Induction of Cancer Cell Apoptosis by Flavonoids Is Associated with Their Ability to Inhibit Fatty Acid Synthase Activity*

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The consumption of food products containing high amounts of flavonoids has been reported to lower the risk of various cancers. The mechanisms underlying the cancer-protective effects of these naturally occurring polyphenolic compounds, however, remain elusive. Based on our previous finding that the cytotoxic effect of the flavanol epigallocatechin-3-gallate on prostate cancer cells correlates with its ability to inhibit fatty acid synthase (FAS, a key lipogenic enzyme overexpressed in many human cancers), we examined the antilipogenic effects of a panel of 18 naturally occurring polyphenolic compounds. In addition to epigallocatechin-3-gallate, five other flavonoids, more particularly luteolin, quercetin, kaempferol, apigenin, and taxifolin, also markedly inhibited cancer cell lipogenesis. Interestingly, in both prostate and breast cancer cells, a remarkable dose-response parallelism was observed between flavonoid-induced inhibition of fatty acid synthesis, inhibition of cell growth, and induction of apoptosis. In support for a role of fatty acid synthesis in these effects, the addition of exogenous palmitate, the end product of FAS, markedly suppressed the cytotoxic effects of flavonoids. Taken together, these findings indicate that the potential of flavonoids to induce apoptosis in cancer cells is strongly associated with their FAS inhibitory properties, thereby providing a new mechanism by which polyphenolic compounds may exert their cancer-preventive and antineoplastic effects.

Flavonoids constitute the largest and most important group of polyphenolic compounds in plants. They are widely distributed in many frequently consumed beverages and food products of plant origin such as fruit, vegetables, wine, tea, and cocoa (1, 2). The molecular structure of flavonoids consists of two aromatic rings (A ring and B ring), that are linked by a three-carbon bridge (Fig. 1). Depending on their oxidation state and functional groups, flavonoids are further divided in six subclasses: flavones, flavanones, flavanols, flavonols, isoflavones, and anthocyanidins (1–3).

Intake of beverages or food products containing flavonoids has been frequently associated with a reduced risk for developing various cancers (2, 4–6). Consumption of onions and/or apples, two major sources of the flavonol quercetin (1, 3), was inversely associated with the incidence of cancer of the prostate, lung, stomach, and breast (7–10). Frequent consumption of tea, an important source of both flavanols (1, 11) and flavonols (1), has been correlated with a lower incidence of cancer of the breast, prostate, bladder, lung, pancreas, colon, stomach, esophagus, and oral cavity (11–13). In addition, moderate wine drinkers also seem to have a lower risk to develop cancer of the lung, endometrium, esophagus, stomach, and colon (14–18). Besides anthocyanidins, red wine contains relatively high levels of both flavanols and flavonols (1, 19). Furthermore, also consumption of olives and/or olive oil, containing relatively large amounts of the flavones luteolin and apigenin, has been associated with a lower risk to develop cancer of the breast, ovary, and colon (20–22).

Although several molecules and pathways have been proposed as targets of flavonoids (3, 6), the precise mechanism by which these compounds exert their cancer-protective effects are poorly understood. Recently, we have shown that the flavanol EGCG, the main polyphenolic compound of green tea, inhibited fatty acid synthase (FAS) activity and lipogenesis in prostate cancer cells, an effect that was strongly associated with growth arrest and cell death (23). FAS is a key metabolic enzyme that catalyzes the synthesis of long chain fatty acids (24). In contrast to most normal tissues, which show low FAS expression, expression of FAS is markedly increased in various human cancers, including cancer of the prostate, breast, ovary, endometrium, colon, and lung (25–27). Up-regulation of FAS occurs early in tumor development and is further enhanced in more advanced tumors. In addition, high FAS expression levels often predict a poor outcome for cancer patients. We have previously demonstrated that RNA interference-mediated silencing of FAS severely inhibits lipogenesis and induces growth arrest and apoptosis in prostate cancer cells (28). Furthermore, blockage of FAS by the chemical inhibitors cerulenin and C75 also inhibits proliferation and is cytotoxic for various tumor cell lines in vitro and/or tumor xenografts in vivo (29–33). In the present work we have investigated whether, in addition to EGCG, other naturally occurring flavonoids also inhibit FAS activity and lipogenesis in cancer cells and whether this inhibition correlates with the effects of these polyphenolic compounds on cancer cell growth and survival.

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† The abbreviations used are: EGCG, epigallocatechin-3-gallate; EC, epicatechin; ECG, epicatechin-3-gallate; EGC, epigallocatechin; FAS, fatty acid synthase; RNAi, RNA interference; siRNA, small interfering RNA.

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**EXPERIMENTAL PROCEDURES**

**Cell Culture**—The human LNCaP prostate cancer cell line and MDA-MB-231 breast cancer cell line were obtained from the American Type Culture Collection (Manassas, VA). Nonmalignant skin fibroblasts were kindly provided by Prof. Dr. J. J. Cassiman (Center for Human Genetics, K. U. Leuven, Leuven, Belgium). Cells were cultured as previously described (23). Quercetin, kaempferol, luteolin, apigenin, taxifolin, eriodictyol, naringenin, myricetin, galangin, flavone, 3-OH-flavone, genistein, resveratrol, (±)-catechin, (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechin-3-gallate (EGCG) were purchased from Sigma; cells were exposed to various concentrations (0–100 μM) of polyphenols or to vehicle (Me₂SO) for the indicated periods. For culturing cells in the presence of palmitate-bovine serum albumin complex, palmitate (Sigma) was first complexed to fatty acid-free bovine serum albumin (Invitrogen) as described (34). Briefly, 4 volumes of a 4% bovine serum albumin solution in 0.9% NaCl were added to 1 volume of 5 mM palmitate in ethanol and incubated at 37 °C for 1 h to obtain a 1 mM stock solution of bovine serum albumin-complexed palmitate.

**RNA Interference**—Transfection of LNCaP cells with small interfering (si) RNA was carried out as previously described (28); siRNA oligonucleotides were purchased from Dharmacon (Lafayette, CO). The siRNA oligonucleotides targeting FAS were: sense, CCCUGAGAUCC-CAGCGCGUUdTdT; antisense, CAGCGCUUGGGAUCUCAGGGdTdT. As a control, the GL2 luciferase siRNA was used as previously described (35). Transfections of LNCaP cells were performed with 0.66 nmol of siRNA duplex in 60-mm dishes (0.4 × 10⁶ cells/dish) using Oligofectamine (Invitrogen). Morphological analysis and [2-¹⁴C]acetate incorporation assays were performed 72 h after transfection.

**[2-¹⁴C]Acetate Incorporation Assay and TLC Analysis**—After 1 or 20 h of exposure to polyphenols or at 72 h after transfection with siRNA, 2-¹⁴C-labeled acetate (57 mCi/mmol; 2 μCi/dish; Amersham Biosciences) was added to the culture medium of LNCaP cells, MDA-MB-231 cells, or fibroblasts. After 4 h of incubation, culture medium and

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**TABLE I**

<table>
<thead>
<tr>
<th>Polyphenol class</th>
<th>% Lipogenesis of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (0 μM)</td>
<td>100.0 ± 9.6</td>
</tr>
<tr>
<td>Luteolin</td>
<td>24.0 ± 9.0*</td>
</tr>
<tr>
<td>Quercetin</td>
<td>38.1 ± 7.0*</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>49.2 ± 8.3*</td>
</tr>
<tr>
<td>Apigenin</td>
<td>68.4 ± 6.7*</td>
</tr>
<tr>
<td>Taxifolin</td>
<td>74.6 ± 11.4*</td>
</tr>
<tr>
<td>EGCG</td>
<td>80.7 ± 6.7*</td>
</tr>
<tr>
<td>Galangin</td>
<td>82.5 ± 12.5</td>
</tr>
<tr>
<td>ECG</td>
<td>85.1 ± 9.3</td>
</tr>
<tr>
<td>Myricetin</td>
<td>87.2 ± 5.1</td>
</tr>
<tr>
<td>Eriodictyol</td>
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</tr>
<tr>
<td>Catechin</td>
<td>91.6 ± 9.0</td>
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<tr>
<td>Naringenin</td>
<td>92.3 ± 8.4</td>
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<tr>
<td>Genistein</td>
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<tr>
<td>EC</td>
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<tr>
<td>Resveratrol</td>
<td>96.9 ± 5.7</td>
</tr>
<tr>
<td>3-OH-flavone</td>
<td>98.3 ± 7.1</td>
</tr>
<tr>
<td>EGC</td>
<td>99.3 ± 4.9</td>
</tr>
<tr>
<td>Flavone</td>
<td>99.3 ± 8.7</td>
</tr>
</tbody>
</table>

*Significantly different from control cells (0 μM) by the Tukey test.
FIG. 2. Flavonoid-mediated inhibition of lipid biosynthesis in cancer cells. A–B, [2-14C]acetate incorporation into cellular lipids of LNCaP cells after 5 h (A) or 24 h (B) exposure to 3-OH-flavone (3-OH-flav), luteolin (lut), quercetin (que), kaempferol (kae), or EGCG. C, [2-14C]acetate incorporation into cellular lipids of MDA-MB-231 cells after 24 h exposure to 3-OH-flavone, luteolin, quercetin, or kaempferol. D, impact of flavonoids on the enzymatic activity of FAS in vitro. Protein extracts from LNCaP cells were incubated with vehicle (ctrl) or with 100 μM 3-OH-flavone, kaempferol, quercetin, luteolin, or EGCG, and FAS activity was measured by quantitation of 2-14C-labeled malonyl-CoA incorporation into fatty acids in vitro. Data are the means ± S.D. (n = 5–16). *, significantly different (p < 0.05) from control (0 μM) by the Tukey test.
cells were collected, and cells were pelleted by centrifugation and re-suspended in 0.8 ml of PBS. Lipids were extracted using the Bligh Dyer method as previously described (36); [2-14C]acetate incorporation into cellular lipids was quantitated by scintillation counting. 

RESULTS

Comparative Analysis of the Inhibitory Activity of Naturally Occurring Polyphenolic Compounds toward Cancer Cell Lipogenesis—To investigate the ability of polyphenolic compounds to inhibit lipogenesis, LNCaP prostate cancer cells were treated with different polyphenols (25 μM) for 5 h, and incorporation of [2-14C]-labeled acetate into cellular lipids was quantitated. Of 18 naturally occurring polyphenolic compounds (Fig. 1), luteolin, quercetin, and kaempferol inhibited lipogenesis by more than 50% (Table I). Apigenin, taxifolin, and EGCG also displayed inhibitory activity on lipogenesis, but their effect was less pronounced. No significant inhibitory effects were observed for the other compounds at a concentration of 25 μM (Table I).

Because luteolin, quercetin, and kaempferol turned out to be the most efficient inhibitors, the effects of these compounds on lipogenesis in LNCaP prostate cancer cells and MDA-MB-231 breast cancer cells were studied in more detail. EGCG, the main polyphenolic compound in green tea which was previously demonstrated to inhibit lipogenesis in LNCaP cells (23), and 3-OH-flavone, a polyphenol that did not affect cancer cell lipogenesis, were used as a positive and negative reference, respectively. 2-14C-Labeled acetate incorporation assays showed that after 5 h of exposure of LNCaP cells to luteolin, quercetin, or kaempferol, a significant decrease in lipid synthesis was already evident at a concentration of 6 μM. Higher concentrations further reduced lipogenesis in a dose-dependent way (Fig. 2A). A further decline was observed after 24 h of exposure (Fig. 2B). At 12 μM, luteolin reduced lipogenesis to less than 10% of the control levels, thereby making luteolin the most efficient inhibitor of the studied flavonoids. EGCG was clearly less active when compared to luteolin, quercetin, and kaempferol (Table I).

Fig. 3. Effect of flavonoid-induced inhibition of lipogenesis on different lipid classes of LNCaP cells. After 20 h of exposure to 3-0H-flavone or quercetin, LNCaP cells were exposed to [2-14C]acetate for 4 h. After lipid extraction, different lipid species were separated by TLC, and incorporation of [2-14C]acetate was quantitated by scintillation counting. PL, phospholipids; TG, triglycerides; Chol, cholesterol. Data are the means ± S.D. (n = 4–11). * significantly different (p < 0.05) from control (0 μM) by the Tukey test.
Luteolin, quercetin, and kaempferol had similar inhibitory effects on lipid synthesis in MDA-MB-231 cells (Fig. 2C), a breast cancer cell line that displays about 50–70% of the lipogenic activity observed in LNCaP cells. 3-OH-flavone did not affect lipid synthesis in LNCaP or MDA-MB-231 cells (Fig. 2A–C), even not at a concentration of 100 μM (data not shown).

**Luteolin, Quercetin, and Kaempferol Decrease Lipogenesis in Prostate and Breast Cancer Cells via Inhibition of FAS Activity**—To confirm that the selected flavonoids act as FAS inhibitors, protein extracts from LNCaP cells were pretreated with these polyphenolic compounds, and incorporation of [14C]-labeled malonyl-CoA into fatty acids was quantitated. A concentration of 100 μM luteolin reduced the in vitro FAS activity to 14%, and 100 μM quercetin caused a reduction to 19%, whereas 100 μM kaempferol caused a reduction to 32% (Fig. 2D). EGCG decreased enzymatic FAS activity to less than 1% at a concentration of 100 μM, thereby making it, at least in vitro, a more efficient inhibitor of FAS enzymatic activity than luteolin, quercetin, and kaempferol (Fig. 2D). In agreement with its effects on cancer cell lipogenesis, 100 μM 3-OH-flavone did not inhibit enzymatic activity of FAS (Fig. 2D). Western blot analysis for FAS revealed that luteolin, quercetin, and kaempferol did not influence FAS protein levels in LNCaP cells (data not shown), thereby confirming that inhibition of lipid synthesis was the direct result of inhibition of enzymatic FAS activity and was not caused by decreased FAS expression. Consistent with their FAS inhibitory effect, inhibition of lipogenesis by luteolin, quercetin, and kaempferol affected the synthesis of phospholipids (including phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, and sphingomyelin) and of triglycerides in LNCaP cells (Fig. 3; data for quercetin are shown; similar results were obtained for luteolin and kaempferol) and MDA-MB-231 cells (data not shown); 3-OH-flavone had no effect on any of these lipid classes (Fig. 3). As previously observed for EGCG and cerulenin (23, 38, 39), luteolin, quercetin, and kaempferol also decreased the synthesis of cholesterol in LNCaP (Fig. 3) and MDA-MB-231 cells (data not shown). Cholesterol synthesis, however, accounts for less than 10% of the total lipogenic activity in LNCaP and MDA-MB-231 cancer cells, whereas ~75 and 15% of the lipogenic activity in these cells represents phospholipid and triglyceride synthesis, respectively.
Luteolin, Quercetin, and Kaempferol Induce Morphological Changes in LNCaP Cells Comparable with Those Observed after RNAi-mediated FAS Inhibition—Microscopic analysis showed that concentrations of quercetin (not shown), luteolin, and kaempferol (Fig. 4) that efficiently inhibited lipogenesis also induced marked morphological changes of LNCaP cells, including loss of cell-cell contacts and formation of astrocyte-like extrusions. Similar changes of LNCaP cell phenotype were observed after specific RNAi-mediated FAS inhibition (which caused a reduction of lipogenesis to 34% of the normal levels, i.e. a FAS inhibitory effect comparable with that of 6–12 μM luteolin or 25–50 μM quercetin or kaempferol) (Fig. 4), suggesting that the changes in cell morphology induced by luteolin, quercetin, and kaempferol were direct results of FAS inhibition. These phenotypical changes were not observed in 3-OH-flavone-treated LNCaP cells (not shown).

Luteolin, Quercetin, and Kaempferol Induce Growth Arrest of Prostate and Breast Cancer Cells—Increasing concentrations of luteolin, quercetin, and kaempferol gradually inhibited proliferation of both LNCaP and MDA-MB-231 cells (Fig. 5). Already at a concentration of 6 μM, growth inhibition was observed. For all three flavonoids, a remarkable parallelism was observed between the dose-response curves reflecting growth inhibition and those reflecting inhibition of lipogenesis. 3-OH-flavone also slightly inhibited proliferation of LNCaP cells and MDA-MB-231 cells (Fig. 5), but only at high concentrations (25–50 μM).

Luteolin, Quercetin, and Kaempferol Induce Apoptosis in Prostate and Breast Cancer Cells—Trypan blue staining revealed that exposure to luteolin, quercetin, or kaempferol for 24 or 48 h induced cell death of both LNCaP and MDA-MB-231 cells (Fig. 6). Remarkably, for all three flavonoids, induction of cell death occurred at flavonoid concentrations that also inhibited lipid synthesis. In contrast, 3-OH-flavone did not affect viability of LNCaP or MDA-MB-231 cells (Fig. 6). After 72 h of exposure, cytotoxic effects were even more pronounced, since also relatively low doses (6–12 μM) of luteolin, quercetin, and kaempferol induced significant death of LNCaP and MDA-MB-231 cells (Fig. 6).

To investigate the pathway by which luteolin, quercetin, and kaempferol induced cancer cell death, stainings with Hoechst 33342 and Western blot analysis for poly-ADP-ribose polymerase were performed on LNCaP cells. Hoechst staining showed that luteolin (12 μM), quercetin (50 μM), and kaempferol (50 μM) induced chromatin condensation and fragmentation of the nuclei into oligonucleosomes (Fig. 7, A–D). Apoptosis and induction of caspase activity was confirmed by Western blot analysis showing cleavage of poly-ADP-ribose polymerase (PARP) (Fig. 7E).
Palmitate Suppresses Cytotoxicity of Luteolin, Quercetin, and Kaempferol in Cancer Cells—To confirm that the cancer cell cytotoxicity induced by flavonoids was related to FAS inhibition, LNCaP cells were exposed for 24 h to luteolin (25 μM), quercetin (50 μM), kaempferol (50 μM), or EGCG (100 μM) in the presence of 75 μM palmitate. Palmitate markedly reduced the cytotoxic effect of all four flavonoids, as the percentage of trypan blue-positive LNCaP cells was significantly decreased after the addition of exogenous palmitate (Fig. 8).

Correlation between the Cytotoxic Effects of Natural Flavonoids and Their Ability to Inhibit Lipid Synthesis in Cancer Cells—To examine whether the lipogenesis inhibitory effect of polyphenols correlates with their cytotoxic effects on cancer cells, viability of LNCaP cells was analyzed after 24 h of exposure to 25 μM concentrations of 18 different polyphenolic compounds (listed in Fig. 1 and Table I). At this concentration, only luteolin, quercetin, kaempferol, apigenin, and taxifolin proved cytotoxic to LNCaP cells. The percentages of trypan blue-positive cells were: for luteolin, 26.9 ± 4.5%; for quercetin, 16.7 ± 2.9%; for kaempferol, 16.2 ± 1.3%; for apigenin, 12.8 ± 1.7%; for taxifolin, 11.7 ± 0.9% (p < 0.05 for all 5 flavonoids versus control LNCaP cells). Other polyphenols had no impact on LNCaP cell viability at a concentration of 25 μM, as percentages of trypan blue-positive cells did not exceed 8% (not significantly different from the control condition). Importantly, a striking correlation was observed between the lipogenesis inhibitory effect of the polyphenolic compounds and their cytotoxic effect on cancer cells (Pearson’s correlation coefficient r = −0.95; p < 0.001) (Fig. 9).

Flavonoid-mediated FAS Inhibition Does Not Affect Growth or Viability of Non-malignant Fibroblasts—To explore whether cell death induced by flavonoids is selective for malignant cells overexpressing FAS, we also investigated the effect of luteolin, quercetin, and kaempferol on non-malignant cells. Fibroblasts were used as a non-cancerous cell type, since contrary to cancer cells, these fibroblasts are characterized by low FAS expression and activity even when cultured in vitro (fibroblast FAS activity was 20 times lower than that observed in LNCaP cells) (28). Although we observed that EGCG acted as a more potent FAS inhibitor than luteolin, quercetin, and kaempferol in vitro, this stronger FAS inhibitory effect of EGCG was not reflected in vivo. Similarly, we observed that myricetin, which was demon-
strated by Li and Tian to display a stronger FAS inhibitory activity than taxifolin in vitro (40), was a less potent inhibitor of lipogenesis than taxifolin in intact cells. Possible explanations for the observed differences between in vitro and in vivo data include differences in uptake, sequestration, and/or oxidation rate of flavonoids.

With regard to the structure-activity relationship of flavonoids, it can be noted that those flavonoids that significantly inhibit lipogenesis (luteolin, quercetin, kaempferol, apigenin, taxifolin, and EGCG) all contain hydroxy groups on the 5 and 7 position of the A-ring, whereas flavone and 3-OH-flavone, which lack these 5,7-hydroxyls, do not affect lipogenesis. Furthermore, the four 5,7-hydroxyflavonols (myricetin, quercetin, kaempferol, and galangin) and the two 5,7-hydroxyflavones (luteolin and apigenin) studied, which inhibited lipid synthesis or at least showed a trend to inhibit lipogenesis, all contain a C-2,3 double bond and a 4-ketone function. In addition, within each flavonoid subclass, the 3',4'-dihydroxyflavonoids (with 2 hydroxyls on the B ring) are most active (luteolin > apigenin; eriodictyol > naringenin; quercetin > kaempferol, galangin, and myricetin). Taken together, it can be concluded that the presence of a C-2,3 double bond, a 4-ketone function, and hydroxyl groups on positions 5, 7, 3', and 4' favor the potential of flavonoids to inhibit lipid synthesis in intact cells. Wang et al. (41) previously demonstrated that the presence of a galloyl moiety was essential for FAS inhibition by catechins: only EGCG and ECG (but not EC or EGC) inhibited FAS activity in vitro. In addition, they also observed that both EGCG and ECG showed irreversible slow binding activity, whereas other flavonoids (including quercetin and kaempferol) only showed reversible fast binding inhibition of FAS (41, 42), thereby suggesting that EGCG and ECG may inhibit lipogenesis by different mechanisms than other flavonoids.

At least six of the studied polyphenolic compounds (EGCG (23), luteolin, quercetin, kaempferol, apigenin, and taxifolin) have marked effects on cancer cell growth and survival. Several of our findings suggest that these effects are related to the ability of flavonoids to inhibit fatty acid synthesis. First, a comparative analysis of 18 naturally occurring flavonoids revealed that their ability to inhibit lipogenesis markedly correlated with their cytotoxic effects in cancer cells. Second, the dose-response curves of luteolin, quercetin, and kaempferol, reflecting the inhibition of total cancer cell lipogenesis as well as those reflecting the inhibition of phospholipid and triglyceride synthesis, show a striking parallelism with the dose-response curves reflecting the impact of these compounds on proliferation and viability of cancer cells. Several of these flavonoids also inhibit cholesterol synthesis, but this effect correlates less well with the effects on viability. For some compounds, such as galangin, marked inhibition of cholesterol synthesis (5-fold reduction) was observed at concentrations (12–25 μM) that did not affect phospholipid or triglyceride synthesis nor cell viability (data not shown). Third, specific blockage of FAS using RNA interference technology also re-

**Fig. 9.** Correlation analysis of flavonoid-induced lipogenesis inhibition and cytotoxicity in cancer cells. LNCaP cells were exposed to 25 μM concentrations of 18 different flavonoids (listed in Fig. 1 and Table I) for 24 h. For each polyphenol, the percentage of cell death (calculated by using the trypan blue exclusion assay) was plotted in function of the observed lipogenesis (expressed as the percentage lipogenesis of the control condition (0 μM)). The Pearson’s correlation coefficient (r) and the coefficient of determination (r²) are indicated. Significant cytotoxic effects on LNCaP cells were observed for luteolin (L), quercetin (Q), kaempferol (K), apigenin (A), and taxifolin (T). Data are the means ± S.D. (n = 4–8).

**Fig. 10.** Impact of flavonoids on lipid synthesis and viability of nonmalignant fibroblasts. A, lipid biosynthesis of fibroblasts treated with 3-OH-flavone (3-OH-flav), luteolin (lut), quercetin (que), or kaempferol (kae) for 24 h, as analyzed by quantitating [2-14C]acetate incorporation into cellular lipids. B, cell death of fibroblasts treated with different concentrations of luteolin, quercetin, or kaempferol for 24 or 48 h, as revealed by counting the percentage of dead cells using the trypan blue exclusion assay. Data are means ± S.D. (n = 6). *p < 0.05 from control (0 μM) by the Tukey test.
sulted in growth arrest and cell death of cancer cells (28) and induced comparable morphological changes in LNCaP cells as observed after treatment with luteolin, quercetin, or kaempferol. Finally, the addition of exogenous palmitate, the most important end product of FAS activity, suppressed the cytotoxic effects of polyphenolic compounds, thereby providing evidence that FAS inhibition is a common denominator in the effects of these agents on cancer cells.

With respect to the potential use of luteolin, quercetin, and kaempferol as cancer-preventive or chemotherapeutic agents, it is worth mentioning that these compounds display very low toxicity in humans. Daily intake of relatively high doses of quercetin (1 g) or luteolin/apigenin (140 mg) for several weeks did not induce any side effects (43, 44). Moreover, the growth inhibitory and cytotoxic effects of these flavonoids may be relatively selective for malignant cells expressing high levels of FAS. In fact and again analogous to what we observed after selective inhibition of FAS by the use of RNAi (28), the proliferation rate and viability of nonmalignant fibroblasts expressing low levels of FAS was not affected by luteolin, quercetin or kaempferol and this despite a further lowering of lipogenesis in these cells. Whether this selectivity also applies in an *in vivo* situation requires further investigation.

The potential contribution of flavonoids to the cancer-preventive effects of polyphenol-rich diets of course depends on the daily intake and uptake of these compounds. The average flavonoid intake in the western world has been estimated to 1000 mg per day (2, 3). However, it is obvious that significantly higher flavonoid intake of food products containing quercetin or kaempferol may be reached by the use of polyphenol-rich diets. Several studies in humans have demonstrated that intake of food products containing quercetin or kaempferol results in plasma concentrations ranging from 0.6 to 6 μM (43, 45–49). In addition, the half-life of quercetin in humans is 20–30 h, suggesting that frequent consumption may result in accumulation of flavonoids in plasma and tissues (2), as previously observed for green tea polyphenols (50). Importantly, our data demonstrate that luteolin, quercetin, and kaempferol already inhibit FAS activity in cancer cells at relatively low concentrations (6–12 μM), which also induce significant cancer cell death. Thus, according to the pharmacokinetic studies discussed above and taking into account that different flavonoids may have additive effects and that regular intake may result in accumulation, flavonoid concentrations affecting FAS activity may be reached by the use of polyphenol-rich diets.

It should be mentioned that flavonoids may also contribute to cancer prevention by other mechanisms such as radical scavenging (2, 6), detoxification of mutagenic xenobiotics (6, 51, 52), and inhibition of topoisomerases (6, 53, 54), cyclin-dependent kinases (55, 56), and protein kinases (including phosphatidylinositol 3-kinase) (57). The question may be asked as to whether some of these alternative effects of flavonoids could be secondary to inhibition of lipogenesis. Indeed, we have recently demonstrated that RNAi-mediated FAS inhibition in prostate cancer cells decreases the synthesis of phospholipids partitioning into detergent-resistant membrane microdomains (58), membrane structures involved in signal transduction and cell migration (59). As a consequence, flavonoid-mediated inhibition of FAS activity in cancer cells may cause multiple downstream effects, resulting from disturbed membrane functions. Together, our findings show that flavonoids constitute interesting candidate molecules for cancer-preventive and/or antiinflammatory therapies and that interference with endogenous lipogenesis may be an important mechanism underlying their effects.