[Gerber et al (1989), *Cancer* 64, 2347-2353] or in the tumor tissues of other cancer sites [Di Ilio et al (1987), *Carcinogenesis* 8, 281-284]. Other studies demonstrate that PUFA were cytotoxic for tumor cells [Begin et al (1988), *J Natl Cancer Inst* 80, 188-194]. Furthermore, it was reported such a cytotoxic effect of PUFA in highly proliferating normal cells which was opposed by antioxidants [Cogrel et al (1993), *Lipids* 28, 115-119]. These findings suggest that the balance between oxidants and antioxidants might take a part in the proliferation regulation of normal as well as transformed cells.

We investigated this hypothesis with a clinical approach asking: 1) whether the profile 'low peroxidation products/high antioxidants' is associated with all cancers, food related cancers, and/or hormono-dependent cancers; 2) whether it could explain the slow evoluting cancers in older patients, since aged people are known to show a higher level of lipid peroxidation.

We conducted a case-control study to answer the first question with 269 hospital-based controls (34 to 86 years old) and 146 cases (43 to 86 years old). We assessed tumor aggressiveness based on tumor size, node invasion and metastasis. Cholesterol, triglycerides, vitamine E, and malon-dialdehyde (MDA) were measured in plasma.

The change in oxidant-antioxidant status was not associated with a specific cancer site, but observed in advanced cancers: vitamin E concentration increased and MDA decreased with tumor size and node invasion. We conducted a transversal study on 365 breast cancer cases to answer the second question. Assessment of tumor aggressiveness was based on pathology, estrogen receptors (ER), tumor size and node invasion. There was no metastatic patients (DNA and proliferation index are recorded but not yet analyzed). Biochemical measurements were done before therapy: cholesterol, triglycerides, vitamine E, glutathion peroxidase, selenium, and MDA. The analysis of this transversal study on breast cancer confirmed the association of the profile 'low peroxidation products/high antioxidants' with tumor aggressiveness and/or progression, based on pathology diagnosis, size and ER. It showed also that less aggressive tumors are prevalent in aged patients, compared with young ones, and that the profile 'low peroxidation products/high antioxidants' is always less marked in aged than in young patients. However, this association does not imply a causal relationship.

As a whole, these data suggest that tumor growth is reflected in the plasma by the profile 'low peroxidation products/high antioxidants'. This seems to be true for all tumor sites. This may be the consequense of an adaptative response of tumor cell towards a selective growth advantage.

To what extent exogenous (nutritional) oxidants and anti-oxidants might interfere? In experimental studies very high intake of vitamin E opposed the regulatory effect of increased lipid peroxydation on tumor growth [Gonzales et al (1991), *Carcinogenesis* 12, 1231-1235]. In epidemiological ecological [Kaiser et al (1989) *Nutr Cancer* 12, 61-68] and prospective [Vatten et al (1990), *Int J Cancer* 46, 12-15] studies, high intake of fish, which contains large amount of *n*–3 PUFAs, was associated with a lower incidence of cancers.

Retinoic acid inhibits insulin-induced cyclin D1 gene expression in T47D breast cancer cells. L Razanamahefa, C Costa, S Bardon *(Laboratoire de nutrition et sécurité alimentaire, Inra, 78350 Jouy-en-Josas, France).*

The vitamin A derived retinoids are evaluated as preventive and therapeutic agents for breast cancer. They have been shown to inhibit the in vitro proliferation of several breast carcinoma cell lines, but the mecha-

nisms of this effect remain to be elucidated. [Moon et al (1992) *Cancer Detect Prev* 16, 73-79; Fontana et al (1992) *Cancer Res* 50, 1977-1982]. In the present study, we examine the effect of all-trans retinoic acid on insulin-induced cyclin D1 gene expression, in parallel with cell growth, in T47D breast cancer cell line. Cyclin D1 is a cell cycle regulator and a candidate proto-oncogene implicated in mammary tumorigenesis the expression of which is associated with changes in the proliferation rate of breast cancer cells.

T47D breast cancer cells in exponential growth phase were grown for 19 h in serum-deprived RPMI medium for northern-blot analysis. All-trans retinoic acid dissolved in ethanol was then added in culture medium at concentrations over the range 10^{-10}-10^{-6} M, in presence of insulin 10 µg/mL. The final concentration of ethanol in medium was 0.1% and control cells received vehicle only. After 5 h, RNA was extracted from triplicate petri dishes by a guanidium-isothiocyanate procedure and northern-blot analysis was performed with 20 to 30 µg of total RNA per lane. Filters were hybridised with a human cyclin D1 cDNA (obtained from D Beach, Cold Spring Harbor, NY, USA), labelled with α-^{32}P dCTP by random primer extension. mRNA abundance was quantitated by electronic autoradiography (Packard Instant Imager). For growth experiments, T47D cells were plated in 3% fetal calf serum (FCS) supplemented medium, in 24-well plates. Twenty-four hours later, cells were treated with retinoic acid and insulin 10 µg/mL, as described above, in medium containing 1% FCS during 7 days. Cells were fixed with methanol and the amount of DNA was evaluated in situ by measuring the specific reaction fluorescent product of desoxyribose and diaminobenzoic acid [DABA assay; Kissane and Robins (1958) *J Biol Chem* 233, 184-188].

Evaluation of DNA cell content showed that retinoic acid (RA) inhibited insulin-induced T47D cell proliferation in a dose dependent manner:

	0	ins	RA10^{-10}	RA10^{-9}	RA10^{-8}	RA10^{-7}	RA10^{-6}
µg DNA per well	3.5	5.9	5.4	4.9	2.3	2.2	1.1

After northern-blot analysis, we observed that retinoic acid caused a concentration-related inhibition of insulin-induced cyclin D1 gene expression. Results are expressed as a percentage of cyclin D1 mRNA signal from insulin treated cells:

	ins	RA10^{-10}	RA10^{-9}	RA10^{-8}	RA10^{-7}	RA10^{-6}
% of mRNA cyclin D1	100	90	83	65	61	51

In conclusion, we have shown that retinoic acid was able to inhibit cyclin D1 gene expression in parallel with its inhibition action on cell growth. Current studies are focused on characterizing the retinoic acid effect on cyclin D1 gene expression, in order to determine if it acts at transcriptional or translational level. We will compare the RA response of several human breast tumor cell lines.

Influence of high concentrations of extracellular calcium on the proliferation and differentiation of porcine osteoblasts in culture. E Eklou-Kalonji, I Denis, A Pointil-