

POLYUNSATURATED FATTY ACIDS (PUFAs)

BOTH ω -3 AND ω -6 PUFAs HAVE CRUCIAL ROLES IN:

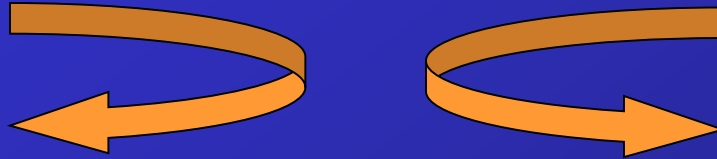
- ▶▶ MEMBRANE BIOLOGY**
- ▶▶ SIGNALING PROCESSES**

**IN MEMBRANES, LENGTH AND INSATURATION NUMBER
MODULATE:**

- ☐ FLUIDITY**
- ☐ FLEXIBILITY**
- ☐ SELECTIVE PERMEABILITY**
- ☐ ACTIVITY OF MEMBRANE-ASSOCIATED
ENZYMES**



LIGAND-RECEPTOR INTERACTIONS



GROWTH

VIABILITY

TUMOR TRANSFORMATION IS CHARACTERISED BY SEVERAL CHANGES IN LIPID COMPOSITION:

- ❖ INCREASE IN CHOLESTEROL/PHOSPHOLIPID RATIO
- ❖ DECREASE OF PUFA CONTENT IN PHOSPHOLIPIDS (arachidonic and docosahexaenoic acid)
- ❖ CHANGES IN THE PERCENTAGES OF DIFFERENT PHOSPHOLIPID CLASSES

ALL THESE CHANGES TAKE PART IN DETERMINING

- INCREASED CELL PROLIFERATION RATE**
- DECREASED SUSCEPTIBILITY TO APOPTOSIS**
- MODIFICATIONS IN COMMUNICATION OR SIGNALING BETWEEN CELLS**
- APPEARANCE OF RESISTANCE TO SOME ANTI-TUMOR DRUGS**

EXPERIMENTAL STUDIES INVESTIGATING THE EFFECTS OF PUFAs ON TUMOR CELLS HAVE OBTAINED CONTROVERSIAL RESULTS, EVEN IF

- ❖ **ω -3 HAVE ABOVE ALL ANTICARCINOGENIC PROPERTIES**
- ❖ **ω -6 HAVE ABOVE ALL CARCINOGENIC PROPERTIES**

OUR AIM

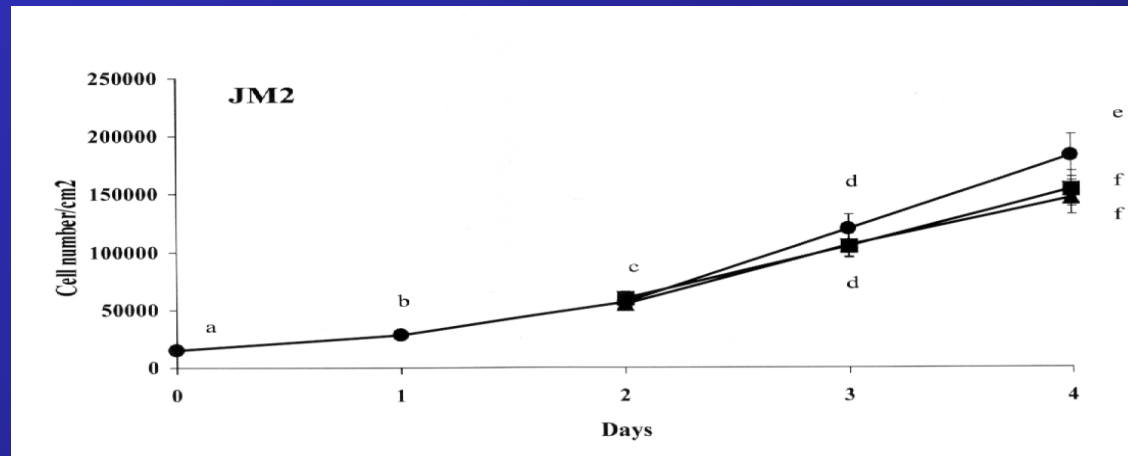
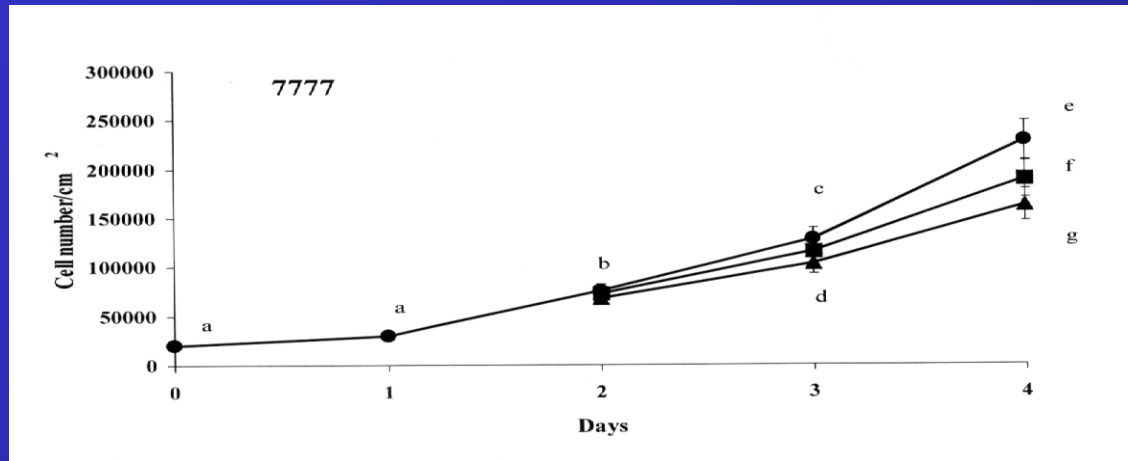
- **DELUCIDATE THE EFFECTS OF PUFAs IN TUMOR CELLS WITH DIFFERENT DEGREE OF MALIGNANCY**
- **IDENTIFY THE METABOLIC PATHWAYS INVOLVED IN THESE EFFECTS**

IN RAT HEPATOMA CELLS ENRICHMENT WITH ARACHIDONIC ACID:

- ▶▶ RESTORES PERCENTAGE CONTENT SIMILAR TO THAT PRESENT IN PHOSPHOLIPIDS OF NORMAL HEPATOCYTES

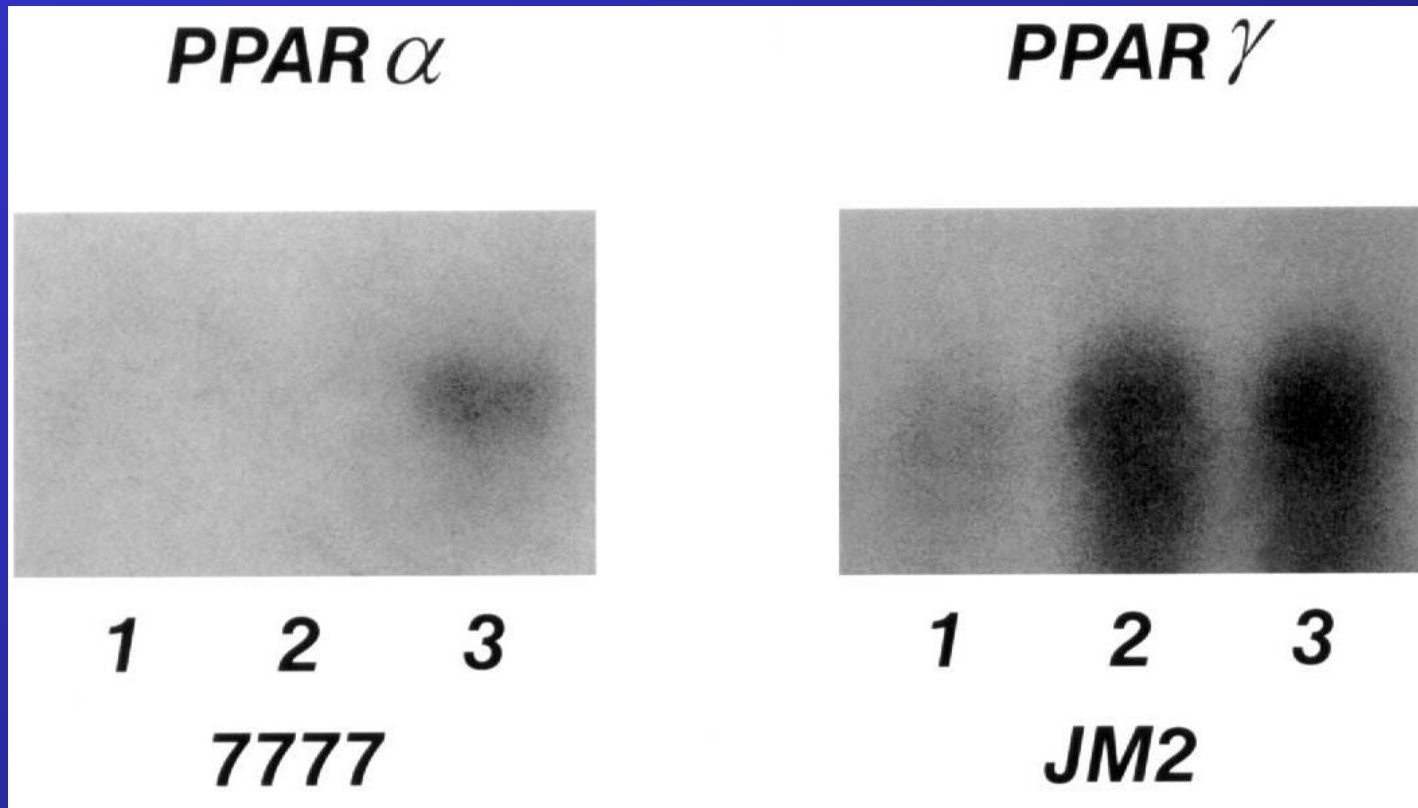
CELLS	TREATMENT		
	None	Arachidonic Acid (nmoles/10 ⁶ cells)	
		250	400
Hepatocytes	23.49 ± 1.91a		
7777 hepatoma	5.43 ± 2.01b	18.97 ± 2.88c	24.62 ± 1.98a
JM2 hepatoma	4.82 ± 0.93b	16.50 ± 5.66c	24.50 ± 2.67a

▶ RESTORED NORMAL ARACHIDONIC ACID CONTENT DECREASES CELL PROLIFERATION IN A DOSE-DEPENDENT MANNER

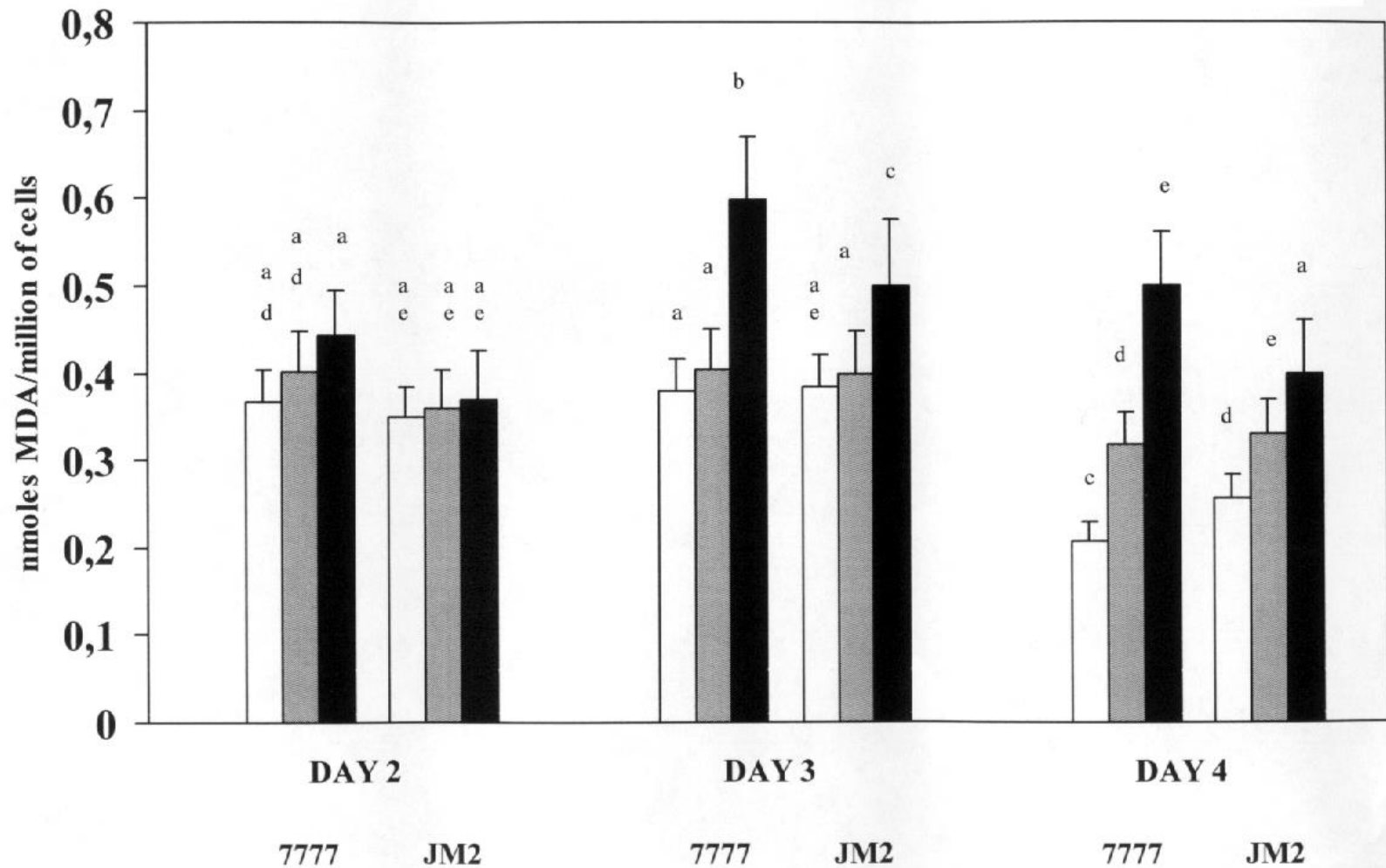


▶▶ RESTORATION OF NORMAL ARACHIDONIC ACID CONTENT IS COUPLED WITH

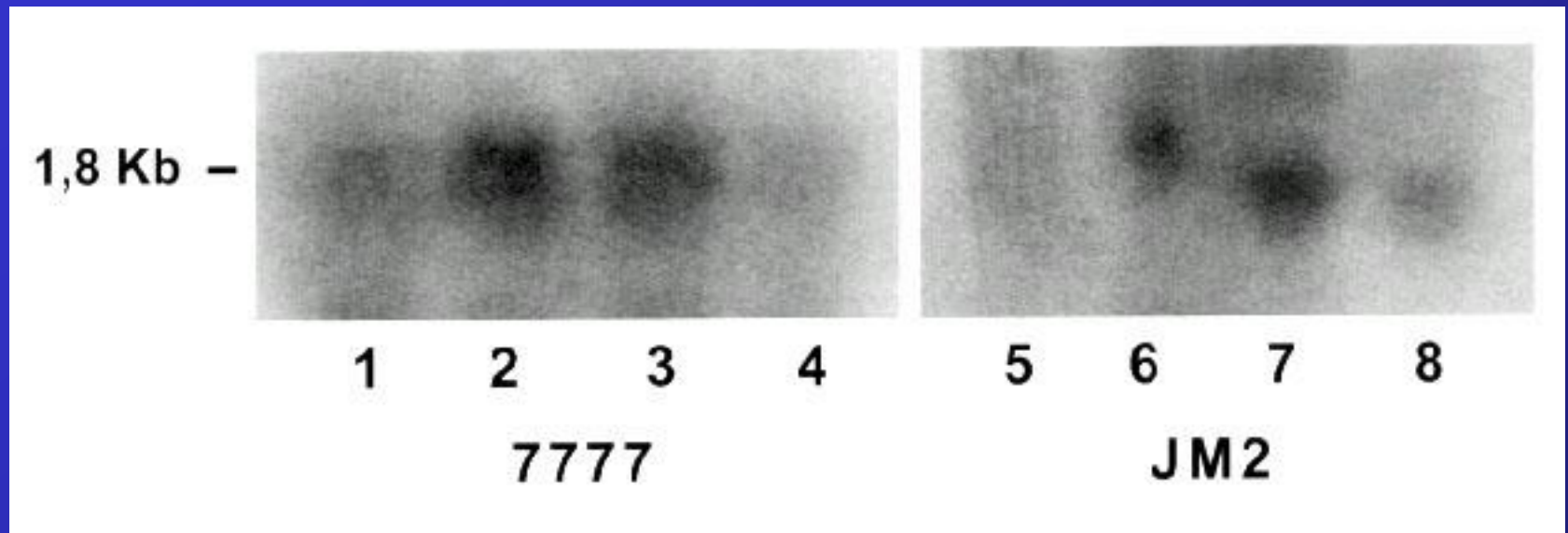
- 1) INCREASED EXPRESSION OF PPAR α OR PPAR γ



2) INCREASED LIPID PEROXIDATION



3) REVERTED TUMOR PHENOTYPE OF CLASS 2 ALDEHYDE DEHYDROGENASE



Lanes 1 and 5, untreated cells; lanes 2 and 4, cells treated with arachidonic acid (250 nmoles/10⁶ cells); lanes 3 and 6, cells treated with arachidonic acid (400 nmoles/10⁶ cells); lanes 4 and 8, cells treated with arachidonic acid (600 nmoles/10⁶ cells)

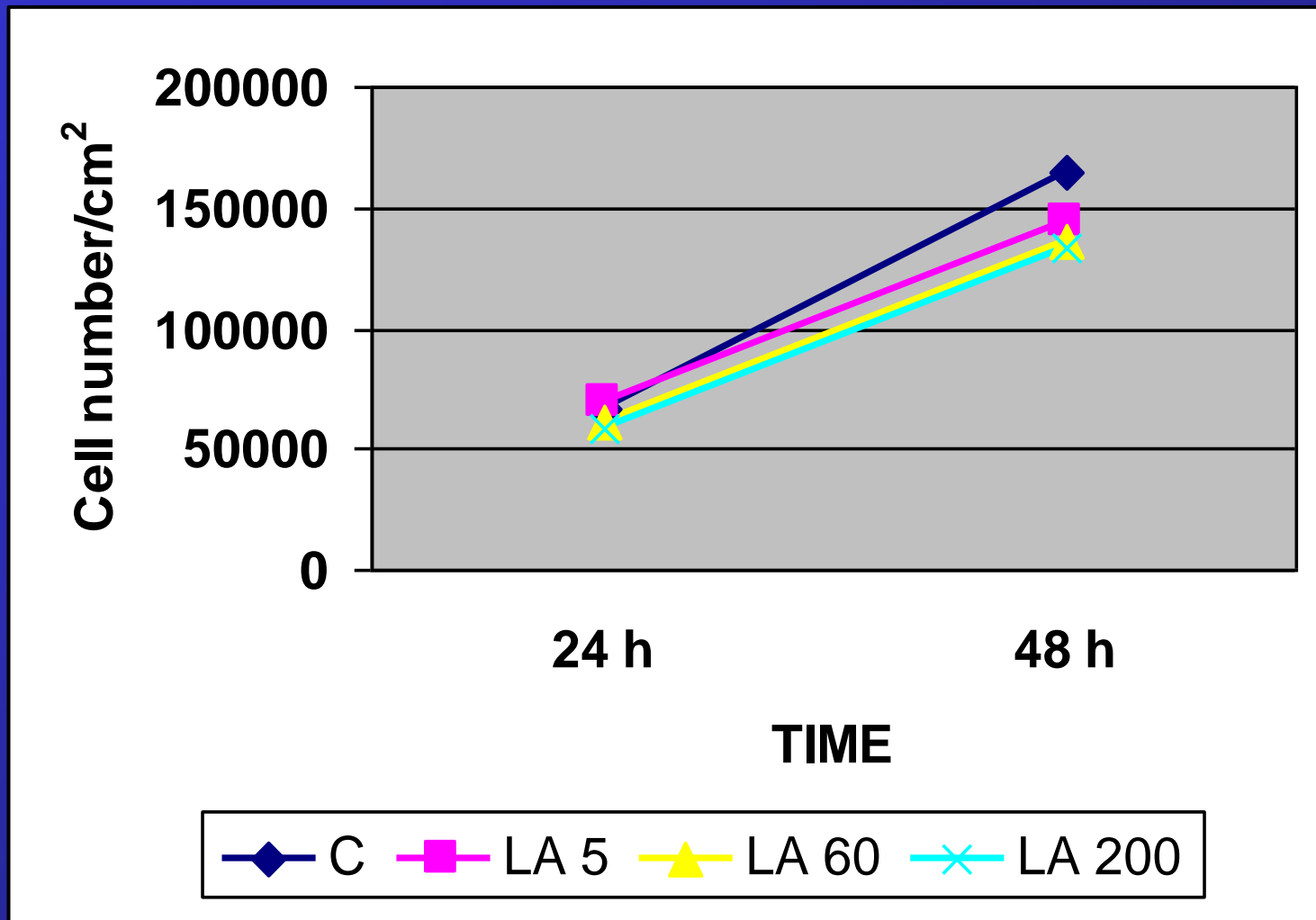
Aldehyde dehydrogenase activity in mitochondria isolated from hepatoma cell lines enriched or not with arachidonic acid

CELLS	DAYS AFTER ARACHIDONIC ENRICHMENT		
	2	3	4
7777	0.57 ± 0.11a	0.93 ± 0.19b	0.60 ± 0.21ab
7777 + ARA	1.91 ± 0.09c	2.82 ± 0.21d	2.65 ± 0.41d
JM2	2.18 ± 0.48a	2.13 ± 0.26a	1.65 ± 0.29a
JM2 + ARA	4.60 ± 0.31b	5.79 ± 0.31c	4.23 ± 0.24b

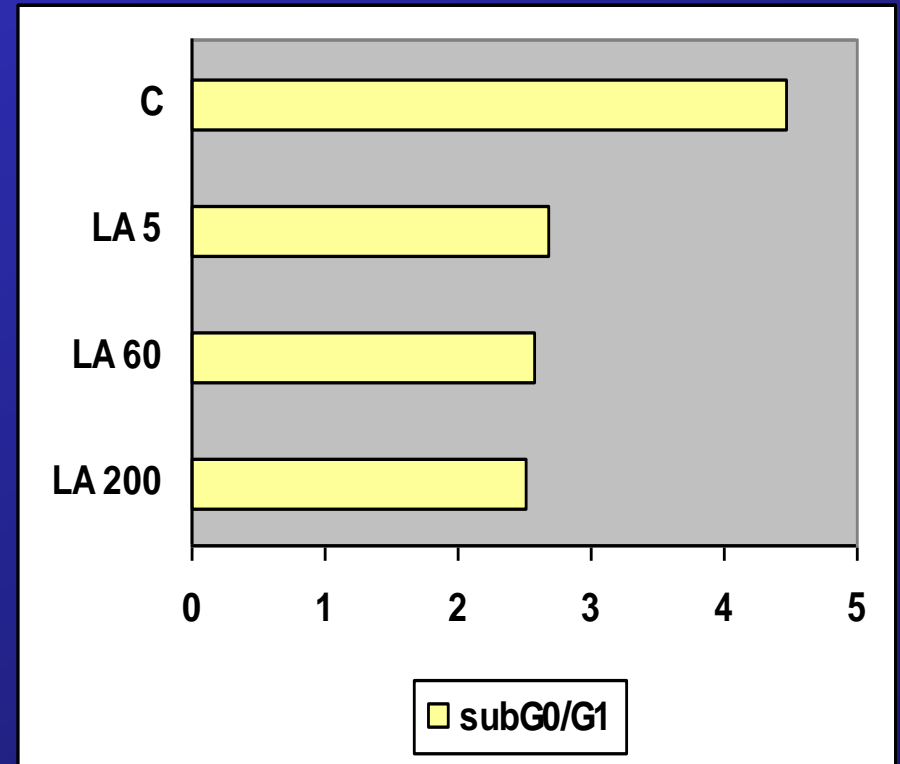
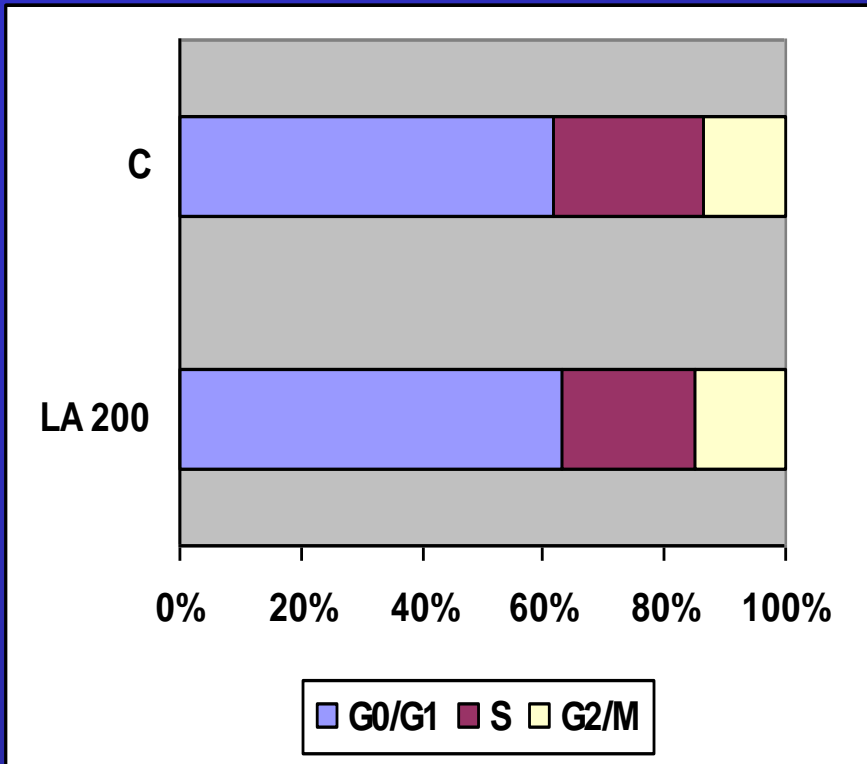
ARA, arachidonic acid (400 nmoles/10⁶ cells)

Data are expressed as nmoles of NADH produced/min/mg of protein

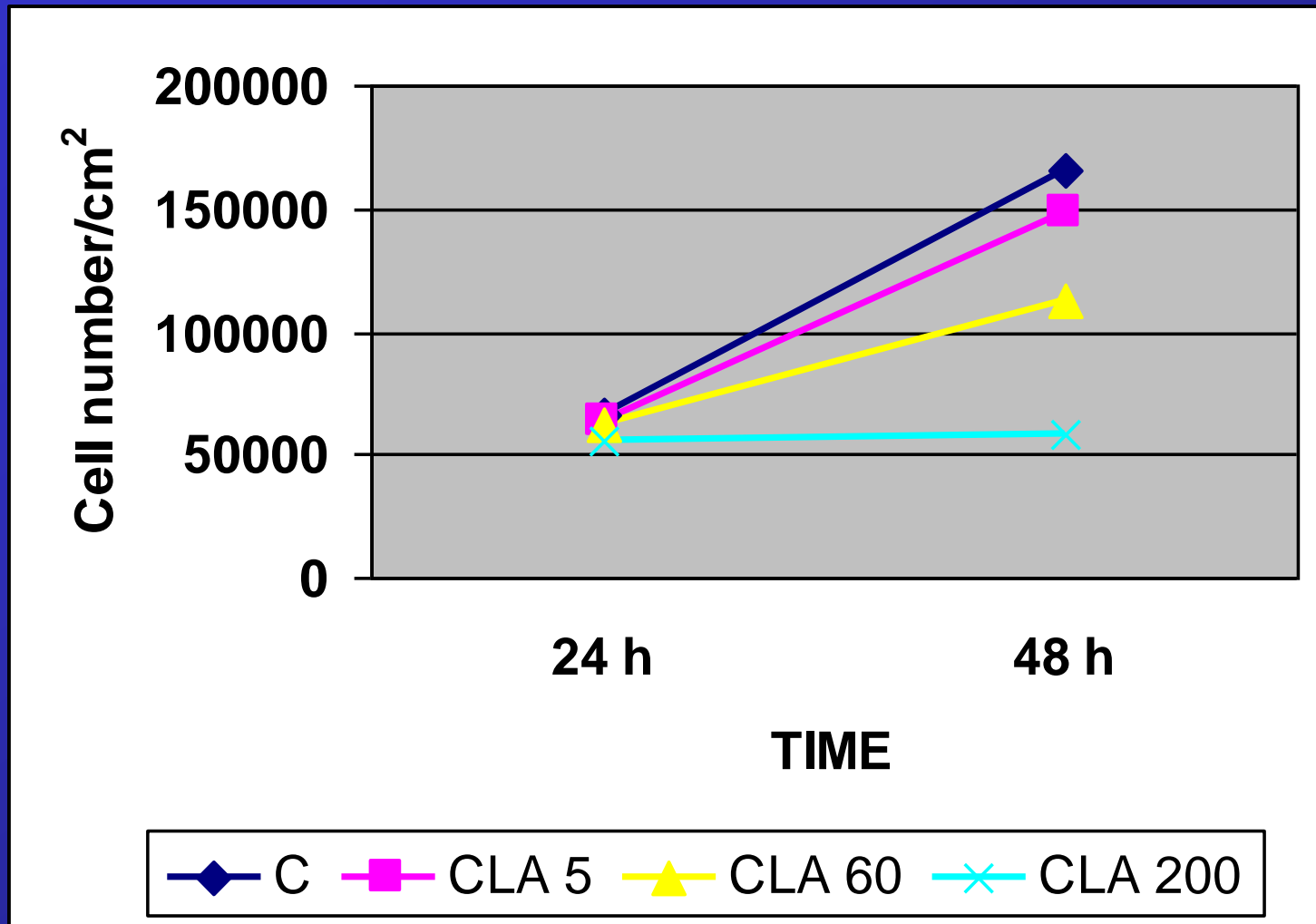
EFFECT LINOLEIC ACID (μM) ON GROWTH OF HepG2 HUMAN HEPATOMA CELL LINE



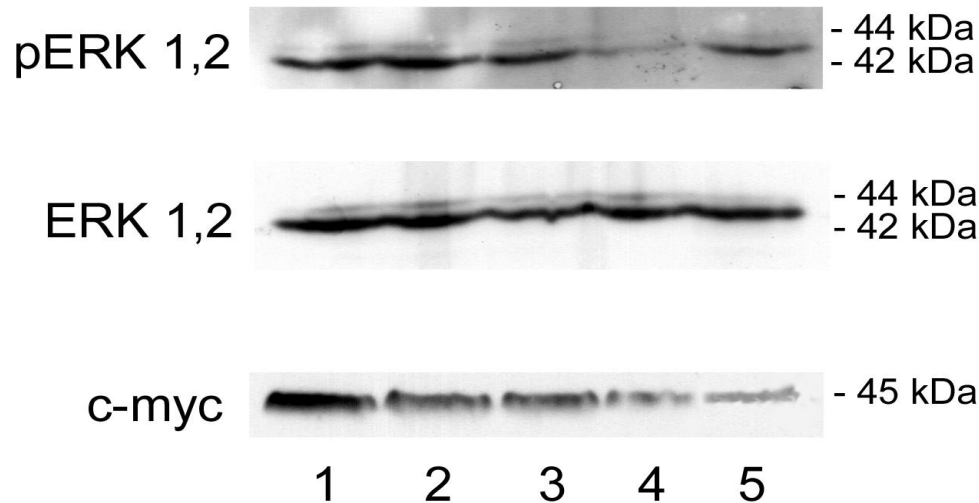
EFFECT OF LINOLEIC ACID (μM) ON DNA DISTRIBUTION AND APOPTOSIS IN HUMAN HepG2 HEPATOMA CELL LINE



EFFECT CONJUGATED LINOLEIC ACID (μM) ON GROWTH OF HepG2 HUMAN HEPATOMA CELL LINE

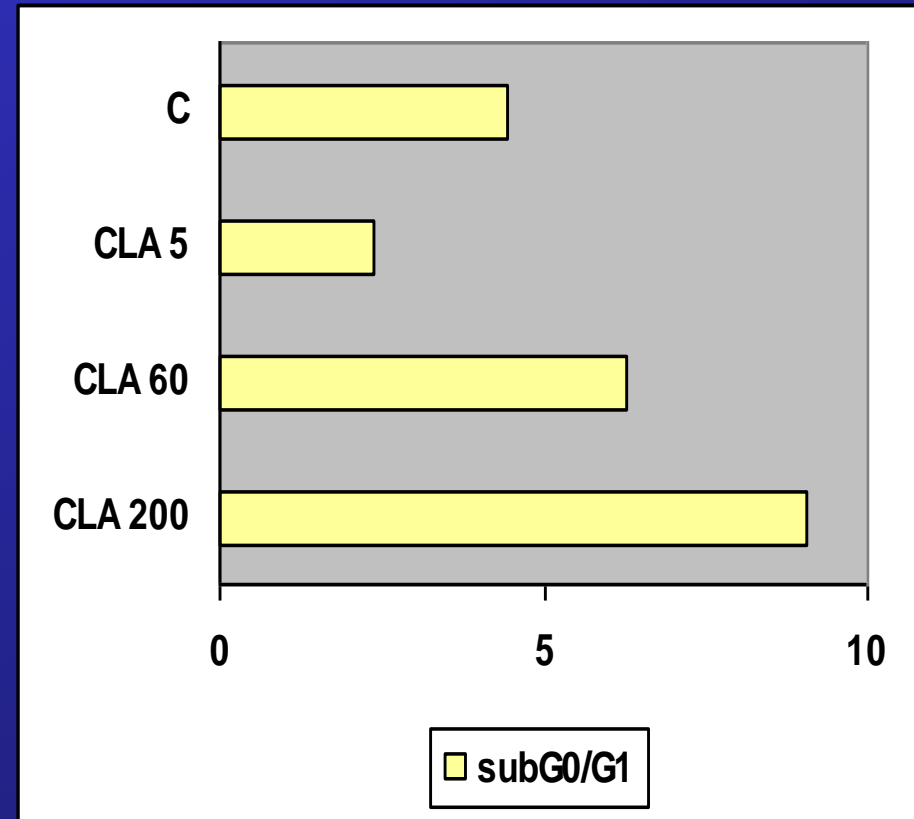
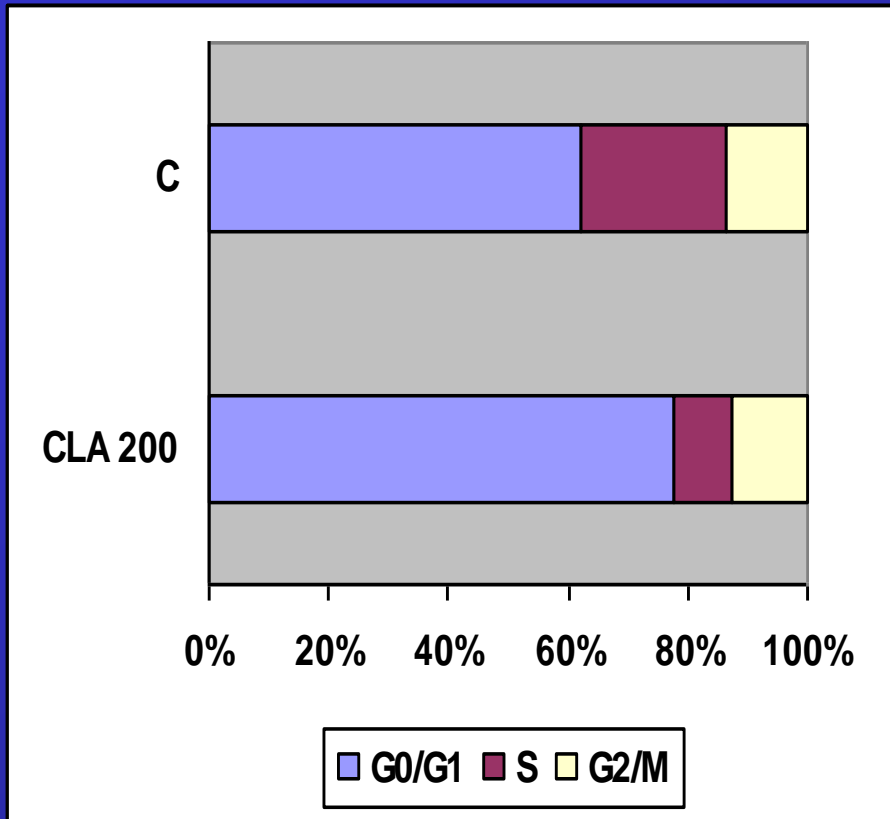


EFFECT OF PUFAs ON PROTEIN CONTENT OF ERK, pERK and c-myc in HUMAN HepG2 HEPATOMA CELL LINE

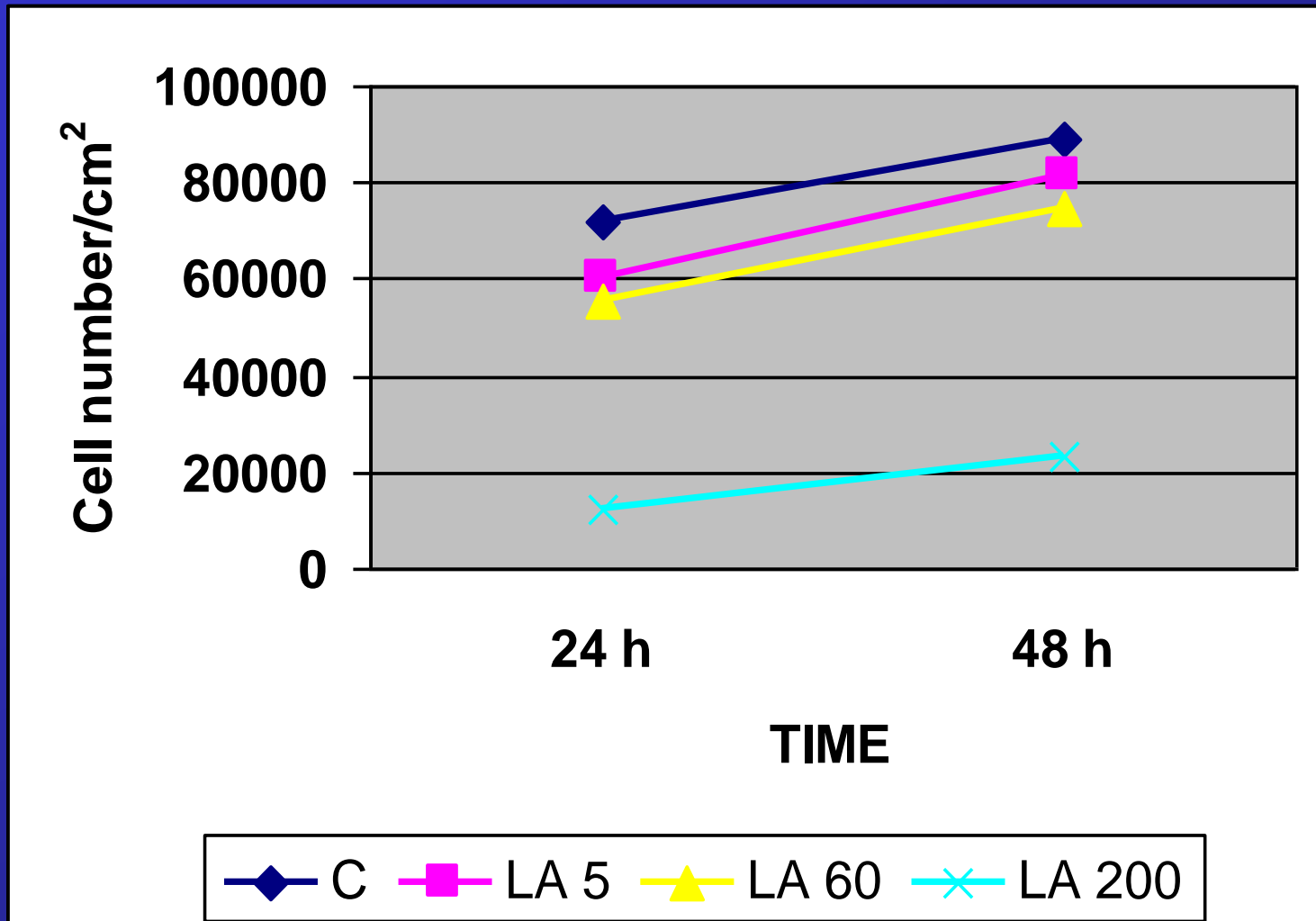


lane 1, control cells; lane 2, 60 μ M LA; lane 3, 200 μ M LA;
lane 4, 60 μ M CLA; lane 5, 200 μ M CLA
LA, linoleic acid; CLA, conjugated linoleic acid

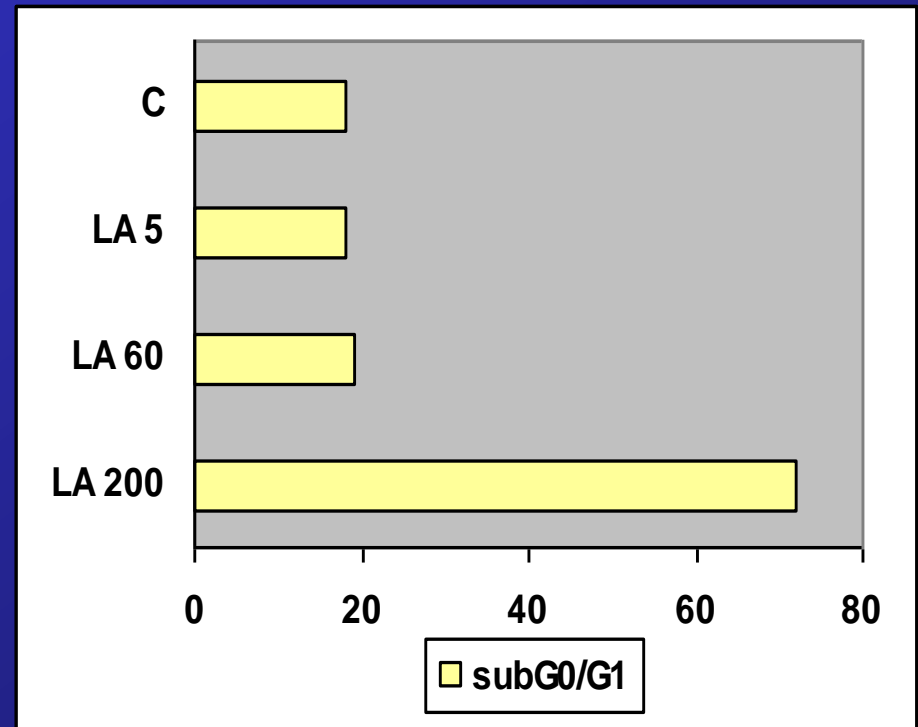
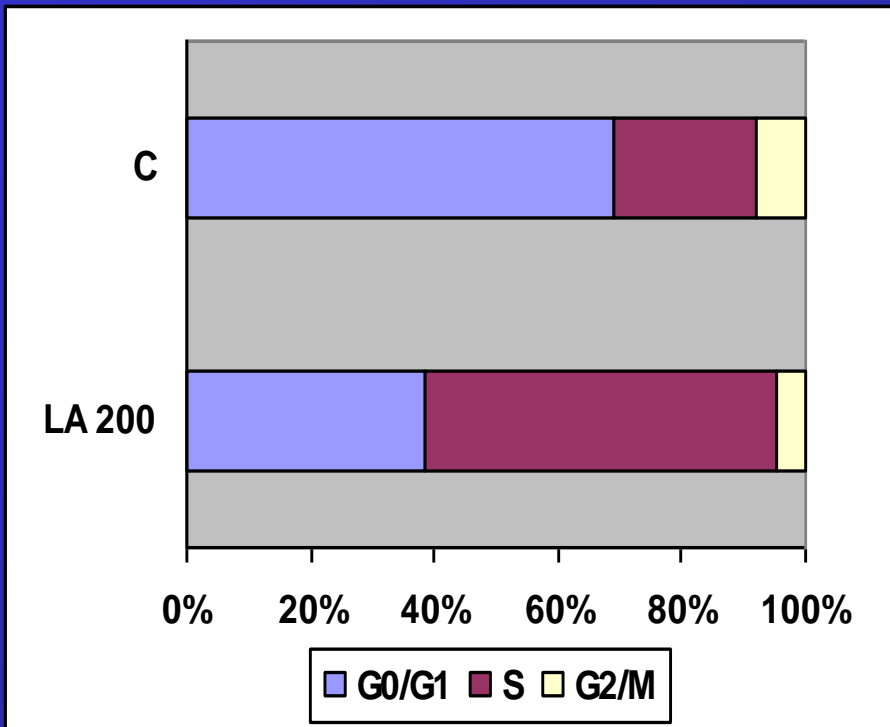
EFFECT OF CONJUGATED LINOLEIC ACID (μM) ON DNA DISTRIBUTION AND APOPTOSIS IN HUMAN HepG2 HEPATOMA CELL LINE



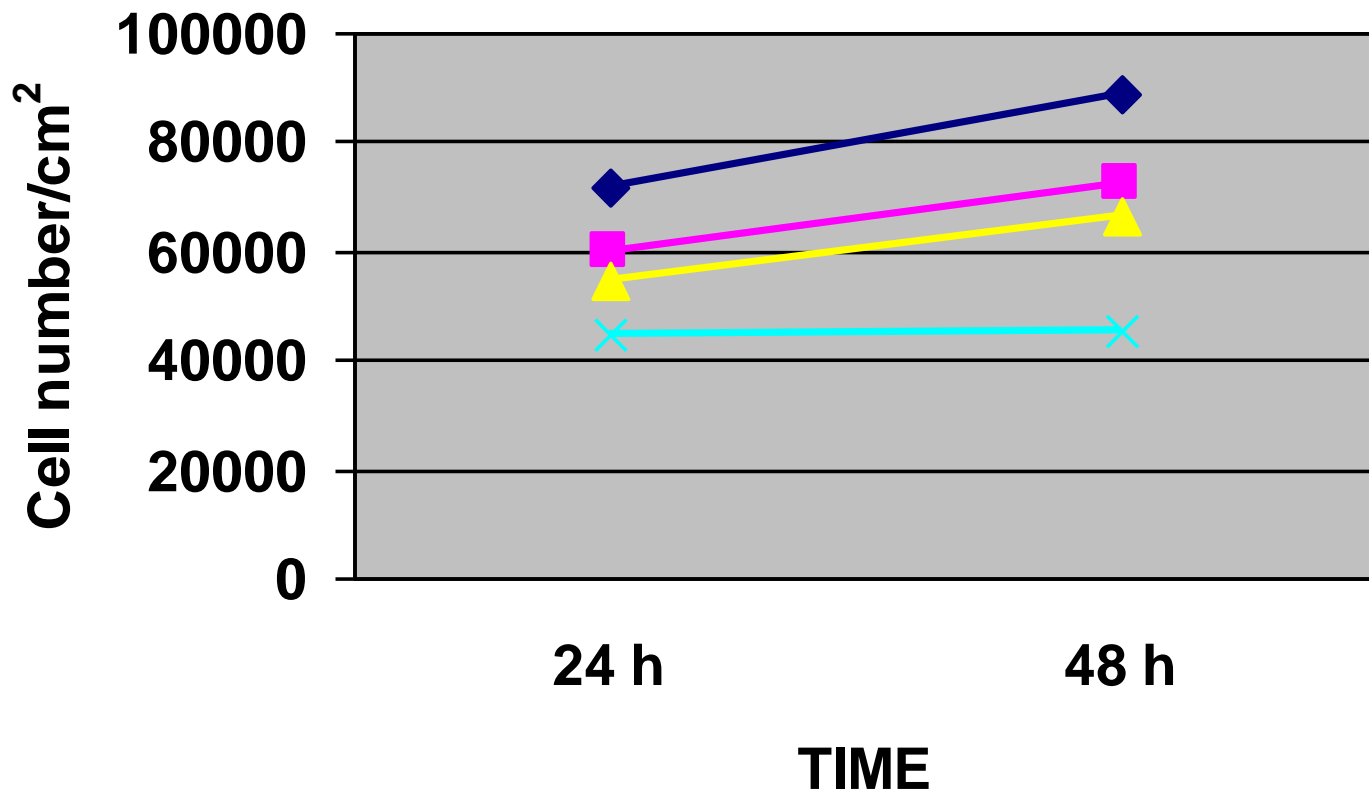
EFFECT OF LINOLEIC ACID (μM) ON GROWTH OF HUMAN SK-Hep-1 HEPATOMA CELL LINE



EFFECT OF LINOLEIC ACID (μM) ON DNA DISTRIBUTION AND APOPTOSIS IN HUMAN SK-Hep-1 HEPATOMA CELL LINE

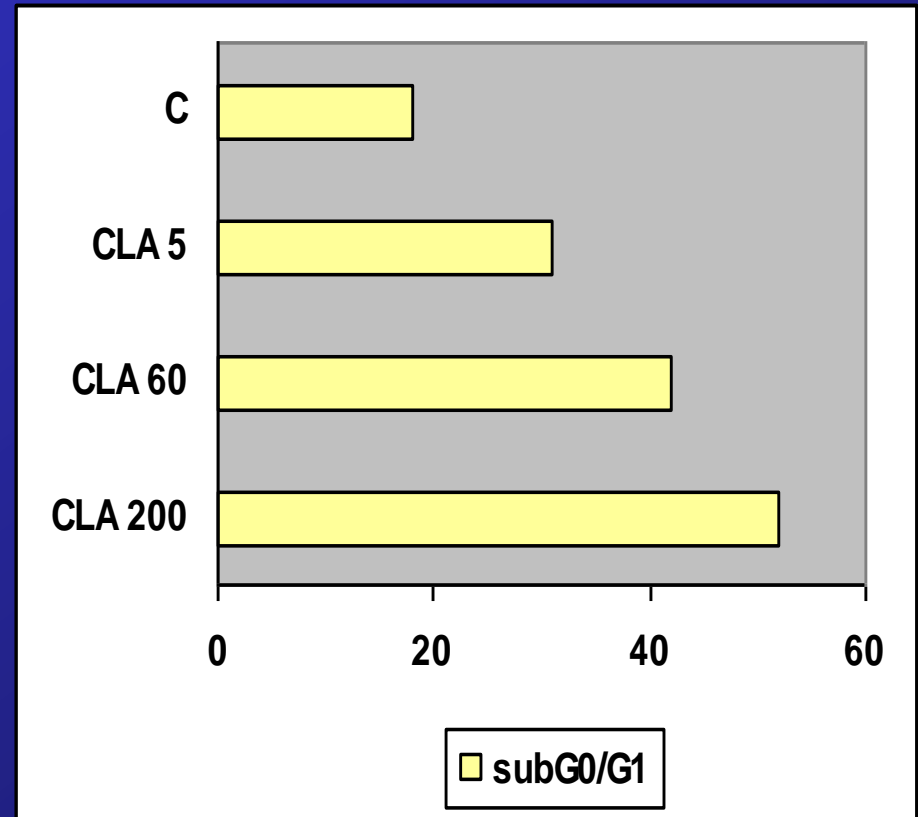
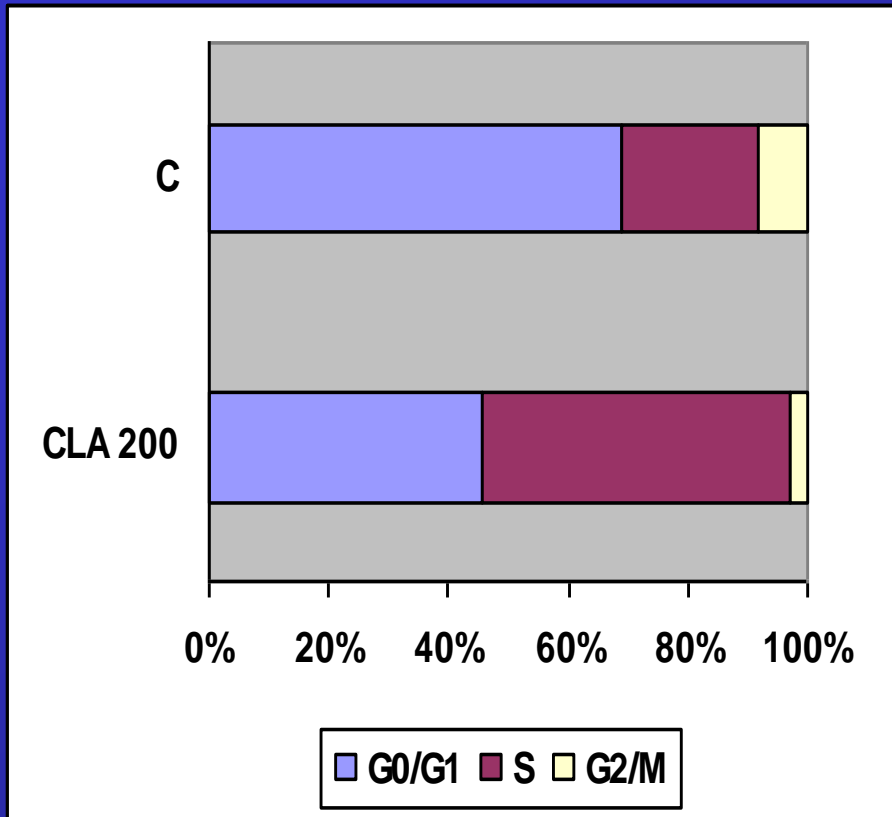


EFFECT OF CONJUGATED LINOLEIC ACID (μM) ON GROWTH OF HUMAN SK-Hep-1 HEPATOMA CELL LINE

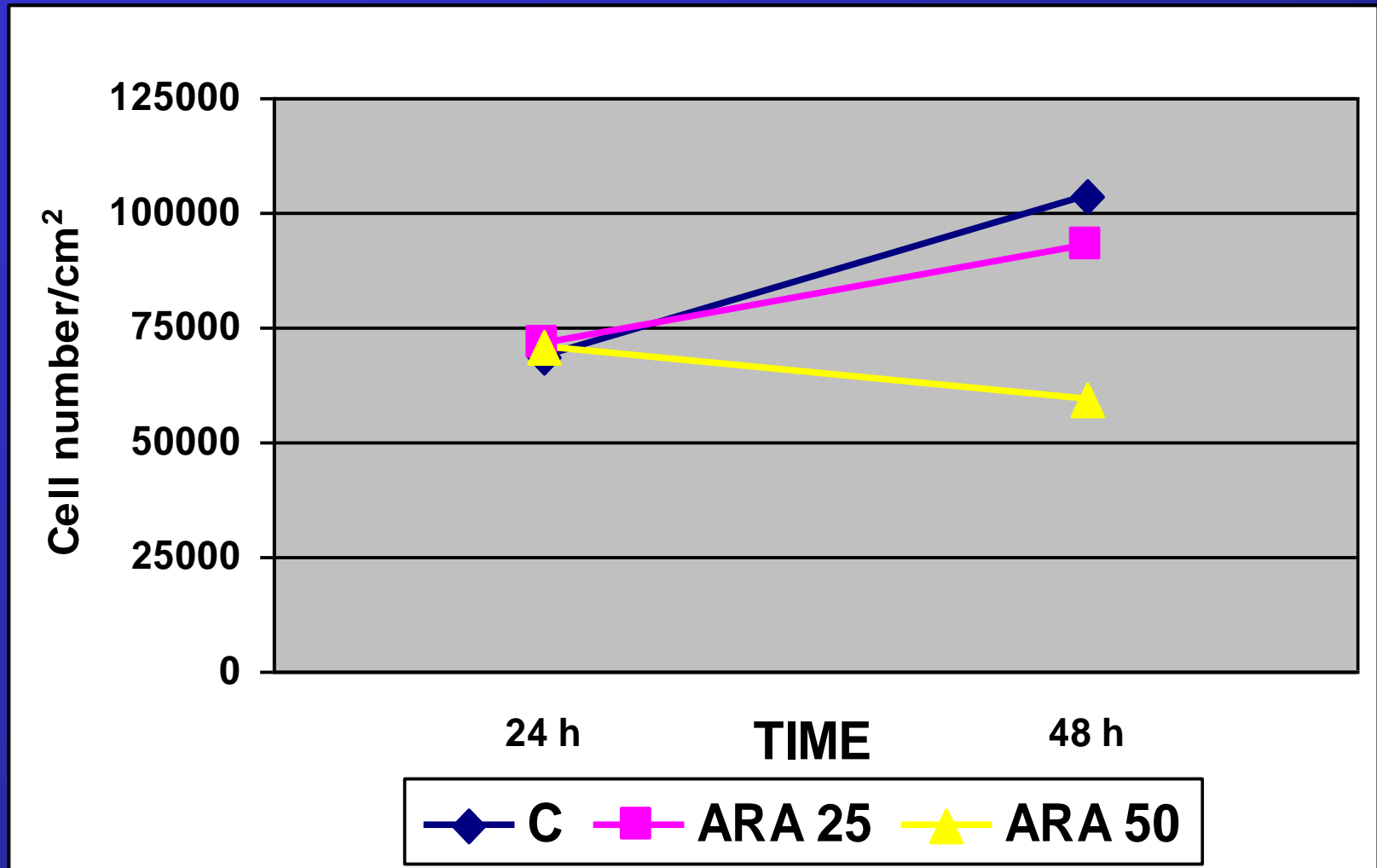


—◆— C —■— CLA 5 —▲— CLA 60 —×— CLA 200

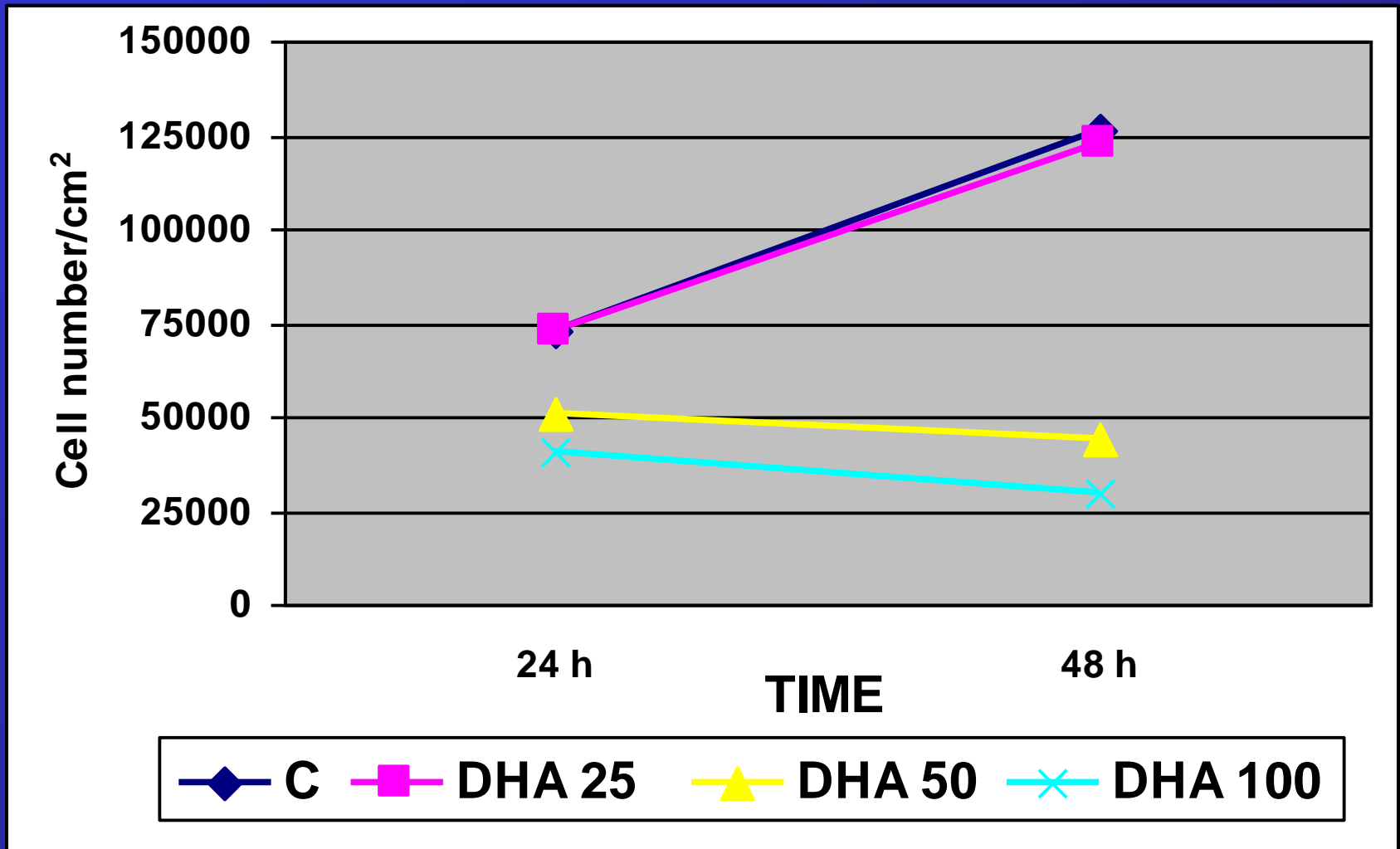
EFFECT OF CONJUGATED LINOLEIC ACID (μM) ON DNA DISTRIBUTION AND APOPTOSIS IN HUMAN SK-Hep-1 HEPATOMA CELL LINE



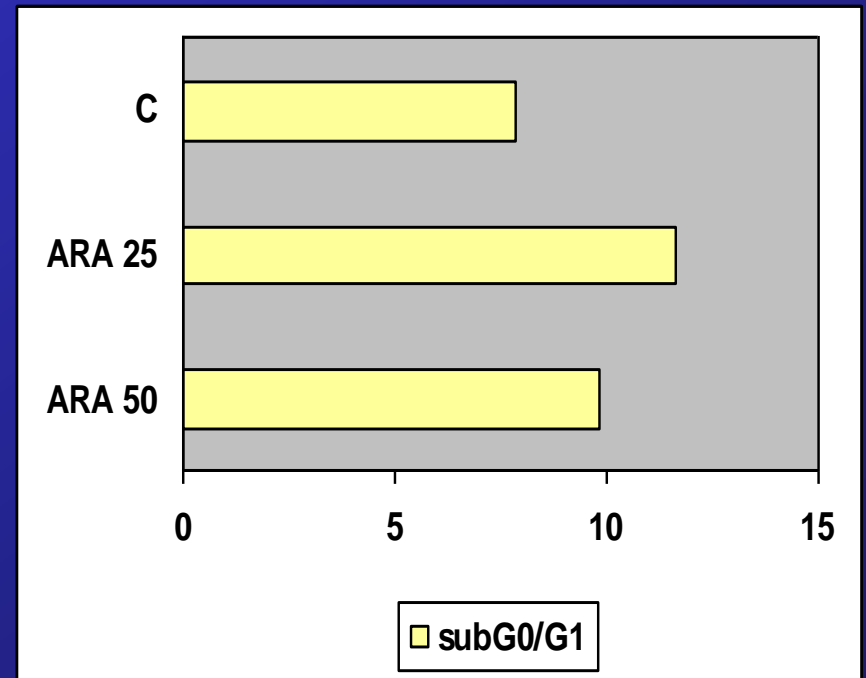
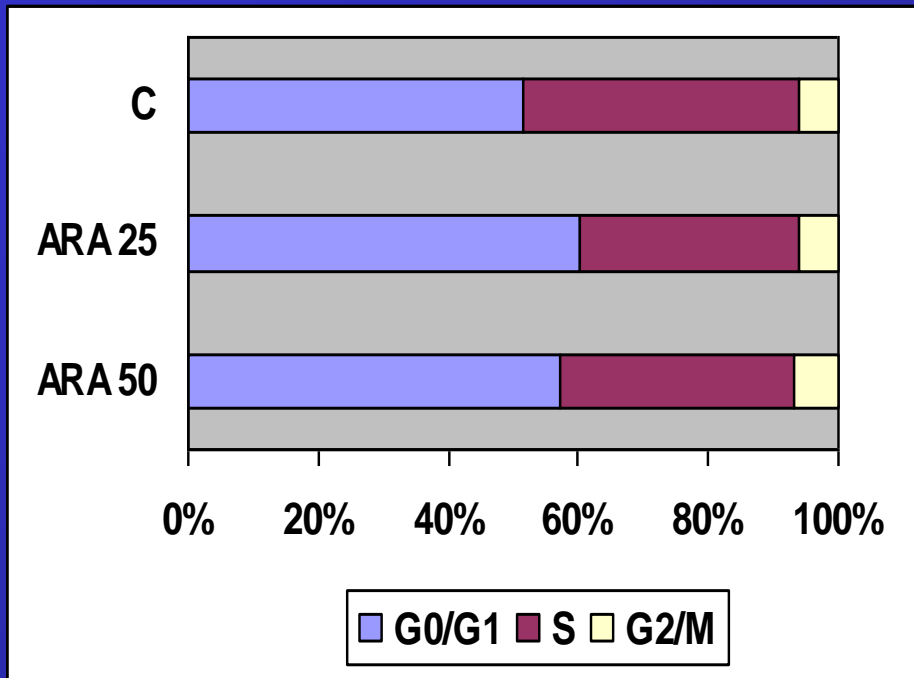
EFFECT OF ARACHIDONIC ACID (μM) ON GROWTH OF HUMAN A549 LUNG TUMOR CELL LINE



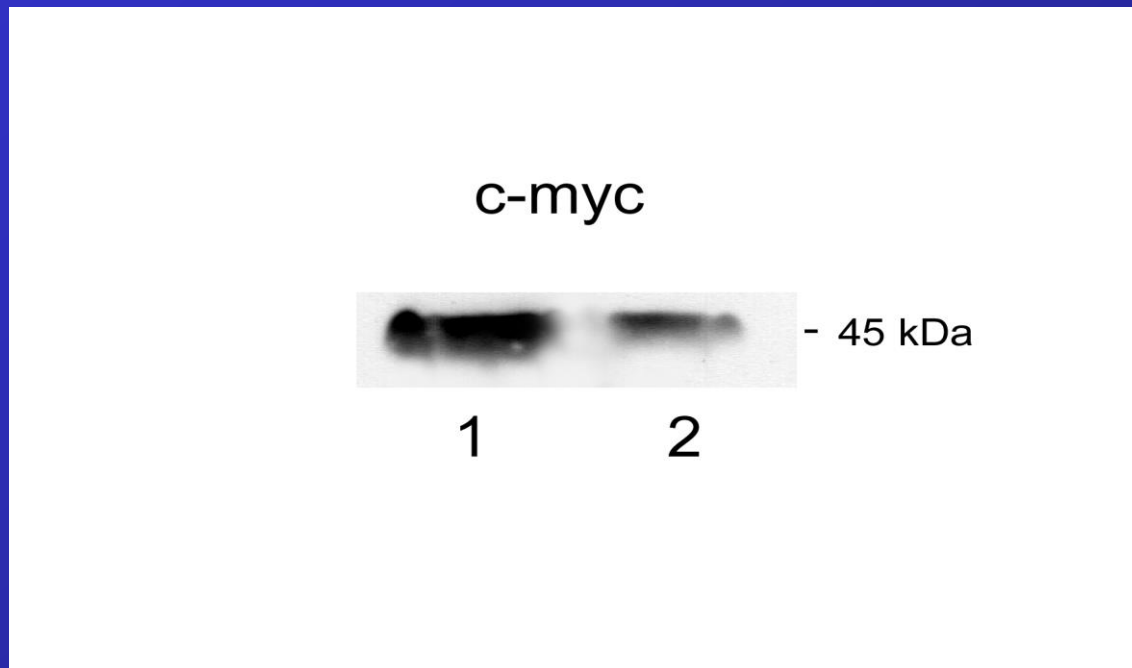
EFFECT OF DOCOSAHEXAENOIC ACID (μM) ON GROWTH OF HUMAN A549 LUNG TUMOR CELL LINE



EFFECT OF ARACHIDONIC ACID (μM) ON DNA DISTRIBUTION AND APOPTOSIS IN HUMAN A549 LUNG TUMOR CELL LINE



EFFECT OF PUFA ON PROTEIN CONTENT OF c-myc IN HUMAN A549 LUNG TUMOR CELL LINE



lane 1, control cells; lane 2, 50 μ M arachidonic acid

CONCLUSIONS

- ✓ **BOTH ω -3 AND ω -6 PUFAs AFFECT TUMOR CELL PROLIFERATION OR VIABILITY IN A DOSE-DEPENDENT MANNER**
- ✓ **IN HEPATOMA CELL LINES THE TYPE OF EFFECT IS PROBABLY RELATED TO THE DEGREE OF MALIGNANCY**
 - **IN WELL DIFFERENTIATED, NON-TUMORIGENIC HepG2 CELLS PUFAs MAINLY DECREASE CELL PROLIFERATION WITHOUT AFFECTING CELL VIABILITY**
 - **IN TUMORIGENIC SK-Hep-1 CELLS PUFAs INDUCE APOPTOSIS**

✓ **THE DECREASE OF CELL PROLIFERATION IN HEPATOMA TUMOR CELLS IS:**

- **COUPLED WITH AN INCREASED PERCENTAGE CONTENT OF CELLS IN G0/G1 PHASE**
- **MEDIATED BY THE DECREASE OF pERK1,2 AND c-myc CONTENT**

✓ **IN WELL DIFFERENTIATED HUMAN A549 LUNG TUMOR CELL LINE PUFAs DECREASE CELL PROLIFERATION WITHOUT AFFECTING CELL VIABILITY**

- **THIS EFFECT IS MEDIATED BY THE DECREASE OF c-myc CONTENT**

TO BE VERIFIED:

- **IS CITOSTATIC EFFECT OF PUFAs IN WELL DIFFERENTIATED HEPATOMA AND LUNG TUMOR CELLS MEDIATED BY :**
 - **CHANGES IN PUFA CONTENT IN PHOSPHOLIPID CLASSES OF MEMBRANES?**

OR BY

- **CHANGES IN THE PRODUCTION OF DERIVATIVES OF ARACHIDONIC ACID (PROSTAGLANDINS, LEUCOTRIENES)**

OR BY

- **DIRECT INTERACTION OF PUFAs WITH PPARs**

➤ **IS APOPTOSIS INDUCED BY PUFAs IN TUMORIGENIC HEPATOMA CELLS MEDIATED BY:**

- **CHANGES IN THEIR CONTENT IN PHOSPHOLIPID CLASSES OF MEMBRANES?**

OR BY

- **INCREASED CELLULAR CONTENT OF CERAMIDE?**