Aberrant crypt focus promotion and glucose intolerance: correlation in the rat across diets differing in fat, n-3 fatty acids and energy

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McKeown-Eyssen (Cancer Epidemiol. Biomarkers Prevent., 3, 687–695, 1994) and Giovannucci (Cancer Causes Control, 6, 164–179, 1995), noting the striking similarity in lifestyle risk factors for colorectal cancer and insulin resistance, proposed that the hyperinsulinemia, glycemia and hypertriglyceridemia associated with insulin resistance promotes colon cancer. To compare the effect of diet on colon cancer promotion and insulin resistance in the F344 rat, we assessed the effect of fat, n-3 fatty acids and energy in pairwise comparisons on average size of aberrant crypt foci (ACF) and on glucose intolerance in the same animals in a single experiment. Diets high in fat and energy increased and diets with increased n-3 fatty acids and calorie restriction decreased both ACF growth and glucose intolerance compared with control diets. The measures of promotion of colon cancer and insulin resistance were strongly correlated (n = 98, r = 0.67, P < 0.001). In addition, both were highly correlated with daily energy intake (r = 0.62 and 0.66) and were also correlated with basal (post-prandial) insulin, glucose and triglycerides (r = 0.31–0.53, P < 0.01). We concluded that ACF growth and glucose intolerance are correlated for a wide range of diets and that increased circulating energy (glucose and triglycerides) may lead to both colon cancer promotion and insulin resistance.

Introduction

McKeown-Eyssen (1) and Giovannucci (2) have recently proposed a broadly based mechanism that links diet with insulin resistance and colorectal cancer. This insulin resistance hypothesis was prompted by the striking similarities of the dietary risk factors for insulin resistance and colorectal cancer. It proposed that the dietary risk factors first lead to hyperinsulinemia, glycemia and hypertriglyceridemia, measures associated with insulin resistance. The hyperinsulinemia then acts as a growth promoter to promote tumor development (1,2) and/or the glycemia and hypertriglyceridemia promote the growth of tumors as a result of the increased available energy (1).

Insulin resistance is a metabolic condition in which muscle and other insulin-sensitive cells become less responsive to insulin. Insulin becomes less effective at stimulating glucose absorption and in reducing blood glucose. This leads to glucose intolerance, hyperinsulinemia, glycemia and hypertriglyceridemia and, in some individuals, to non-insulin-dependent diabetes mellitus (3). Insulin resistance can be measured directly with the hyperinsulinemic, euglycemic clamp (4). It can also be assessed by measuring plasma glucose and insulin in the fasting state or after an oral glucose challenge (4,5).

In animal studies, dietary factors that affect insulin resistance measured with the euglycemic clamp also appear to affect tumor promotion. Diets high in saturated fat and energy increase both insulin resistance (6) and colon tumor promotion (7), whereas diets providing high levels of n-3 fatty acids and those with caloric restriction reduce both insulin resistance (8,9) and tumor promotion (10,11).

We recently compared the effects of a high and a low fat diet on measures of the development of insulin resistance and colon cancer promotion in the same animals and found some evidence supporting the insulin resistance hypothesis (12). Rats were given a colon carcinogen and were then randomized into a low or high saturated fat diet. One hundred days later, the rats consuming the high fat diets had larger aberrant crypt foci (ACF) than those on the low fat diet. As ACF are thought to be an early stage in the development of colon cancer in the rat (13) and increased ACF size is closely related to tumor promotion (7,14,15), ACF size is used as an early measure of colon cancer promotion (16). The animals on the high fat diet already had evidence of insulin resistance prior to this evidence of promotion. They showed glucose intolerance, with elevated plasma glucose and insulin after an oral glucose challenge, before 100 days, though their fasting glucose and insulin were not significantly elevated. This result was consistent with the temporal sequence suggested by the insulin resistance hypothesis. However, the absence of fasting hyperinsulinemia and glycemia suggested that the high fat diet did not lead to promotion indirectly through insulin resistance, but rather that the diet led to both insulin resistance and promotion through a common path (12).

Here we report further comparisons of the effect of diet on insulin resistance and promotion. We used diets with high and low dietary fat as before, diets with increased n-3 fatty acid content and diets with energy restriction and excess and we determined the effects of these diets on both glucose intolerance and ACF size. First, we replicated the earlier studies in which the effect of these diets on insulin resistance and tumor promotion had been measured directly. Then, as glucose intolerance and ACF size were measured in the same animals and the comparisons made in a single experiment, we examined the correlation between our measures of insulin resistance and tumor promotion across the wide range of diets. The results showed that the dietary factors that lead to insulin resistance are closely related to those that lead to promotion. Finally, we

Abbreviations: ACF, aberrant crypt foci; AOM, azoxymethane; a.u.c., total area under the glucose tolerance curve; CAF, cafeteria diet; CAF, cafeteria control diet; CR, calorie-restricted diet; HF, high fat diet; HF3, high fat n-3 diet; IR, insulin resistance; LF, low fat diet; LF3, low fat n-3 diet; OGTT, oral glucose tolerance test; %en, percent energy.

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The low fat n-3 diet (LF3) was identical to the LF except that all the corn oil were at the same concentration as in the LF diet on an energy density basis. 40% of those calories provided by beef tallow. Vitamins, minerals and protein 12%en as fat (corn oil). The high fat diet (HF) provided 60%en as fat, with 70%en as carbohydrate, largely corn starch, and energy. They are detailed in Table I.

suggest possible paths that might link diet with both the development of insulin resistance and increased cell proliferation and promotion.

Materials and methods

Animals
Ninety-eight male 8-week-old Fischer 344 rats (Harlan, Sprague–Dawley, Indianapolis, IN) weighing ~180 g were housed individually in wire-bottom cages in a temperature and humidity controlled environment (22°C and 50% respectively). The cages were arranged randomly in the room to minimize possible confounding environmental effects, such as light and noise. The room was maintained on a 12 h dark/light cycle, with the dark cycle extending from 7:00 p.m. to 7:00 a.m. Tap water from an automated system was provided ad libitum. Care of the animals conformed to the guidelines of the Canadian Council on Animal Care and the protocol was approved by the University of Toronto Animal Care Committee.

Design
The protocol scheme is summarized in Figure 1. After 12–16 days acclimatization, when the animals consumed Rodent Chow (Ralston Purina International, Strathroy, Canada), the rats were individually identified and given an abbreviated oral glucose tolerance test (OGTT) (0 and 120 min only). Four to eight days later the rats were initiated with the colon carcinogen azoxymethane (AOM) (Sigma, St Louis, MO) at a dose of 20 mg/kg body weight between 9:00 and 11:00 a.m. One week later, they were randomized to the seven dietary groups (see below) of 14 animals each, in groups that were initially designed to be compared in pairs. The average weights and the OGTT values obtained from the earlier determinations were found to be the same for each of the groups. Animal weights were then measured weekly and food consumption was measured over a week close to the end of the study. After 65 days on the diets, the OGTT was repeated. On days 82 and 87 blood was collected from the orbital sinus under light halothane anesthesia for determination of basal insulin, glucose and triglyceride and fasting insulin values respectively. On day 91, the animals were killed by cervical dislocation and their colons were removed for assay of ACF.

Diets
Seven diets were developed to test the association of insulin resistance (IR) and ACF promotion with changes in dietary fat, n-3 fatty acids and dietary energy. They are detailed in Table I.

In the LF diet (LF) was based on the high corn starch AIN-76 diet (17), which is frequently referred to as AIN-76 D-65, and provided 18% energy (%en) as protein (casein), 70%en as carbohydrate, largely corn starch, and 12%en as fat (corn oil). The high fat diet (HF) provided 60%en as fat, with 40% of those calories provided by beef tallow. Vitamins, minerals and protein were at the same concentration as in the LF diet on an energy density basis. The low fat n-3 diet (LF3) was identical to the LF except that all the corn oil in the LF was replaced with flax oil, an oil known to contain a large fraction of linolenic acid (18). The high fat n-3 diet (HF3) was identical to the HF except that half of the beef tallow in the HF was replaced with flax oil. These diets were compared with the LF and HF respectively. A low energy, calorie-restricted diet (CR) provided 60% of the energy of animals on the LF. Feeding of the CR was based on consumption in the LF group and the results for the CR were compared with those on the LF. A high energy, cafeteria diet (CAF) provided the animals with a day-to-day variety of flavors and textures which encouraged the rats to increase their food and energy intake (19). This diet provided an average of 62% of calories as fat. The results obtained with this diet were compared with that of a separate cafeteria control diet (CAFc). The CAFc was developed based on the food intake results from pre-testing the CAF. It was prepared as a homogenized mixture of the components that matched the feeding preferences of the rats, so that animals on the CAFc received the same dietary components as animals in the cafeteria group without the constantly changing flavours and textures.

All of the semi-purified diets were provided by Dyets (Bethlehem, PA). Flaxseed oil used in LF3 and HF3 was provided by Dyets from ACE Hardware (Bethlehem, PA). Cafeteria food was bought from local grocery stores.

Measures of glucose, triglycerides and insulin
For the OGTT, the animals were fasted for 7 h (7:00 a.m. to 2:00 p.m.) and were then given an oral glucose gavage (4 mg anhydrous glucose/g body wt). Blood glucose from the tail vein was determined by a glucometer (Medisense Canada, Toronto, Canada), prior to and 30, 60 and 120 min after gavage. The total area under the glucose tolerance curve (a.u.c.) was calculated from 0 to 120 min with the trapezoid rule (20).

The basal blood was collected at 8:00 a.m., 1 h after food was removed, and this served as a sample of convenience after the animals’ usual early morning meal (21). Coded blood samples, obtained by orbital sinus and cardiac puncture, were centrifuged at 1800 r.p.m. for 20 min at 4°C after addition of EDTA. Glucose was measured by the glucose oxidase method (Kit 510; Sigma) and triglycerides by an enzymatic method (Kit 337; Sigma). Insulin was measured by radioimmunassay using rat-specific antibody (RAT Insulin RIA kit; Linco Research, St Charles, MO) and a modification of the protocol described by Marban et al. (22) with the sample plasma volumes further reduced to 20 µl. As described previously, plasma samples and standards were diluted 1:5 with assay buffer, and rat-specific antibody (125I tracer) and precipitating agent were diluted 1:2 with assay buffer. Linearity was established to a dilution of 1:2 and the assay was assessed for inter-assay and intra-assay variation (coefficient of variation 13%).

Measure of ACF promotion
ACF were assayed as previously described (16). In brief, the colons were removed, rinsed in Krebs–Ringer bicarbonate buffer, cut longitudinally and fixed flat between filter papers in 10% buffered formalin. They were then stained briefly with 0.2% methylene blue and examined mucosal surface-up under a light microscope at ×40 magnification for ACF. These were identified on the basis of darkly stained and enlarged crypts that appeared to bulge from

Fig. 1. Diagrammatic representation of the experimental protocol with time in days. All animals received AOM (20 mg/kg body wt) 7 days before assignment to experimental diets. LF, low fat; HF, high fat; LF3, low fat, high n-3; HF3, high fat, high n-3; CR, calorie-restricted; CAF, cafeteria; CAFc, cafeteria control; OGTT, oral glucose tolerance test; TG, triglycerides; ACF, aberrant crypt foci, assay for aberrant crypt focus number and size.
Diet, insulin resistance and colon cancer promotion

### Table I. Composition of the seven experimental diets

<table>
<thead>
<tr>
<th></th>
<th>LF</th>
<th>HF</th>
<th>LF3</th>
<th>HF3</th>
<th>CR</th>
<th>CAF*</th>
<th>CAFC*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (casein)</td>
<td>18</td>
<td>24.6</td>
<td>18</td>
<td>24.6</td>
<td>30</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.3</td>
<td>0.4</td>
<td>0.3</td>
<td>0.4</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat (corn oil)</td>
<td>5</td>
<td>6.8</td>
<td>6.8</td>
<td>8.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat (beef tallow)</td>
<td>30</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat (flaxseed oil)</td>
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<td>15</td>
<td></td>
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<td>Starch (corn starch)</td>
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<td>64</td>
<td>20.86</td>
<td>40</td>
<td>27</td>
<td>27</td>
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<tr>
<td>Sucrose</td>
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<td>6.8</td>
<td>6.8</td>
<td>8.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fiber (cellulose)</td>
<td>3</td>
<td>4.1</td>
<td>4.1</td>
<td>5</td>
<td></td>
<td></td>
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<td>4.8</td>
<td>5.8</td>
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<td></td>
<td></td>
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<tr>
<td>Vitamin mix</td>
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<td>1</td>
<td>1.37</td>
<td>1.7</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Choline bitartrate</td>
<td>0.2</td>
<td>0.27</td>
<td>0.27</td>
<td>0.3</td>
<td>0.9</td>
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<tr>
<td>TBHQ*</td>
<td>0.001</td>
<td>0.007</td>
<td></td>
<td></td>
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<tr>
<td>Total weight (g)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Protein</td>
<td>18</td>
<td>18</td>
<td>18</td>
<td>18</td>
<td>30</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Fat</td>
<td>12</td>
<td>60</td>
<td>12</td>
<td>60</td>
<td>20</td>
<td>62</td>
<td>62</td>
</tr>
<tr>
<td>Complex CHO</td>
<td>16</td>
<td>64</td>
<td>16</td>
<td>64</td>
<td>16</td>
<td>40</td>
<td>27</td>
</tr>
<tr>
<td>Simple CHO</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy (kcal/100 g)</td>
<td>401.5</td>
<td>552</td>
<td>401.5</td>
<td>552</td>
<td>403</td>
<td>426.2</td>
<td>426.2</td>
</tr>
</tbody>
</table>

*LF, low fat diet; HF, high fat diet; LF3, low fat with high n-3 fatty acids diet; HF3, high fat with high n-3 fatty acids diet; CR, 40% calorie-restricted diet based on composition of LF diet; CAF, cafeteria diet, consisting of seven food items (peanut butter, weiners, cheese puff, Ritz cracker, kit kat, bologna and wafer) and a diet supplement, food provided in three containers, three food items provided daily, one new item at a time and diet supplement every day. The diet supplement was designed from previous feeding studies so as to provide minerals, vitamins and fiber in amounts similar to the other experimental diets; CAFC, cafeteria control diet, consisting of the food items and supplement used in cafeteria diet mixed together in order to reduce variety in texture and flavor, the proportion of which in the diet was the same as that of the cafeteria diet and was calculated from food intake in a pilot study in our laboratory. All diets except CAF and CAFC were provided by Dyets (Bethlehem, PA). The estimated ratio of n-6/n-3 fatty acids for LF, HF, LF3, HF3 and CR were 56, 16.6, 0.3, 0.8 and 56 respectively.

*TBHQ, t-butylhydroquinone.*

### Results

The measure of promotion (size of ACF at 100 days), measures of insulin resistance (a.u.c. and basal insulin, glucose and triglyceride) and energy intake and body weight for the seven groups of animals on the different diets are detailed in Table II (upper panel). The curves of OGTT used for calculating the a.u.c. for the seven groups are shown in Figure 2.

**Pairwise comparisons of ACF size, measures of IR and energy consumption and weight**

The comparisons of pairs of diets designed to differ in fat, n-3 fatty acids and energy are shown in Table II (lower panel). The HF resulted in an increased ACF size, an increased a.u.c., a higher energy intake and a greater weight gain than the LF. The corresponding comparisons with the HF3 and LF3, containing increased amounts of n-3 fatty acids, resulted in similar, though less marked, differences. The substitution of n-3 fatty acids in the HF (HF3 versus HF) resulted in reduced ACF growth, a.u.c. and basal triglyceride as well as energy intake and final weight. The CR resulted in decreased a.u.c., basal insulin, energy intake and final weight, as compared with the LF. The CAF resulted in increased ACF size, a.u.c., basal insulin, energy intake and final weight than its control (CAFC).

The average number of ACF per colon and fasting insulin were not different in any of the pairwise comparisons (data not shown).

**Correlations of the measures**

It was evident from the data and comparisons in Table II that there are interesting associations between average ACF size and measures of insulin resistance. A plot of ACF size as a function of a.u.c. showed the close association between ACF size and a.u.c. (Figure 3).

To examine the associations further, the raw correlation coefficients were determined (Table III). From these correlations it is clear that ACF size and a.u.c. are strongly associated with each other ($r = 0.67, P < 0.001$). ACF size is also associated with the basal measures of insulin, glucose and triglyceride concentrations ($r = 0.38–0.40, P < 0.01$). In addition, energy intake and final body weight are strongly correlated with ACF size ($r = 0.67$) and with a.u.c. ($r = 0.63$). Even with partial correlations, calculated to assess the significance of the correlations between variables not due to the diet groups (not shown in detail), there are still substantial correlations between ACF size and a.u.c. and between ACF size and final body weight ($r = 0.23$ and 0.24 respectively, $P < 0.05$). These results suggest that ACF size depends both on dietary energy (associated with final weight) and on a.u.c., which also depends on dietary energy.
Table II. Mean values of factors measured in the experiment (upper) and pairwise comparisons of groups (lower)

<table>
<thead>
<tr>
<th>Group</th>
<th>Measure of promotion</th>
<th>Measures of insulin resistance</th>
<th>Energy intake and weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ACF size (AC/ACF)</td>
<td>Basal insulin (µIU/ml)</td>
<td>Basal glucose (mmol/l)</td>
</tr>
<tr>
<td></td>
<td>a.u.c. (mmol/l×h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LF</td>
<td>2.54 ± 0.10</td>
<td>15.3 ± 0.30</td>
<td>355 ± 45</td>
</tr>
<tr>
<td>HF</td>
<td>3.59 ± 0.11</td>
<td>16.7 ± 0.29</td>
<td>413 ± 46</td>
</tr>
<tr>
<td>LF3</td>
<td>2.46 ± 0.10</td>
<td>14.8 ± 0.18</td>
<td>315 ± 25</td>
</tr>
<tr>
<td>HF3</td>
<td>2.79 ± 0.10</td>
<td>15.6 ± 0.21</td>
<td>280 ± 30</td>
</tr>
<tr>
<td>CR</td>
<td>2.33 ± 0.07</td>
<td>14.1 ± 0.20</td>
<td>172 ± 22</td>
</tr>
<tr>
<td>CAF</td>
<td>3.56 ± 0.15</td>
<td>17.4 ± 0.41</td>
<td>473 ± 35</td>
</tr>
<tr>
<td>CAFC</td>
<td>3.09 ± 0.11</td>
<td>16.3 ± 0.27</td>
<td>335 ± 27</td>
</tr>
<tr>
<td>ANOVA</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Fat comparisons
- HF versus LF: + <0.001, +0.003 NS NS NS +0.008 +0.003
- HF3 versus LF: +0.003 +0.008 NS NS NS +0.01 NS +0.04

n-3 Fatty acid comparisons
- LF3 versus LF: NS NS NS NS NS NS NS NS
- HF3 versus LF: - <0.001 -0.006 -0.03 NS NS -0.008 -<0.001 -<0.001 NS NS

Energy comparisons
- CR versus LF: -0.03 -0.005 -0.002 NS NS NS -<0.001 -<0.001 NS NS
- CAF versus CAFC: +0.01 +0.03 +0.005 NS NS NS +<0.001 +<0.001 NS NS

(Upper) Mean values of factors measured in the experiment ± SEM for each of the seven dietary groups (n = 98). ACF size, the average number of aberrant crypts/aberrant crypt foci; AC, aberrant crypt; ACF, aberrant crypt foci; a.u.c., total area under the OGTT curve (0–2 h); TG, triglycerides; LF, low fat diet; HF, high fat diet; LF3, low fat, high n-3 diet; HF3, high fat, high n-3 diet; CR, calorie-restricted diet; CAF, cafeteria diet; CAFC, cafeteria control diet. Average number of ACF per colon and serum fasting insulin concentrations were measured. ANOVA revealed no significant difference between groups.

(Lower) Pairwise comparisons of comparable groups as tested by Student's t-test, direction of the difference between means of two groups and the P value. For those groups tested more than once, only P < 0.01 is considered significant.
Table III. Correlations between colon cancer promotion, insulin resistance and energy intake and weight

<table>
<thead>
<tr>
<th>Factor</th>
<th>ACF size</th>
<th>a.u.c.</th>
<th>Basal insulin</th>
<th>Basal glucose</th>
<th>Basal TG</th>
<th>Energy intake</th>
<th>Final weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACF size</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>a.u.c.</td>
<td>0.67</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal insulin</td>
<td>0.39</td>
<td>0.40</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal glucose</td>
<td>0.40</td>
<td>0.38</td>
<td>0.37</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal TG</td>
<td>0.38</td>
<td>0.31</td>
<td>0.24</td>
<td>0.39</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy intake</td>
<td>0.66</td>
<td>0.62</td>
<td>0.53</td>
<td>0.40</td>
<td>0.35</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Final weight</td>
<td>0.67</td>
<td>0.64</td>
<td>0.55</td>
<td>0.45</td>
<td>0.28</td>
<td>0.84</td>
<td>1.00</td>
</tr>
</tbody>
</table>

ACF size, the average number of aberrant crypts/aberrant crypt foci; a.u.c., total area under the OGTT curve (0–2 h); TG, triglyceride. Correlations are based on data for all animals (n = 98). Values >0.26 are statistically significant (P < 0.01).

**Fig. 2.** Results of the oral glucose tolerance test. Values are means ± SEMs. Tail vein blood glucose was measured before and 30, 60 and 120 min after glucose gavage (4 mg/g body wt). Areas under the curves (a.u.c.) were calculated as described in Materials and methods.

**Fig. 3.** ACF size (aberrant crypts/aberrant crypt foci) as a function of a.u.c. (average area under curve of the OGTT) for all of the seven experimental dietary groups from Table II. Error bars larger than the symbols used are shown in both directions. The line is the best mean square fit to the data.

**Discussion**

To determine whether diet affects the process of insulin resistance and colon cancer promotion in the same way, we compared the effect of diets low and high in fat, n-3 fatty acids and energy on markers of insulin resistance and promotion in a single experiment with F344 rats. The measurements were facilitated by the use of glucose intolerance (a.u.c.) and ACF growth (aberrant crypts/ACF at 100 days) as the markers of insulin resistance and tumor promotion. The results of comparisons made with these markers (Table II, lower panel) are consistent with the results of earlier studies in which each of these factors was examined separately in individual studies with single dietary factors with the hyperinsulinemic euglycemic clamp or tumor promotion assays. The study thus confirms that insulin resistance and tumor promotion are affected in a similar way by diet over a wide range of dietary variables: for fat comprising 12–60% en, for n-3 fatty acids comprising ~1–15% fat and for energy consumption of 40–100 kcal/rat/day. The study also shows a striking correlation between the measures of insulin resistance and promotion (Figure 3 and Table II). It shows further that both measures are associated with energy consumption and the final weight of the animals and that both are associated with basal (post-prandial) levels of insulin, glucose and triglycerides. Furthermore, the partial correlations show that glucose intolerance and ACF size are correlated even after taking into account the effect of the dietary groups. Thus, the major conclusion of our study is that glucose intolerance and ACF size, our measures of insulin resistance and colon cancer promotion, are closely correlated for a wide range of diets.

The insulin resistance hypothesis with increased concentrations of insulin, glucose and triglycerides could explain the correlation between our measures of insulin resistance and colon cancer promotion. Exogenous insulin increases the growth of ACF and promotes colon cancer in rats (23,24). However, this promotion was observed at high insulin concentrations, well out of the usual physiological range, and may not be the most important factor. Glucose is an important source of colonic cell energy and is known to effect cell proliferation, perhaps especially when the cells are at high density, as they are in ACF or as tumors (25,26). Hypertriglyceridemia can affect both insulin resistance and cell proliferation. Triglycerides administered i.v. as liposomes together with heparin can increase levels of free fatty acids and lead to insulin resistance in both humans and rats within a period of a few hours (27). Lipoproteins containing triglycerides increase proliferation of several cell lines in vitro (28). Alternatively, hyperinsulinemia, glycemia and hypertriglyceridemia could act together to increase levels of intracellular fuels. Recently, Prentki and Corkey (29) have reviewed evidence suggesting that elevated levels of intracellular carbohydrate and lipid, ‘glucolipoxia’, give rise to a ‘signal of plenty’, with second messengers, including diacyl glycerol and calcium, that in
some cells induce insulin resistance. In other cells, these same signals might be expected to increase proliferation, reduce apoptosis and promote carcinogenesis.

The nature of the mechanism that leads from diet to energy consumption is not known. In this experiment, energy reduction was enforced by design with the CR, but with the other diets, the wide range of energy intakes was a consequence of appetite and satiety (30). Variety in the CAF can increase consumption (19); beef tallow in the HF can increase caloric intake (31); addition of n-3 fatty acids to the HF can decrease intake (32,33). These same factors may be affecting food consumption in the human diet. They could be responsible for increased energy consumption, for hyperinsulinemia, glyceremia and hypertriglyceridemia and for the long-term increase in both insulin resistance and tumor promotion.

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