Alterations of Serum Lipids in Breast Cancer: Effects of Disease Activity, Treatment, and Hormonal Factors

Martyn L. Knapp,1 Said Al-Sheibani,1 and Pamela G. Riches2

Fasting venous blood collected from 83 patients with breast cancer was analyzed for triglycerides; total, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) cholesterol; tumor necrosis factor (TNFα); glucose; creatinine; insulin; glucagon; growth hormone; cortisol; and thyrotropin. Patients with stage IV disease had significantly higher (P <0.05) triglyceride concentrations and significantly lower (P <0.05) concentrations of total and HDL cholesterol than did patients with less advanced disease or age-matched controls. Furthermore, LDL cholesterol concentrations in patients with bone metastases were significantly lower (P <0.05) than concentrations in patients with liver or liver plus bone metastases or in controls. These results could not be attributed to smoking habits, alcohol consumption, or treatment. We observed no correlations between serum concentrations of lipid and concentrations of TNFα, insulin, glucose, creatinine, cortisol, growth hormone, or thyrotropin. However, there was a significant (P <0.05) negative correlation between total cholesterol and glucagon and between LDL cholesterol and glucagon for patients with stage II, III, and IV disease, suggesting that glucagon may reduce LDL cholesterol concentrations by an as-yet-unidentified mechanism.

Additional Keyphrases: triglycerides • lipoproteins • cholesterol • tumor necrosis factor • glucose • creatinine • insulin • glucagon • somatotropin • cortisol • thyrotropin

Several studies have demonstrated changes in serum lipids in cancer patients (1). In human colon cancer, patients with advanced disease have significantly lower concentrations of serum total cholesterol than do controls matched for age and sex, whereas patients with early disease show no difference from controls (2). Rats with chemically induced colon cancer develop a significant increase in serum triglycerides with disease progression, which cannot be attributed to changes in diet (3).

Spiegel et al. (4) showed that patients with acute leukemia and non-Hodgkin lymphoma had a decrease in serum high-density lipoprotein (HDL) cholesterol and an increase in triglycerides, which returned to normal after successful therapy.3 Similarly, in juvenile chronic granulocytic leukemia, patients reportedly developed an increase in serum triglyceride and a decrease in total and HDL cholesterol (5).

Studies in patients with breast cancer have demonstrated an increase in serum triglyceride concentrations with disease progression (6, 7), which may also be accompanied by a decrease in serum HDL cholesterol (8). It has been postulated that changes in the concentrations of serum lipids in breast cancer could result from increased production of tumor necrosis factor α (TNFα) by activated macrophages in response to the tumor (7). TNFα has been shown to antagonize some of the actions of insulin, including inhibition of adipose tissue lipoprotein lipase by inhibiting expression of the lipoprotein lipase gene (9). This would impair catabolism of VLDL, leading to an increase in serum triglyceride and a decrease in HDL cholesterol through the reduced availability of apoproteins for HDL formation (10).

Currently, no study has investigated the relationship between the concentrations of serum lipids and TNFα in a group of cancer patients. Furthermore, the possible role of other hormonal factors in the etiology of lipid alterations in cancer has not been investigated. Therefore, in the present study, we examined the following: (a) the association between serum lipid concentrations and stage of disease in breast cancer, taking into consideration the effects of treatment, weight loss, smoking, and alcohol consumption; (b) the frequency of increases of serum TNFα in breast cancer and its relationship to the serum lipid concentrations in breast cancer patients; and (c) the possible role of insulin, glucagon, growth hormone (somatotropin), cortisol, and thyrotropin in any alterations of serum lipid concentrations in these patients.

Materials and Methods

Patients and Samples

We collected blood samples from 83 patients with histologically proven breast cancer who were attending the oncology clinic at the Westminster Hospital, London, or the breast clinic at St. Peter's Hospital, Chertsey. Ethical Committee approval was obtained for the collection of samples from these patients. Patients were staged according to the Tumor–Node–Metastases (TNM) classification (11).

We excluded from this study patients with known diabetes, with thyroid disease, being treated for hyperlipidemia, receiving parenteral nutrition, or with clinical evidence of infection, and dieting or anorexic patients. None of the patients studied was pregnant, had

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3 Nonstandard abbreviations: HDL, LDL, VLDL, high-, low-, and very-low-density lipoprotein, respectively; and TNFα, tumor necrosis factor α.

Received March 7, 1991; accepted October 9, 1991.
nephrotic syndrome, or had a chronic inflammatory disorder such as rheumatoid arthritis. The clinical information obtained for the patients is summarized in Table 1.

Venous blood (10 mL) was collected between 0830 and 1030 h into plain glass tubes (7.5 mL) and tubes containing lithium heparin (2.5 mL) after a minimum 12-h fast. We centrifuged the heparinized sample without delay at 1000 × g for 15 min and stored it at −70 °C until assay. The serum sample was allowed to clot for 15 min at 4 °C then similarly was centrifuged and stored at −70 °C in 1-mL aliquots until analysis. We also collected fasting venous blood (5 mL) into plain glass tubes from age- and sex-matched control subjects who did not have malignant disease or any serious pathology, but who were attending the hospital for treatment of relatively minor conditions (in terms of blood chemistry), e.g., varicose vein stripping and total knee replacement. We centrifuged and stored the samples as previously described.

Analyses

We performed the following analyses on the samples from the breast cancer patients: triglyceride, total cholesterol, HDL cholesterol, LDL cholesterol, glucose, creatinine, immunoreactive TNFα, glucagon, insulin, growth hormone, cortisol, and thyrotrphin. On the samples from the age- and sex-matched controls, we performed the analyses on the following substances: triglyceride, total cholesterol, HDL cholesterol, and (calculated) LDL cholesterol.

All assays were performed with serum samples, except for glucagon, which was assayed in heparinized plasma.

Triglyceride and total cholesterol were measured with the Boehringer Hitachi 705 analyzer (Boehringer Mannheim, Lewes, Sussex, U.K.) by standard coupled enzymatic procedures (12, 13). For triglyceride, between-batch imprecision (CV) was 2.5% at 1.2 mmol/L; the reference range for fasting subjects was ≤2.3 mmol/L. For total cholesterol, between-batch imprecision was 2.1% at 4.8 mmol/L, with the reference range extending to 6.5 mmol/L.

HDL cholesterol was determined by the dextran sulfate/MgCl₂ precipitation method (14) with use of reagents from Technicon Diagnostics (Basingstoke, U.K.). After precipitation, the supernate was immediately analyzed for cholesterol as described above. The between-batch imprecision was 3.5% at 1.9 mmol/L; the reference range was 0.9–1.9 mmol/L.

LDL cholesterol was calculated from the measured concentrations of total cholesterol, HDL, and triglyceride, according to the formula of Friedewald et al. (15). The reference range for LDL cholesterol was ≤5.0 mmol/L.

Glucose was measured by a glucose oxidase procedure on the YSI glucose analyzer (Clandon Scientific Ltd., Aldershot, U.K.). The between-batch imprecision was 2.6% at 9.8 mmol/L.

Creatinine was measured by a kinetic Jaffé procedure with the Astra 8 analyzer (Beckman Instruments, High Wycombe, U.K.). The between-batch imprecision was 4.1% at 120 μmol/L.

Immunoreactive TNFα was measured by an enzyme-linked immunosorbent assay on microtiter plates. Synthetic recombinant human TNFα standard (lot 87/650), rabbit polyclonal anti-TNFα antiserum (lot R332), and mouse monoclonal anti-TNFα antiserum (lot 1014) were provided by Dr. A. Meager, National Institute of Biological Standards and Controls, South Mimms, U.K. Alkaline phosphatase-linked sheep anti-mouse IgG (Sigma Chemical Co., Poole, U.K.) was the labeled antibody. The assay showed no detectable cross-reactivity with

<table>
<thead>
<tr>
<th>Table 1. Clinical Information from the Breast Cancer Patients</th>
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<tbody>
<tr>
<td>Stage</td>
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<tr>
<td>n Patients with recurrent disease, n (%)</td>
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<tr>
<td>History of weight loss, n (%)</td>
</tr>
<tr>
<td>Alcohol intake, mL/week, mean (range)</td>
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<tr>
<td>Current smokers, n (%)</td>
</tr>
<tr>
<td>Treatment, n (%)</td>
</tr>
<tr>
<td>No current therapy</td>
</tr>
<tr>
<td>Radiotherapy</td>
</tr>
<tr>
<td>Chemotherapy</td>
</tr>
<tr>
<td>Anti-estrogen</td>
</tr>
<tr>
<td>Tamoxifen</td>
</tr>
<tr>
<td>Aminoglutethimide</td>
</tr>
<tr>
<td>Medroxyprogesterone</td>
</tr>
<tr>
<td>Steroid</td>
</tr>
<tr>
<td>Dexamethasone</td>
</tr>
<tr>
<td>Hydrocortisone</td>
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* Bony metastases, 21 patients; liver metastases, seven patients; bone + liver metastases, eight patients; other sites, 11 patients. a Loss of body weight >10% during previous six months. N/A, not applicable.
TNFα or interleukin 1 and gave results that correlated with those by a cytotoxic bioassay for recombinant TNFα. The detection limit was 7 units/mL, with between-batch imprecision of 9.5% at 25 units/mL. With this assay, serum TNFα was undetectable (<7 units/mL) in normal, healthy individuals.

The following reagents were required: coating buffer (sodium carbonate, pH 9.6, 50 mmol/L); assay/wash buffer (phosphate buffer, pH 7.2, 50 mmol/L), containing sodium chloride (150 mmol/L) and Tween 20 (5 mL/L); blocking buffer (assay/wash buffer plus human serum albumin, 10 g/L); and substrate buffer (diethanolamine, pH 9.8, 0.5 mmol/L, containing MgCl₂, 0.5 mmol/L).

Rabbit polyclonal anti-TNFα antiserum was diluted 10-fold with coating buffer before use. Mouse monoclonal anti-TNFα was diluted 100-fold with assay/wash buffer before use.

Recombinant TNFα standard was diluted in assay/wash buffer to give final concentrations of 8, 16, 31, 63, 125, 250, 500, and 1000 units/mL.

The assay protocol was as follows:
1. Add 50 µL of diluted polyclonal anti-TNFα to each microtiter plate well and incubate at 4 °C overnight.
2. Wash the wells three times with assay/washing buffer.
3. Add blocking buffer, 100 µL/well, and incubate at 37 °C for 3 h.
4. Wash, as in step 2.
5. Add sample or standard, 50 µL/well in duplicate, and incubate at 4 °C overnight.
6. Wash (step 2).
7. Add monoclonal anti-TNFα, 50 µL/well, and incubate at 4 °C overnight.
8. Wash (step 2).
9. Add alkaline phosphatase-linked sheep antimouse IgG (diluted 100-fold in assay/wash buffer), 100 µL/well, and incubate at 20 °C for 3 h.
10. Wash (step 2).
11. Add 4-nitrophenyl phosphate (10 mmol/L in substrate buffer), 100 µL/well. After incubation at 20 °C for 1 h, stop the reaction by adding 3 mol/L NaOH, 50 µL/well.
12. Measure the absorbance of the microtiter plate at 405 nm (we used a Titertek Multiskan plate reader).
13. Read the concentration of TNFα in the samples from the standard curve, plotted on log linear paper.

Glucagon and insulin were determined by double-antibody RIA with reagents from ICN Biomedicals Inc., Costa Mesa, CA. For the glucagon assay, the antibody cross-reaction with enteroglucagon, insulin, and gastrin was <0.002%. The between-batch imprecision (CV) was 11.3% and 8.3% at glucagon concentrations of 20 and 150 pmol/L, respectively; the detection limit was 5 pmol/L, and the fasting reference range was ≤40 pmol/L. For the insulin assay, the antibody cross-reaction with proinsulin, C peptide, and glucagon was 19%, 0.0003%, and 0.09%, respectively. The between-batch imprecision was 12.3% and 7.1% at insulin concentrations of 7.5 and 25.1 milli-units/L, respectively; the detection limit was 1 milli-unit/L, and the fasting reference range was ≤45 milli-units/L.

Growth hormone was measured by a two-site immunoradiometric assay, with reagents supplied by Immunodiagnostic Systems (IDS) Ltd., Tyne & Wear, U.K. The between-batch imprecision was 12.3% and 9.2% at concentrations of 5 and 20 milli-int. units/L, respectively; the detection limit was 1 milli-int. unit/L, and the fasting reference range was ≤15 milli-int. units/L.

Cortisol was estimated by RIA with reagents supplied by IDS Ltd. The between-batch imprecision was 7.1% and 9.8% at cortisol concentrations of 175 and 500 nmol/L, respectively. The reference range for serum cortisol at 0800 h was 150–650 nmol/L.

Thyrotropin was determined by a solid-phase, two-site fluorimmunometric assay "Delfia" with reagents supplied by Pharmacia Ltd., Milton Keynes, U.K. The between-batch imprecision was 5.4% and 3.5%, respectively, at thyrotropin concentrations of 1.1 and 11.3 milli-int. units/L; the detection limit was 0.02 milli-int. unit/L with a reference range 0.4–5.0 milli-int. units/L.

Statistical Analysis

We used Student's paired t-test to assess the significance of any differences in serum lipid concentrations between the breast cancer patients and the age- and sex-matched controls. We used the Mann–Whitney U-test to assess the significance of any changes in analyte concentration associated with stage of disease, site of metastases, treatment, or weight loss in the breast cancer patients.

Correlations between the various analytes were assessed by both the Spearman rank correlation and least-squares linear regression. Deming's correction for slope was not applied because Sx/Sy was <0.2 (16). When all of the data had been analyzed, we found no discrepancies in statistical outcome between these two tests; i.e., a significant correlation by the Spearman rank test was invariably also significant by least-squares linear regression, and vice versa. Therefore, for the sake of clarity, the correlation data presented in Results refer only to the results obtained by least-squares linear regression. However, no difference in the outcome of the results analysis would have occurred if the Spearman rank correlation data were also shown.

Results

Association between fasting serum lipid concentrations and stage of disease in breast cancer. When fasting serum triglyceride concentrations in breast cancer patients were compared with the concentrations in age- and sex-matched control subjects (Figure 1), only patients with stage IV disease had significantly higher concentrations than the controls did (0.02 < P < 0.05). Median fasting serum triglyceride concentrations increased with stage of disease, which was statistically significant for stage IV compared with stage I patients (0.01 < P < 0.025). The site of metastases had no significant effect on fasting serum triglyceride concentrations in stage IV patients.
Serum total cholesterol tended to decrease with increasing stage of disease (Figure 2). This was significant for stage IV patients vs patients with stage I disease and controls (0.01 < P < 0.025) and for stage III patients vs patients with stage I or II disease and controls (0.025 < P < 0.05). Furthermore, the site of metastases in stage IV patients had a significant effect on total cholesterol concentration: the patients with bone metastases had significantly less total cholesterol than did patients with liver metastases or bone plus liver metastases (0.025 < P < 0.05).

Patients with stage IV disease had significantly lower concentrations of HDL cholesterol than did controls or patients with stages I, II, or III disease (0.001 < P < 0.01) (Figure 3). The site of metastases had no significant effect on the serum HDL cholesterol concentration in stage IV patients.

Although patients with stage IV breast cancer as a group had significantly lower LDL cholesterol concentrations than did patients with stage II disease (0.02 < P < 0.05), they did not have significantly lower LDL cholesterol concentrations than patients with stage I or stage III disease (Figure 4). However, patients with bone metastases had significantly lower LDL cholesterol concentrations than did controls and patients with either liver metastases or bone plus liver metastases (0.02 < P < 0.05). LDL cholesterol concentrations in patients with metastases at other sites were not significantly different from concentrations in controls.

Analysis of correlation between fasting triglycerides and total, HDL, and LDL cholesterol in breast cancer patients. We found no significant correlation between the serum concentrations of fasting triglycerides and total, HDL, or LDL cholesterol for the breast cancer patients, whether the data were analyzed separately for each stage of disease or for all stages combined. There was, however, a significant positive correlation between total (y) and LDL cholesterol (x) for all stages of disease (y = 2.27 + 1.01x; r = 0.81; P < 0.001) and between total (y') and HDL cholesterol (x) for stage IV patients (y' = 4.22 + 0.61x; r = 0.32; 0.02 < P < 0.05).

We also found a significant negative correlation between HDL (y) and LDL cholesterol (x) for stage I patients (y = 3.50 - 0.42x; r = -0.56; 0.01 < P < 0.02),
but not for other patients in other stages.

*Influence of smoking, alcohol consumption, and weight loss on serum lipid concentrations.* There was no significant difference in fasting serum concentrations of triglycerides, total cholesterol, HDL cholesterol, and LDL cholesterol between smokers and nonsmokers for any stage of disease ($P > 0.05$).

Patients with stage I disease who consumed $> 50$ mL of alcohol per week had significantly higher concentrations of HDL cholesterol (median 2.1 mmol/L) than did patients who consumed $< 50$ mL of alcohol per week (median 1.8 mmol/L; $0.02 < P < 0.05$). This effect of alcohol consumption was not observed for triglyceride, total, or LDL cholesterol, or in patients with more advanced disease.

We investigated the influence of weight loss on serum lipid concentrations in breast cancer patients only for patients with stage IV disease because only three patients with less advanced disease had considerable weight loss, whereas 24 of 47 patients with stage IV disease had considerable weight loss. There was no significant difference between fasting serum concentrations of triglyceride, total cholesterol, and LDL cholesterol in those patients who had considerable weight loss than in those who did not ($P > 0.05$). However, serum concentrations of HDL cholesterol were significantly lower in patients who had weight loss (median 0.95 mmol/L) than in those who did not (median 1.40 mmol/L; $0.01 < P < 0.025$). The HDL cholesterol concentrations in stage IV patients without weight loss were nevertheless still significantly less than the HDL cholesterol concentrations in patients with stage I, II, or III disease ($0.025 < P < 0.05$).

*Relationship between stage of disease in breast cancer and fasting serum concentrations of hormones and immunoreactive TNFα.* Fasting serum concentrations of immunoreactive TNFα were significantly higher in patients with stage III or IV disease than in patients with stage I or II disease ($0.02 < P < 0.05$). Similarly, fasting plasma concentrations of glucagon were significantly higher in stage IV patients than in patients with less advanced disease ($0.02 < P < 0.05$). We found no significant difference in the fasting serum concentrations of insulin, glucose, growth hormone, cortisol, and thyrotropin between patients with stage I, II, III, or IV disease (Table 2). Stage IV patients showed no significant difference in the fasting serum concentrations of hormones and TNFα according to metastatic site.

We observed no significant correlations between fasting concentrations of serum immunoreactive TNFα, glucagon, insulin, cortisol, growth hormone, or thyrotropin in the breast cancer patients whether the data were analyzed separately by stage of disease or by all stages combined. There was, however, a significant positive correlation between fasting serum glucose ($\gamma$) and insulin ($\alpha$) for all stages of disease ($\gamma = 4.37 + 0.021\alpha$; $r = 0.54; P < 0.001$). The concentrations of fasting serum glucose did not correlate with those of any other analyte.

*Effect of treatment on fasting serum concentrations of lipids, hormones, and TNFα.* Fasting serum concentrations of lipids, hormones, and immunoreactive TNFα in patients receiving radiotherapy or chemotherapy were not significantly different from concentrations in patients who had not received radiotherapy or chemotherapy. Similarly, there was no significant difference in the fasting concentrations of the above analytes between patients receiving anti-estrogen therapy or steroid therapy and those not receiving this therapy ($P > 0.1$). This

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**Table 2. Relationship between Stage of Disease and Fasting Serum Concentrations of Various Analytes**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Stage I (median (range))</th>
<th>Stage II (median (range))</th>
<th>Stage III (median (range))</th>
<th>Stage IV (median (range))</th>
</tr>
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<tbody>
<tr>
<td>TNFα, units/mL</td>
<td>&lt;7 (&lt;7–26)</td>
<td>&lt;7 (&lt;7–41)</td>
<td>16 (&lt;7–81)*</td>
<td>16 (&lt;7–500)*</td>
</tr>
<tr>
<td>Glucagon, pmol/L</td>
<td>7 (&lt;5–23)</td>
<td>6 (&lt;5–20)</td>
<td>12 (&lt;5–18)</td>
<td>12 (&lt;5–130)*</td>
</tr>
<tr>
<td>Insulin, milli-units/L</td>
<td>18 (2–82)</td>
<td>18 (10–39)</td>
<td>12 (2–86)</td>
<td>18 (8–170)</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>4.4 (3.2–8.0)</td>
<td>4.7 (3.3–7.0)</td>
<td>3.3 (2.1–6.9)</td>
<td>5.1 (2.8–9.1)</td>
</tr>
<tr>
<td>Growth hormone, milli-int. units/L</td>
<td>3.3 (1.9–17.0)</td>
<td>2.5 (1.5–35.0)</td>
<td>7.0 (2.3–9.0)</td>
<td>4.0 (1.3–38.0)</td>
</tr>
<tr>
<td>Cortisol, mmol/L</td>
<td>320 (50–820)</td>
<td>350 (290–910)</td>
<td>360 (150–810)</td>
<td>370 (30–1060)</td>
</tr>
<tr>
<td>Thyrotropin, milli-int. units/L</td>
<td>0.9 (0.4–7.5)</td>
<td>1.5 (0.6–6.6)</td>
<td>1.8 (0.5–4.7)</td>
<td>2.1 (0.4–6.6)</td>
</tr>
</tbody>
</table>

*Significantly higher than in patients with stage I or II disease ($P < 0.05$). *Significantly higher than in patients with stage I, II, or III disease ($P < 0.05$).

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remained true whether the data were analyzed separately by stage of disease or analyzed by all stages combined.

Analysis of correlation between fasting serum concentrations of lipids, hormones, and TNFα. There was no correlation between fasting serum concentrations of triglycerides, total cholesterol, HDL cholesterol, or LDL cholesterol and serum concentrations of immunoreactive TNFα, whether the data were analyzed separately for each stage of disease or for all stages combined (Table 3). Similarly, there was no correlation between fasting serum triglycerides or total, HDL, or LDL cholesterol and serum insulin, glucose, creatinine, cortisol, growth hormone, or thyrotropin in the breast cancer patients, whether the data were analyzed separately for each stage of disease or for all stages combined (data not shown).

There was, however, a significant negative correlation between fasting serum total cholesterol and plasma glucagon concentrations and between serum LDL cholesterol and plasma glucagon concentrations for patients with stage II, III, or IV disease and for all stages combined (Figure 5). In Figure 5, three of the five patients with plasma glucagon concentrations >40 pmol/L had boney metastases; the remaining two had liver metastases. All five patients had lost >10% of their body weight over the previous six months. However, in all other respects, nothing in the clinical status of these patients made them appear to be any different from the other patients with stage IV disease.

When we re-analyzed the data without the results from these five patients, there was no significant negative correlation between serum total cholesterol and plasma glucagon or between serum LDL cholesterol and plasma glucagon for stage IV patients. However, the negative correlation between serum total cholesterol (y) and plasma glucagon (x) for all stages combined remained significant (y = 6.35 - 0.049x; 0.02 < P < 0.05).

We found no correlation between fasting serum triglycerides and glucagon or serum HDL cholesterol and glucagon.

Discussion
In this study, we have confirmed that patients with stage IV breast cancer have significantly higher concentrations of triglyceride and significantly lower concentrations of HDL cholesterol in fasting serum than do patients with less advanced disease or age- and sex-matched controls.

Serum total cholesterol decreased with increasing stage of disease; for stages III and IV patients, these concentrations were significantly lower than in age- and sex-matched controls. Serum total cholesterol concentrations appear to be lowest in those patients with stage IV disease who have boney metastases. Furthermore, serum LDL cholesterol concentrations in patients with boney metastases appear to be significantly lower than the concentrations in controls and patients with liver metastases or liver plus bone metastases.

The reduction in serum total cholesterol with increasing stage of disease can be partly attributed to a reduction in HDL cholesterol. Total cholesterol in patients with boney metastases may also be further decreased by a reduction in LDL cholesterol.

These changes in lipid concentrations with stage of disease could not be attributed to smoking habits, alcohol consumption, or therapy, and only part of the decrease in serum HDL cholesterol observed in stage IV patients was associated with weight loss.

When the data were examined for correlations between serum lipid concentrations and concentrations of TNFα, insulin, glucose, creatinine, glucagon, cortisol, growth hormone, and thyrotropin, no correlations were observed other than a significant negative correlation between total cholesterol or LDL cholesterol and plasma glucagon. No evidence suggested that TNFα had a role in the etiology of any of the serum lipid changes in the breast cancer patients.

The changes in serum lipid concentrations observed in this study are similar to those documented by other workers in breast cancer (6–8), leukemia and lymphoma (4, 5), and experimental colonic cancer in rats (3).

Except for breast cancer, most previous studies have demonstrated a reduction in serum total cholesterol with disease progression (1, 2, 5). However, in breast cancer, the one study in which total cholesterol was estimated (7) showed a significant increase in total cholesterol and disease progression, a finding not confirmed in the present work.

<table>
<thead>
<tr>
<th>Table 3. Correlation between Fasting Serum Lipid Concentrations and Immunoreactive TNFα in Breast Cancer Patients</th>
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<tbody>
<tr>
<td>Stage</td>
</tr>
<tr>
<td>Serum triglyceride</td>
</tr>
<tr>
<td>(r = 0.13, P &gt; 0.5)</td>
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<tr>
<td>Serum total cholesterol</td>
</tr>
<tr>
<td>(r = 0.026, P &gt; 0.5)</td>
</tr>
<tr>
<td>Serum HDL cholesterol</td>
</tr>
<tr>
<td>(r = -0.24, P &gt; 0.2)</td>
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<tr>
<td>Serum LDL cholesterol</td>
</tr>
<tr>
<td>(r = 0.16, P &gt; 0.4)</td>
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* y = 0.6.001x, immunoreactive TNFα.
In our study, we found no significant effects of steroid or anti-estrogen therapy on serum lipids. Currently, evidence on the effects of steroids is conflicting. Ettinger et al. (17) found that patients with systemic lupus erythematosus who were treated with prednisone had higher concentrations of serum triglyceride, LDL cholesterol, and total cholesterol than did patients not on steroid therapy, whereas Lakatos and Harsoygi (18) observed no significant differences in serum triglycerides or in total, HDL, or LDL cholesterol in 73 patients with rheumatoid arthritis who were receiving corticosteroid therapy, compared with 56 patients receiving nonsteroidal anti-inflammatory drugs.

With regard to the effect of anti-estrogen therapy on serum lipid concentrations, Rosner and Wallgren (19) found that patients with breast cancer who were treated with tamoxifen after surgery demonstrated a significant decrease in serum LDL cholesterol and an increase in triglycerides. Bertelli et al. (20) also found a significant decrease in LDL cholesterol in breast cancer patients treated with tamoxifen after surgery, compared with patients not treated with tamoxifen. There was no significant change in serum triglyceride or HDL. However, the patients in these two studies were all clinically free of disease at the time the samples were taken, so the results are not strictly comparable with those in the present study, in which all patients had clinical evidence of disease.

Among previous explanations proposed to account for an increase in serum triglyceride and a decrease in HDL cholesterol because of the reduced availability of apoproteins for HDL formation (10).

Adipose tissue lipoprotein lipase activity might be reduced by several mechanisms, including increased production of TNFα in response to the tumor (7, 9), decreased secretion of insulin (21), or increased production of hormones that antagonize the actions of insulin such as glucagon, growth hormone, cortisol, and catecholamines (22). The present study provides no evidence for the involvement of TNFα, insulin, glucagon, growth hormone, or cortisol, although a possible role for catecholamines cannot be excluded.

Increased dietary triglyceride intake could theoretically produce an increase in serum triglyceride concentrations. However, in the present study, we saw no evidence for increased triglyceride intake in stage IV patients, compared with that in patients with stage I, II, or III disease. Furthermore, the development of hypertriglyceridemia in animal tumor systems is independent of dietary triglyceride intake (23).

In animals whose hypertriglyceridemia developed after tumor implantation, hepatic triglyceride synthesis has been shown to be normal, which weighs against an increase in hepatic synthesis as an explanation (23).

Many tumor cells are rich in intracellular neutral lipids, which might be released into the circulation upon cell lysis (24). However, it is unlikely that the tumor cell population could contribute continuously the amount of triglyceride necessary to markedly increase serum triglyceride. Furthermore, as has been well documented in both animals and man, serum lipid changes may occur when the tumor comprises <1% of the host body weight (25). Therefore, bulk excretion of lipid by the tumor as the primary cause of hypertriglyceridemia is unlikely.

**Fig. 5.** Correlation between (left) fasting serum total cholesterol and plasma glucagon concentrations and (right) serum LDL cholesterol and plasma glucagon concentrations in breast cancer patients. (Left) C, stage I (y = 6.51 – 0.028x; r = −0.15, P > 0.5); A, stages II & III (y = 7.11 – 0.152x; r = −0.55, 0.02 < P < 0.05); O, stage IV (y = 5.73 – 0.033x; r = −0.54, P < 0.01); all stages: y = 5.94 – 0.036x (r = −0.49, P < 0.001). (Right) C, stage I (y = 3.57 – 0.051x; r = −0.23, P > 0.2); A, stages II & III (y = 4.90 – 0.17x; r = −0.57, 0.01 < P < 0.02); O, stage IV (y = 3.64 – 0.032x; r = −0.55, P < 0.001); all stages: y = 3.86 – 0.064x (r = −0.47, 0.001 < P < 0.01)
Mechanisms other than a decrease in adipose tissue lipoprotein lipase activity that could result in the decrease in HDL cholesterol concentrations observed in the stage IV breast cancer patients include increased hepatic catabolism of HDL via increased hepatic lipase activity (26), reduced lecithin:cholesterol acyltransferase activity, or reduced apolipoprotein A-I. The concentrations of apolipoprotein A-I, the cofactor for this acyltransferase enzyme, were decreased in patients with severe burns associated with a reduction in HDL cholesterol (27). This latter study also demonstrated reduced concentrations of apoprotein C-II, the cofactor for adipose tissue lipoprotein lipase associated with hypertriglyceridemia in a subgroup of burn patients, providing a further mechanism by which hypertriglyceridemia could occur via reduced activity of adipose tissue lipoprotein lipase.

The etiology of increased serum triglyceride and reduced HDL cholesterol concentration associated with stage IV breast cancer is probably multifactorial. However, if the inhibition of adipose tissue lipoprotein lipase activity were primarily responsible, one might expect to find a negative correlation between fasting serum triglycerides and HDL. Such a negative correlation was not observed, implying that other, possibly as yet unknown, mechanisms may be more important.

In the present study the decreased concentrations of total cholesterol in patients with stage IV breast cancer can be attributed partly to a corresponding decrease in HDL cholesterol. However, patients with boney metastases had significantly lower LDL cholesterol than did age- and sex-matched controls or patients with liver metastases or bone plus liver metastases. This is the first study in which a change in serum lipids has been associated with a particular metastatic site. Furthermore, because the serum LDL cholesterol concentrations in patients with bone plus liver metastases were not decreased, apparently the presence of liver metastases may in some way oppose the reduction in LDL cholesterol associated with boney metastases.

A decrease in LDL cholesterol could be due to a decreased conversion of intermediate-density lipoprotein to LDL by hepatic lipase (28). Such a mechanism has been postulated to explain the low LDL cholesterol concentrations seen in patients with acute viral and bacterial infections (29).

An increased LDL receptor content has been demonstrated for certain malignancies, but not specifically in breast cancer. This could result in increased cholesterol uptake by the tumor cells, thus lowering serum LDL cholesterol concentrations (30). Perhaps tumor deposits in bone or activated osteoclasts and osteoblasts associated with boney metastases have an increased rate of LDL cholesterol uptake compared with metastatic tumor deposits in other sites. If so, this could explain why patients with boney metastases had lower concentrations of LDL cholesterol.

It is well known that liver metastases may be associated with cholestasis (31). This could result in reduced biliary cholesterol excretion and explain why no significant decrease in LDL cholesterol is observed in patients with liver metastases or bone plus liver metastases.

A potentially significant finding in the present study was the negative correlation between total serum cholesterol and glucagon and between LDL cholesterol and glucagon. Clearly, additional careful work is needed to clarify this finding; at this stage, we cannot state for certain whether there is a causal relationship between these changes or whether some other common factor is influencing both glucagon and cholesterol concentrations. However, perhaps glucagon, by an as-yet-identified mechanism, may decrease LDL cholesterol concentrations—either by increasing LDL uptake/catabolism, by increasing biliary cholesterol excretion, or by reducing the LDL production from VLDL. The observation that fasting plasma concentrations of glucagon were not significantly higher in stage IV patients with boney metastases than in patients with liver or liver plus bone metastases, implies that glucagon lowers LDL cholesterol concentrations by a mechanism independent of the effect of boney metastases on LDL cholesterol concentrations. This argument is strengthened by the fact that the negative correlation between LDL cholesterol and glucagon was also observed for patients with stage II or III disease and for all stages combined, in addition to patients with stage IV disease.

We thank Dr. R. Phillips, Dr. I. Hanham, Mr. C. Anders, and Mrs. J. Champion for assistance with providing the samples from patients with breast cancer. We also thank the British Council for providing funding for S. A.-S., who was studying for the M.Sc. degree in Clinical Biochemistry from the University of Surrey, U.K., and Drs. Heather Freeman and J. Chakraborty for helpful discussion.

References
