

# Non-alcoholic beverages, unknown influence on cell proliferation – an *in vitro* study

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## Abstract

**Introduction and objective.** The aim of the presented study was to check differences between 'Diet' and 'non-Diet' soft drinks on cell proliferation.

**Materials and methods.** Coca Cola and Pepsi Cola of different origin and their dietetic versions were examined at concentrations of 2% and 4%. Fructose and glucose as well as medium alone (control) were examined.

**Results.** Cell number was higher in media supplemented with soft drinks, compared to control. Proliferation depended on the soft drink concentration and its origin, but not on sugar and calorific content.

**Conclusions.** An unknown factor is responsible for the increase in proliferation.

## Keywords

artificial sweeteners, cell proliferation, fibroblasts, obesity, soft drinks.

## INTRODUCTION

Soft drinks (non-alcoholic drinks) are a significant part of diet. High sweeteners contents are mainly responsible for the side-effects of soft drink consumption [1, 2]. Soft drinks might be the source of mitogenic unknown agents. The contents of soft drinks are usually hidden and protected by law; therefore, unknown substances might have an impact on cell proliferation. If this assumption is correct, an excessive intake of soft drinks could increase obesity, type 2 diabetes, atherosclerosis, metabolic syndrome, liver fibrosis, as well as cancer and angiogenesis [3, 4].

The impact of selected sweetened and unsweetened carbonated soft drinks on the proliferation of mesenchymal cells were examined, with 3T3NIH fibroblasts serving as the model.

## MATERIALS AND METHOD

Fibroblasts were cultured in a density of 8,000 cells/cm<sup>2</sup> in DMEM/Ham's F-12 medium, supplemented with 10% Fetal Bovine Serum at 37°C and 5%CO<sub>2</sub>. Coca Cola (USA, Egypt, Mexico, Poland, Canada), Coca Cola Diet (USA), Coca Cola 'Zero' (USA), Pepsi Cola (USA) and Pepsi Cola 'Light' (USA) were diluted in medium at 2% and 4%, decarbonated, and used at pH 7.4. Cells were also incubated with fructose and glucose (1, 2, 4 and 10mg/ml). 2 and 4mg/ml corresponding to 2 and 4% concentrations, respectively. Higher concentrations of the tested soft drinks decreased the pH level, which resulted in a significant acidification of the culture medium even in the presence of buffers. This was caused by the relatively high content of phosphoric acid added to soft drinks. Sugar content should also be within norms for normal cell culture medium. The 2% and 4% concentrations are the most appropriate for evaluating the influence of the tested beverages directly onto

3T3 NIH fibroblast growth, with a good balance between the medium and tested chemical agents. Cells were incubated for 12h in beverages or sugar enriched media, and then in fresh medium for 24h, and finally counted using trypan blue assay. Fresh medium was used as the control. Each experiment was repeated four times. Results were shown as means (±SD, standard deviation). The statistical comparison between culture media and between each concentration was made by paired t-test; P<0.05 was considered significant.

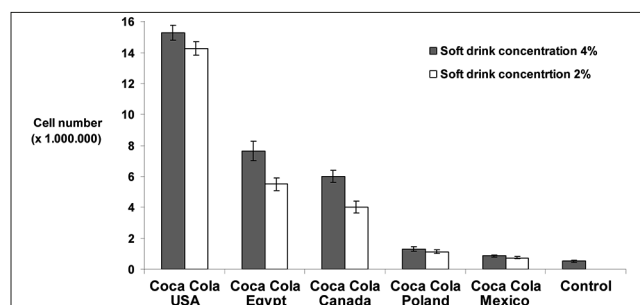
## RESULTS

The number of cells was higher when cultured in media enriched with soft drinks than in the control group (P<0.05) (Fig. 1; Fig. 2), and depended on the soft drink concentration (P=0.01; α=0.05) and soft drink origin (P<0.05). The fibroblast proliferation rate was the highest in medium enriched with Coca Cola (USA), while the lowest in medium enriched with Coca Cola (Mexico) (Fig. 1). No significant differences were observed when cells were cultured with glucose or fructose. Cell number was (x100,000) 8.63±0.16 (P=0.96), 8.02±0.11 (P=0.23), 8.09±0.14 (P=0.33), 8.95±0.09 (P=0.88) for fructose, and 9.08±0.20 (P=0.007), 8.88±0.09 (P=0.56), 10.13±0.10 (P=0.48), 9.87±0.10 (P=0.16) for glucose, respectively, for 1, 2, 4 and 10mg/ml concentrations.

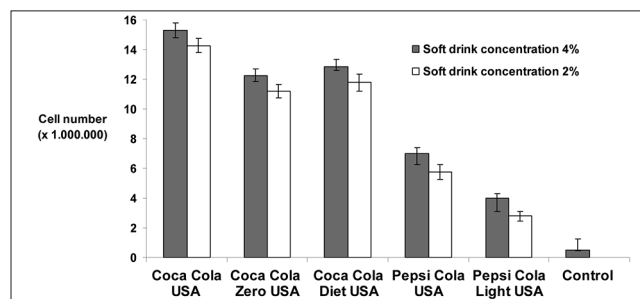
Coca Cola (USA) resulted in the most intensive proliferation (Fig. 2). In contrast, the lowest number of cells among the American drinks was noticed in medium enriched with Pepsi Cola 'Light' (Fig. 2). The cell number was higher in all cultures with sugar-sweetened soft drinks in the Coca Cola and Pepsi Cola groups, respectively (P<0.05), and was significantly higher in the culture supplemented with Coca Cola than in culture supplemented with Coca Cola 'Diet' and Coca Cola 'Zero' (P=0.002; P=0.002; α=0.05) (Fig. 2). The cell number was higher in culture supplemented with Pepsi Cola than with Pepsi Cola 'Light' (P=0.001; α=0.05) (Fig. 2), although cell proliferation was quicker in Coca Cola 'Diet' and 'Zero' than in normal Pepsi Cola.

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**Figure 1.** 3T3 cells in medium enriched with Coca Cola from different regions at concentrations of 2% and 4%



**Figure 2.** 3T3 cells in medium enriched with Coca Cola, Coca Cola "Zero", Coca Cola "Diet", Pepsi Cola and Pepsi Cola "Light" from USA at the concentration of 2% and 4%

## DISCUSSION

The differences in cell proliferation depend on the origin of the soft drink. Fibroblasts proliferated the most intensively in media enriched with Coca Cola originating from the USA, whereas in medium enriched with Mexican Cola, the cell number was the lowest and similar to the control group. In Mexico, the use of High Fructose Corn Syrup (HFCS) is forbidden. In Poland, HFCS is not used in such a high dose as in the USA. It seems that fructose could be one of the elements responsible for cell proliferation.

The tested soft drinks contain sucrose and HFCS in different concentrations and proportions. HFCS is the most controversial because it is suspected of leading to metabolic syndrome [5, 6, 7]. It is well known that in the USA, HFCS replaces sugar in processed food as well as in soft drinks, whereas such a wide-scale replacement of sucrose has not occurred in the countries of the European Union. HFCS and fructose supplementation, according to *in vitro* studies, stimulate fibroblast and other cell proliferation also *in vivo* [8, 9, 10, 11]. On the other hand, the presented study shows that the influence of pure fructose and glucose on the proliferation is similar.

It is hypothesized that this may be a consequence of the different calorific content or the type of sweetener. To investigate this phenomenon, sugar free Coca Cola 'Zero' and 'Diet', and similar soft drinks such as Pepsi Cola and Pepsi Cola 'Light' were checked. The results of the second part of the study showed that sugar-free Coca Cola (USA) differed only slightly from one that was sugar-sweetened in mitogenic activity on fibroblasts. Initially, it was suspected that the high proliferation fibroblasts rate, cultured in media with Coca Cola (USA) and Pepsi Cola (USA), was a consequence of the high calorific level in soft drinks with sugar. The medium calorific level is reflected in the cell proliferation rate. Coca Cola and Pepsi Cola have 97kcal/240ml and 100kcal/240ml, and 27mg and 23mg of carbohydrates, respectively. This implies that the mentioned soft drinks should stimulate cells proliferation in a similar manner; however, no such coincidence was observed.

In the presented study, sugar-free soft drinks cultured in media and labeled as 'Light', 'Diet' or 'Zero' were characterized by high mitogenic activity (Coca Cola), similar to sugar-sweetened ones, not commensurate with the calorific level. Coca Cola 'Diet' and 'Zero' have 1.0 and 0.7kcal/240ml, Pepsi Cola 'Light' has no calories at all; therefore, the cell number in these cultures should be similar to the control. This observation is very interesting and suggests that actual lists of ingredients can vary, or that some ingredients may contain additional substances not mentioned on the labels.

The results of the presented study did not indicate the real factors for 3T3 cell proliferation. To answer this question, analysis of the pure active compounds or chemical classes of Cola should be undertaken. However, the production process of these soft drinks is protected. The essential differences discussed above apply to the concentration of HFCS and sucrose, although the tested sugar-free soft drinks do not contain these substances. Thus, the mitogenic activity of soft drinks may be the consequence of unknown factors.

Unfortunately, there is no information about the influence of soft drinks on cell growth *in vitro*, which is why no comparison can be made with the obtained results. The influence of high-glucose concentrations on cells in culture with regard to diabetes showed a decrease in human aortic endothelial cell viability, and an increase in human retinal endothelial cell viability [12]. In paper presented by Duffy et al., high-glucose conditions increased cell proliferation of bone-marrow mesenchymal stem cells [13]. The presented results show that sugar content in the tested soft drinks was not responsible for high cell proliferation. However, this is still a very interesting result which deserves more experimental work.

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