05 g). This suggests that the loss of body fat mass in NPF mice was due to tumor burden and semistarvation, but in IL-6 mice there was a further drop in fat content attributable to IL-6 secretion. No significant influence of IL-6 was detected on the percentage of lean body mass or on water content during the experiment (Fig. 1, B and C).

Internal organ weight as a percentage of body weight including the tumor was calculated. Percentage of spleen weight was significantly higher in IL-6 mice (Fig. 2B) because of proliferation of hematopoietic cells (data not shown). Percentage of liver weight was also significantly increased in IL-6 mice (Fig. 2). On day 19 liver size of fed mice without tumor and of pair-fed mice was 1.1 ± 0.06 and 0.93 ± 0.06 g, respectively. On days 13 and 14 after tumor inoculation, liver of IL-6 and NPF mice weighed 1.28 ± 0.07 and 1.06 ± 0.05 g, respectively. On days 18 and 19 livers of IL-6 and NPF mice weighed 1.24 ± 0.03 and 1.07 ± 0.07 g, respectively.

Liver morphology. Histological examination of livers from IL-6 and NPF mice revealed a 15-fold increase in mitotic rate in IL-6 mice compared with NPF mice (30/120 vs. 2/120 high-power field). There was no excess accumulation of lipid droplets in the liver of IL-6 mice compared with NPF mice as determined by H & E and oil red O staining (data not shown). These data show that liver enlargement in IL-6 mice is due to hepatocyte proliferation. To establish a correlation between histopathological findings and plasma liver function tests, we determined serum aspartate aminotransferase and alanine aminotransferase in IL-6 and NPF mice. Serum aspartate aminotransferase levels were 225 ± 27 and 335 ± 63 IU/l, and serum alanine aminotransferase levels were 22.0 ± 1.6 and 29.0 ± 2.5 IU/l in IL-6 and NPF mice, respectively, suggesting that there was no hepatocellular damage in IL-6 mice.

Serum analysis. Serum levels of glucose, TG, cholesterol, HDL-C, and FFA are presented in Fig. 3. Blood glucose levels in the IL-6 mice were reduced to 89, 44, and 36% compared with glucose levels in NPF mice on days 6, 13, and 18 after tumor inoculation, respectively (Fig. 3A). Blood glucose levels of pair-fed mice without tumors and NPF mice on day 19 were reduced to 84 and 75% compared with fed mice without tumor. Thus glucose levels in NPF mice were reduced because of semistarvation, with a slight but significant influence of tumor burden. However, the influence of IL-6 on glucose level is evident, and we previously reported the effect of chronic IL-6 secretion on glucose and glycogen metabolism (26).

As expected, pair-fed control mice without tumor had lower levels of TG than fed mice without tumor: 78 ± 10 mg/dl in fed mice without tumor and 49 ± 5 mg/dl after pair feeding on day 19 (Fig. 3B). NPF mice showed the predicted drop in TG levels due to semistarvation. There was no significant difference between TG levels of pair-fed and NPF mice on day 19. However, TG levels were higher in IL-6 than in NPF mice. On days 18 and 19 TG levels in IL-6 mice were 1.5 times those of NPF mice: 51 ± 2 and 88 ± 4 mg/dl in NPF and IL-6 mice, respectively.

On day 19 plasma FFA levels were significantly elevated in pair-fed mice without tumor as a result of starvation: 623 ± 12 and 1,191 ± 132 μmol/ml in fed mice without tumor and pair-fed mice without tumor, respectively (Fig. 3C). During the experiment, FFA levels in NPF mice were similar to those of control fed mice. However, on day 19, plasma FFA levels in NPF mice were significantly elevated (706 ± 42, 392 ± 44, and 315 ± 64 μmol/ml on days 6, 13, and 18, respectively). To understand whether the reduced plasma FFA levels in IL-6 mice were due to inhibition of adipose tissue intracellular lipolysis or reflect the decreased adipose tissue mass, we calculated FFA levels per gram of fat as derived from body composition analysis. FFA levels
intake in pair-fed mice without tumor on day 19 (747 ± 70 \( \mu \text{mol} \cdot \text{ml}^{-1} \cdot \text{g}^{-1} \)) and in NPF and IL-6 mice on day 19/18 (929 ± 83 and 708 ± 107 \( \mu \text{mol} \cdot \text{ml}^{-1} \cdot \text{g}^{-1} \), respectively), but no additional effect of IL-6 secretion was detected (Fig. 3D). Thus the reduction in FFA levels in IL-6 mice reflects the marked reduction in adipose tissue mass. An increase in serum cholesterol in NPF and IL-6 mice was evident 2–3 wk after tumor inoculation compared with control mice, but there was no effect of IL-6 on cholesterol levels (Fig. 3E).

HDL-C levels decreased significantly in IL-6 mice on days 13 and 18 compared with NPF mice. Some decrease in HDL-C was observed in NPF mice on day 19 compared with pair-fed mice without tumor, but there was a further influence of IL-6 (Fig. 3F).

Effects of IL-6 on de novo synthesis of FA and cholesterol and on TG secretion. Lipid de novo synthesis was determined by measuring the incorporation of \( ^3\text{H}_2\text{O} \) into FA and cholesterol in liver, small intestine, adipose tissue on days 18 and 19 after tumor inoculation. In the liver, IL-6 induced an increase in the incorporation of \( ^3\text{H}_2\text{O} \) into FA (3-fold) and into cholesterol (5-fold) compared with lipids synthesized in livers of NPF mice (Table 2). IL-6 had no effect on the rate of cholesterol and FA synthesis in the small intestine or the rate of FA synthesis in the epididymal fat pad. Inasmuch as FA synthesis was enhanced by IL-6 and serum TG levels were higher in IL-6 mice, we measured the TG secretion from the liver using Triton WR-1339 (Fig. 4). TG secretion of IL-6 and NPF mice on day 6 was similar to TG secretion of fed mice without tumor, suggesting no effect of IL-6 on TG secretion. On days 18 and 19 we observed a marked decrease in TG accumulation in the sera of IL-6 and NPF mice compared with fed mice without tumors. Yet there was no difference between TG secretion of IL-6 and NPF mice.

Effect of IL-6 on LPL and G6PDH. LPL activity was measured in the heart muscle, the gastrocnemius muscle, and the epididymis fat pad (Table 3). Because of starvation, a significant reduction in adipose tissue LPL activity was observed in IL-6 and NPF mice compared with controls, but there was no significant difference between the IL-6 and NPF mice. Activity of liver G6PDH in IL-6 mice was 23.5 ± 1.2 \( \text{nmol} \cdot \text{min}^{-1} \cdot \text{mg protein}^{-1} \), twice that in the liver of fed mice without tumor or NPF mice (11.7 ± 1.2 and 12.4 ± 2.1 \( \text{nmol} \cdot \text{min}^{-1} \cdot \text{mg protein}^{-1} \), respectively, \( P < 0.05 \)). However, the semistarvation in the NPF mice did not affect enzyme activity.

DISCUSSION

The data presented here demonstrate that chronic secretion of IL-6 by tumor cells in a mouse model causes anorexia, hypoglycemia, decrease in body fat mass, increase in liver weight due to hepatocyte proliferation, and altered hepatic and plasma lipid metabolism. Mice injected with MCA 102 cells transduced with IL-6 cDNA develop tumors. However, tumors secreting IL-6 weighed twice as much as nonsecreting
tumors, indicating that IL-6 secretion enhances the development of tumor tissues. The metabolic effects of IL-6-secreting tumor are still evident even after twice the numbers of MCA 102 Neo R cells were injected to compensate for the different size of the NPF and IL-6 tumors.

It has been demonstrated that acute administration of inflammatory cytokines to rodents produces elevated serum TG levels as a result of increased hepatic TG secretion. The increased hepatic VLDL secretion has been shown to result from higher levels of plasma FFA derived from TG lipolysis in adipose tissue, higher de novo synthesis of FA by the liver, and uptake of partially lipolyzed (TG-rich) VLDL remnants by the liver (4, 8). Inasmuch as cytokines also affect plasma cholesterol and HDL-C levels, acute administration of tumor necrosis factor and/or interleukin-1 to Syrian hamsters was associated with an increase in plasma cholesterol and a decrease in HDL-C levels (13).

We previously showed that 2–3 wk after tumor inoculation both NPF and IL-6 mice had a similar decrease in body weight. However, in this study we show a significant difference in body composition between NPF and IL-6 mice. Body composition studies in eviscerated mice show no significant changes in percent lean body mass and percent water, but there is a reduction in fat weight due to semistarvation, as well as an additional decrease in fat weight due to tumor burden and a further decrease in fat content induced by chronic secretion of IL-6. Several mechanisms in addition to decreased food intake have been proposed to account for the decrease in body fat in cancer cachexia: 1) tumor burden may induce loss of adipose tissue via production of lipid-mobilizing factor (19). This factor has been shown to increase adipose tissue cAMP concentration, hormone-sensitive lipase activity, and serum FFA levels. In our model we have also observed a significant reduction in body fat content in NPF mice (compared with pair-fed mice without tumor), with an additional effect of IL-6 on body fat content. However, FFA levels (calculated per gram of fat tissue) were similar to those of fed and pair-fed mice without tumor. 2) A decrease in adipose tissue LPL activity would reduce the amount of fatty acids channeled toward adipose tissue storage. However, in our model the reduction in adipose tissue LPL activity in IL-6 and NPF mice is similar. 3) Cytokines including IL-6 can induce elevation of plasma FFA by increasing adipose tissue lipolysis (29). However, in our model, chronic secretion of IL-6 is associated with reduced plasma FFA levels, which reflect the low fat content rather than decreased intracellular lipolysis. 4) Elevated energy expenditure (REE) is a cardinal feature of cachexia but not of starvation. Patients with lung and pancreatic cancer have an increase in REE (6, 10, 35). It is therefore possible that the additional effect of IL-6 on body fat content is mediated via an increase in REE.

Cytokines also enhance in vivo hepatic synthesis of FA and cholesterol in the chronic animal model. The de novo synthesis of FA in the liver of IL-6 mice is probably the main source of plasma TG, inasmuch as
Fig. 3. Effect of IL-6 on levels of serum glucose, serum triglycerides (TG), plasma free fatty acids (FFA), plasma FFA per gram of fat, serum cholesterol, and serum high-density-lipoprotein cholesterol (HDL-C) were measured in mice without tumor (fed and PF) and in NPF and IL-6 mice. Values are means ± SE of 8–10 measurements. *P < 0.05 compared with NPF mice at the same time. **P < 0.05 compared with fed mice without tumor. ***P < 0.05 compared with pair-fed mice without tumor on day 19. #P < 0.05 compared with NPF mice on day 14.

Table 2. Incorporation of $^3$H$_2$O into tissue FA and cholesterol

<table>
<thead>
<tr>
<th>Mice</th>
<th>Day</th>
<th>Liver FA</th>
<th>Liver Cholesterol</th>
<th>Intestine FA</th>
<th>Intestine Cholesterol</th>
<th>Epididymal FA</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPF</td>
<td>19</td>
<td>4.8 ± 0.7</td>
<td>0.9 ± 0.5</td>
<td>3.6 ± 0.3</td>
<td>0.9 ± 0.06</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td>IL-6</td>
<td>18</td>
<td>14.5 ± 1.1*</td>
<td>5.1 ± 1.1*</td>
<td>5.0 ± 0.4</td>
<td>1.0 ± 0.1</td>
<td>1.5 ± 0.3</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed as nmol·h$^{-1}$·g$^{-1}$. Mice were injected intraperitoneally with $^3$H$_2$O 18–19 days after tumor inoculation. After 60 min, mice were killed, and liver, intestine, and epididymal fat were subjected to cholesterol and fatty acid (FA) extraction. *P < 0.05.
food intake is sharply reduced and fat deposits are diminished, leading to low levels of plasma FFA. Despite elevated rate of FA synthesis in the liver of IL-6 mice, there is no IL-6 effect on hepatic TG secretion. However, there is a mild elevation in serum TG. Adipose tissue and skeletal muscle LPL activity per gram of tissue were similar between IL-6 and NPF mice. Inasmuch as adipose tissue mass is decreased by 50% in IL-6 mice, it is conceivable that the removal of TG from the circulation by adipose tissue is affected. The reduction in adipose tissue mass is also the cause of low levels of FFA in the circulation of IL-6 mice.

Liver-derived FA could theoretically be utilized for ketone body production for use by the brain and muscles as an alternative source of energy, given low blood glucose levels in IL-6 mice. However, serum concentrations of β-hydroxybutyrate and acetoacetate were not elevated in IL-6 mice (data not shown), suggesting that the excess production of liver FA is not channeled toward ketone body formation.

Several animal models have been described recently in which fatty livers were found together with reduced adipose tissue mass. Overexpression of a dominant-positive truncated form of sterol regulatory element binding protein (SREBP-1a) in transgenic mice is associated with the development of massive fatty liver with an increase in mRNA levels of enzymes of cholesterol and FA synthesis and a reduction in white adipose tissue mass (34). Transgenic mice expressing LPL only in the liver also develop fatty liver and reduction of adipose tissue mass during the suckling period (25). However, H & E and oil red O stainings indicate that there is no more fat in hepatic tissue of IL-6 mice than in hepatic tissue of NPF mice, and the additional weight of the liver is due to hepatocyte proliferation.

Terminally differentiated hepatocytes are capable of undergoing sustained cell proliferation (27). This process is enhanced by cytokines and, in particular, by IL-6 (30). Liver regeneration is impaired in IL-6-deficient mice (5) and can be restored to normal by treatment with IL-6. Mice expressing human IL-6 cDNA in the liver only (7) show activation of acute-phase genes with no impairment of liver function. Coexpression of human IL-6 and soluble IL-6 receptor results in nodules of hepatocellular hyperplasia progressing toward the formation of liver adenomas (24). In IL-6 mice, we demonstrate hepatocyte proliferation in nondamaged liver.

The significant increase in liver size due to hepatocyte proliferation in IL-6 mice requires cholesterol and phospholipids for membrane formation. Inasmuch as the enhanced hepatic lipid synthesis does not result in elevated TG secretion, ketone body production, or fatty liver formation, it is possible that the primary goal of the increased de novo synthesis of FA and cholesterol by the liver of IL-6 mice is to supply the necessary building blocks for cell membrane formation. The increased FFA synthesis by the liver also compensated for the decreased reflux of FFA from the depleted adipose tissue mass.

We have demonstrated in IL-6 mice that there is an increased uptake of 2-deoxyglucose by the liver, hepatic glycogen content is depleted, phosphoenolpyruvate carboxykinase activity is unaffected, glucose-6-phosphatase activity is inhibited, and G6PDH activity is boosted (26). All these factors indicate that, in the chronic model, glucose-6-phosphate is channeled to the pentose shunt and might contribute to the elevated concentrations of acetyl-CoA and NADPH needed for FA and cholesterol synthesis.

Thus IL-6 induces a complex alteration in lipid and cholesterol metabolism orchestrated for the following purposes: 1) it maintains serum lipid concentration, despite the presence of anorexia. This increase in serum TG has been shown to be linked to host defense

Table 3. LPL activity

<table>
<thead>
<tr>
<th></th>
<th>LPL Activity, mU/g</th>
<th>Fed vs. NPF</th>
<th>IL-6 vs. NPF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fed, mU/g</td>
<td>NPF, mU/g</td>
<td>IL-6, mU/g</td>
</tr>
<tr>
<td>Heart</td>
<td>137.0 ± 4.0</td>
<td>148.0 ± 7.0</td>
<td>183.0 ± 8.0</td>
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<tr>
<td>Muscle</td>
<td>12.1 ± 0.8</td>
<td>10.3 ± 1.2</td>
<td>14.0 ± 1.4</td>
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<tr>
<td>Epididymal fat</td>
<td>470.0 ± 54.0</td>
<td>162.0 ± 15.0</td>
<td>185.0 ± 26.0</td>
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</tbody>
</table>

Values for lipoprotein lipase (LPL) activity are means ± SE of 10–16 mice. At 18–19 days after tumor inoculation, mice were killed, and organs were removed and subjected to determination of LPL activity. NS, not significant.
IL-6 is present in human serum in a variety of pathological conditions, such as infectious or inflammatory disease, and is secreted from malignant tumors (34, 36). The involvement of IL-6 in malignant disease has been investigated in a number of different malignancies, e.g., renal cell carcinoma, malignant melanoma, lung cancer, ovarian carcinoma, and multiple myeloma (23, 28, 33). Serum IL-6 levels up to 100–200 pg/ml [similar to IL-6 levels in our model (26)] correlate with the increased REE and weight loss in lung cancer patients (35). IL-6 is secreted by the tumor cells and serves as an autocrine growth factor for the tumor itself and a prognostic marker for the patient. Our model of IL-6 mice may, therefore, contribute to the understanding of the in vivo biology of such tumors.

REFERENCES


