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## Vitamin E Prevents Lipogenesis Dysregulation in the Liver Cells at Old Age

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No competing interests.

# Vitamin E Prevents Lipogenesis Dysregulation in the Liver Cells at Old Age

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## Abstract

Thyroid hormones play critical roles in lipogenesis regulation. Genomic dependent and genomic independent effects of triiodothyronine on free fatty acids (FFA) synthesis have been determined. The lipogenesis regulation with L-thyroxine (T4) in the liver cells of 3- and 24-month-old rats and effects of vitamin E (?-tocopherol acetate) or N-acetylcystein (NAC) on old liver response to hormone action have been investigated. T4 in both experiments in vivo and in vitro increased lipogenesis in liver cells of young rats, while old hepatocytes were resistant to the hormone action. Vitamin E as well as N-acetylcystein (NAC) increased significantly the liver and hepatocytes sensitivity to the T4 action at old age. However, the vitamin E-dependent induction of FFA synthesis with T4 was not followed by FFA accumulation. The newly synthesized FFA were used for cholesterol and neutral lipids synthesis in old liver cells treated with T4 plus vitamin E or NAC. The data obtained have demonstrated for the first time that the age-dependent lipogenesis dysregulation depends on redox state changes in liver. Vitamin E improved both the long-term and the short-term genomic independent thyroid hormone lipogenesis regulation at old age.

## Introduction

Obesity and associated disorders are the major public health problem. Elevated lipogenesis and subsequent lipid accumulation were demonstrated in several animal models of obesity and insulin-independent diabetes mellitus (Levadovvski et al., 1998). Increased lipogenesis promotes development of human hepatocarcinoma (Calvisi et al., 2011) and nonalcoholic fatty liver disease (NAFLD) (Fabbrini et al., 2008). Elevated peripheral free fatty acids (FFA) and de novo lipogenesis predominantly contribute to the accumulation of lipids in the liver at NAFLD. Inhibition of triacylglycerol (TAG) synthesis via diacylglycerol acyltransferase 2 (DGAT2) antisense oligonucleotides improves liver steatosis (Yamaguchi et al., 2007). Suppression of the genes, which are involved in lipogenesis (ATP-citrate lyase (ACL), acetyl-CoA carboxylase (ACC), fatty acid synthase

(FAS), stearoyl-CoA-desaturase 1 or sterol regulatory element-binding protein 1(SREBP-1)), reduced proliferation and survival of hepatic carcinoma cells (Calvisi et al., 2011).

Senescence-accelerated prone mice exhibit higher expression of FAS, hepatic steatosis with increased hepatic cholesterol (CH) content, plasma TAG, and aspartate aminotransferase levels (Kuhla et al., 2011). Over-expression of the FAS, ACC, ACL and SREBP-1 was determined in both the senescent immortalized normal human hepatocytes (Chang cells) and liver of 24-month-old Fisher 344 rats (Kim et al., 2010). Blocking of lipogenesis with siRNA or FAS-specific inhibitors cerulenin and C75 attenuates the cell senescence. However, fatty acid synthesis, which was assessed by measuring the activities of FAS, ACC, ACL and "malic" enzyme in the liver and adipose tissues, significantly decreased in the 27-month-old Fisher 344 rats (Barakat et al., 1989) and 20-month-old Wistar rats (Nogalska and Swierczynski, 2001). It has been concluded that hormonal dysregulation at old age plays important role in the age-dependent lipogenesis decline (Nogalska and Swierczynski, 2001; Moorodian and Albert, 1999; Fukuda and Iritani, 1992).

Thyroid hormones are well known stimulators of fatty acids, cholesterol and neutral lipids synthesis in the liver and adipose tissues regardless of animal age and dietetic status (Gnoni et al., 1980; Blennemann et al., 1995; Fukuda et al., 1992; Moorodian and Albert, 1999; Araki et al., 2009). However, lipogenic response to L-triiodothyronine (T3) in young rats was significantly greater than that found in aged rats (Moorodian and Albert, 1999). Authors concluded that resistance to thyroid hormone action found in aged animals may explain the reduced hepatic lipogenesis at old age.

Previously we reported that the age-dependent and palmitate-induced disturbances of sphingolipid turnover could be ameliorated in the liver by vitamin E treatments (Babenko et al., 2011). Vitamin E is a well-known chain-breaking antioxidant which protects cell membranes from peroxidative stress. Recently it has been demonstrated that vitamin E therapy is associated with a significant improvement in nonalcoholic steatohepatitis (Sanyal et al., 2010; Tilg and Moschen, 2010). Vitamin E prevented hepatic oxidative stress, toxicity and hyperproliferation in Wistar rats treated with a potent hepatic tumor

promoter ferric nitrilotriacetate (Agarwal et al., 2005). Tocotrienols, important plant vitamin E constituents, are effective suppressors of TAG and CH biosynthesis in human hepatocarcinoma cells (HepG2) and hypercholesterolemic mice (Zaiden et al., 2010). Gamma- and delta-tocotrienol suppressed the upstream regulators of lipid homeostasis genes (DGAT2, APOB100, SREBP-1/2, 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGCR)) and reduced CH, TAG and lipoproteins (VLDL) levels. We observed that L-thyroxin (T4) in both experiments in vivo and in vitro increased lipogenesis in liver cells of young rats, while old hepatocytes were resistant to the hormone action. Vitamin E as well as N-acetylcystein (NAC) increased significantly liver sensitivity to the T4 action at old age. However, vitamin E-dependent induction of FFA synthesis with T4 was not followed by FFA accumulation. The newly synthesized FFA were intensively used for CH and neutral lipids synthesis in old liver cells treated with T4 plus vitamin E/NAC. The data presented demonstrated for the first time that vitamin E improved thyroid hormone lipogenesis regulation at old age.

## Methods

### Animals:

The 3- and 24-month-old male Wistar rats were used in the experiments. They were kept at 24°C on a cycle of 12 h light/12 h darkness and had a free access to a standard chow diet and drinking water ad libitum. Experimental procedures were approved by the Institutional Animal Care and Use Committees at the Kharkov Karazin National University. The 24-month-old animals were divided into two groups. The animals were fed by vitamin E ( $\alpha$ -tocopherol acetate) (100 mg/kg body weight) (experimental group) or corn oil (control group) intragastrically daily for a week. 3- and 24-month-old rats, which had a free access to a standard chow diet, were injected intraperitoneally with 0.2 mg T4/kg body weight or equivalent volumes of hormone vehicle (0.1 N NaOH, controls). The animals were starved overnight prior to experiment. Livers were obtained 24 h after the last feeding of the rats by vitamin E or corn oil, or T4/0.1N NaOH. Livers of 3- and 24-month-old rats, which had a free access to a standard chow diet and drinking water ad libitum, were used for hepatocytes isolation as described below.

### Experiments with Liver:

To study the lipogenesis in vivo 3- and 24-month-old animals were injected with 1 mCi of sodium

[<sup>14</sup>C]acetate intraperitoneally four times every 30 min for 2 h. The liver was perfused with 0.9% NaCl, then removed and washed in Krebs-Henseleit buffer, pH 7.4, containing 2 mM CaCl<sub>2</sub> and 0.2% BSA. The lipids were extracted and analyzed as described below.

### Experiments with hepatocytes:

Hepatocytes were isolated from the 3- or 24-month-old rats by the method described in (Petrenko and Sukach, 1991). Preparation of hepatocytes was started between 9:00 and 10:00 a.m. To study effect of T4 on lipogenesis, hepatocytes (107/ml) were incubated in Eagle medium containing 10% fetal calf serum, 100 units/liter streptomycin, 100 units/liter penicillin, 25.4  $\mu$  Ci/ml sodium [<sup>14</sup>C]acetate and hormone (10 nM) for 1h. Control cells were treated with 0.1 N NaOH for 1h. Hepatocytes isolated from the 24-month-old rats were treated with vitamin E (100  $\mu$ g/ml) or NAC (10 mM) plus T4 (10nM) for 1h. To determine the cells viability, the trypan blue exclusion into hepatocytes has been studied. Before lipid extraction, the cells were washed twice with the Krebs-Henseleit buffer, pH 7.4, containing 2 mM CaCl<sub>2</sub>, 25 mM HEPES, 0.1% BSA. The lipids were extracted and analyzed as described below.

### Extraction and Separation of Lipids:

The lipids were extracted according to the Bligh and Dyer protocol (1959). The chloroform phase was collected and dried under N<sub>2</sub> at 37°C. The lipids were redissolved in chloroform/methanol (1:2, v/v) and applied on TLC plates. For lipids separation the solvent system: hexane/diethyl ether/acetic acid (36.5:12.5:1, v/v) was used. The appropriate standards were applied on each plate for quantification. The contents of phospholipids in chromatographic fractions were determined by the method of Marsh and Weinstein (1966). The gel spots containing [<sup>14</sup>C]lipids were scraped and transferred to scintillation vials. Radioactivity was measured by a scintillation counter.

### Statistical analysis:

Data were analyzed by one-way analysis of variance (ANOVA) followed by post hoc Fisher's protected least significant difference (Fisher PLSD) test. Results shown represent the means  $\pm$  standard error of the mean (SEM) and deemed statistically significant when  $p < 0.05$ .

## Results and Discussion

Thyroid hormones as well as dietetic fat are important regulators of hepatic lipogenesis and fatty acids

turnover (Girard et al., 1994). Thyroid hormones are involved in both the long- and short-term regulation of lipogenic enzymes, such as FAS, a key enzyme in hepatic lipogenesis (Giudetti et al., 2005; Radenne et al., 2008). T3 is able to increase the FAS enzymatic activity and mRNA expression level in chick embryo, rat and human hepatocytes. T3 regulates FAS transcription through a genomic action and can also act via activation of phosphoinositide 3-kinase non-genomically (Radenne et al., 2008). In the present study, we showed, in both in vivo and in vitro, that T4 increased significantly de novo synthesis of FFA, neutral lipids and CH in liver (Illustration 1) and isolated hepatocytes (Illustration 2) of 3-month-old rats. Hormone-stimulated increase of FFA synthesis was not associated with the FFA accumulation and followed by the increase of CH and TAG contents in the liver (Illustration 3) of young animals. However, at old age the ability of T4 to stimulate the lipogenesis was significantly less. T4 addition to the culture media of old hepatocytes did not change significantly the FFA, neutral lipids and CH de novo synthesis (Illustration 4). A single T4 injection to the 24-month-old rats did not change FFA synthesis (Illustration 1) and FFA, CH and TAG levels in the liver (Illustration 5). The results obtained are in line with other investigations on age-dependent liver and adipose tissue resistance to the action of lipogenesis regulatory factors. The significant age-dependent decrease of ACC and FAS mRNA expression and their induction by refeeding has been determined in the liver of fasted rats (Fukuda and Iritani, 1992). Age-related decrease of insulin-stimulated lipogenesis has been determined in the isolated adipocytes from Sprague-Dawley animals and hepatocytes of Wistar rats (Novelli et al., 2004; Babenko and Kharchenko, 2012). Malic enzyme stimulation with T3 in the liver of 25-month-old Fisher 344 rat was lower than in the younger animals (Moorodian and Albert, 1999). Taking into account that lipogenic enzymes expression significantly reduced at old age (Nogalska and Swierczynski, 2001; Nogalska et al., 2005) it may be supposed that age-dependent decline of liver cells response to thyroid hormone action at least partly depends on the low basal level of lipogenesis at old age.

Results, obtained in the present work, demonstrated that T4 induced de novo lipids synthesis in the isolated young hepatocytes during first hour of treatment the experiments in vitro and in vivo, and after 24 h of hormone action in vivo. Recently, it has been determined that among the most rapid nongenomic actions of thyroid hormones in the liver cells was the transcription factor (Nrf2) activation (Romanque et al., 2011). Administration of T3 to Sprague-Dawley rats

triggers cytosol-to-nuclear translocation of Nrf2 in rat liver by a redox-dependent mechanism. Moreover, the role of Nrf2 in the modulation of hepatic lipid homeostasis through lipogenic gene expression has been demonstrated (Huang et al., 2010). Deletion of Nrf2 in mice resulted in a significant reduction of FFA and CH synthesis gene expression, and decrease in FFA content of hepatic TAG, as well as lipoprotein-associated TAG and CH contents. From these results and data on age-related decline in transcriptional activity and mRNA expression of Nrf2 in the liver (Suh et al., 2004; Tomobe et al., 2012) it is possible to suggest that the constitutive loss of Nrf2 affects the lipogenesis dysregulation in aged rats.

Nrf2 knockout studies have demonstrated that  $\alpha$ -tocopherol or NAC addition to the culture media significantly reduce the cell sensitivity to elevated level of oxygen and death (Leung et al., 2003). Different antioxidants exhibit their protective effects through Nrf2 activation (Negi et al., 2011). Vitamin E prevents the inflammation-induced Nrf2 suppression in alveolar macrophages (Dworski et al., 2011), while the  $\gamma$ -tocopherol induces Nrf2 activity in diabetic neuropathy (Pazdro and Burgess, 2010) and  $\alpha$ -tocopherol stimulates Nrf2 expression in both untreated and acrolein-treated human retinal pigment epithelial cells (Feng et al., 2010). Different vitamin E constituents lipid-lowering properties have been demonstrated in cell lines (Parker et al., 1993), animal models (Qureshi et al., 2005) and humans (Qureshi et al., 1991). This suggests that vitamin E may be an useful tool for improvement of lipogenesis dysregulation at old age. Our results show that a single T4 injection to the 24-month-old vitamin E-treated rats did not change the FFA content and increased the CH and neutral lipids levels in the liver (Illustration 6). Vitamin E as well as NAC addition to the culture media of the hepatocytes isolated from 24-month-old rats significantly increased cells sensitivity to hormone action (Illustration 7). T4 increased significantly FFA, CH and neutral lipids synthesis in vitaminE/NAC-treated old hepatocytes.

## Conclusions

In summary, the data above strongly suggest that age-dependent lipogenesis dysregulation mainly depends on redox state changes in liver. Vitamin E as well as NAC can be useful tools for improvement of liver cells sensitivity to thyroid hormone action at old age.

## Authors Contributions

NB conceived of the study and participated in its design, coordination, and manuscript preparation. LH and VG participated in data collection and performed the statistical analysis.

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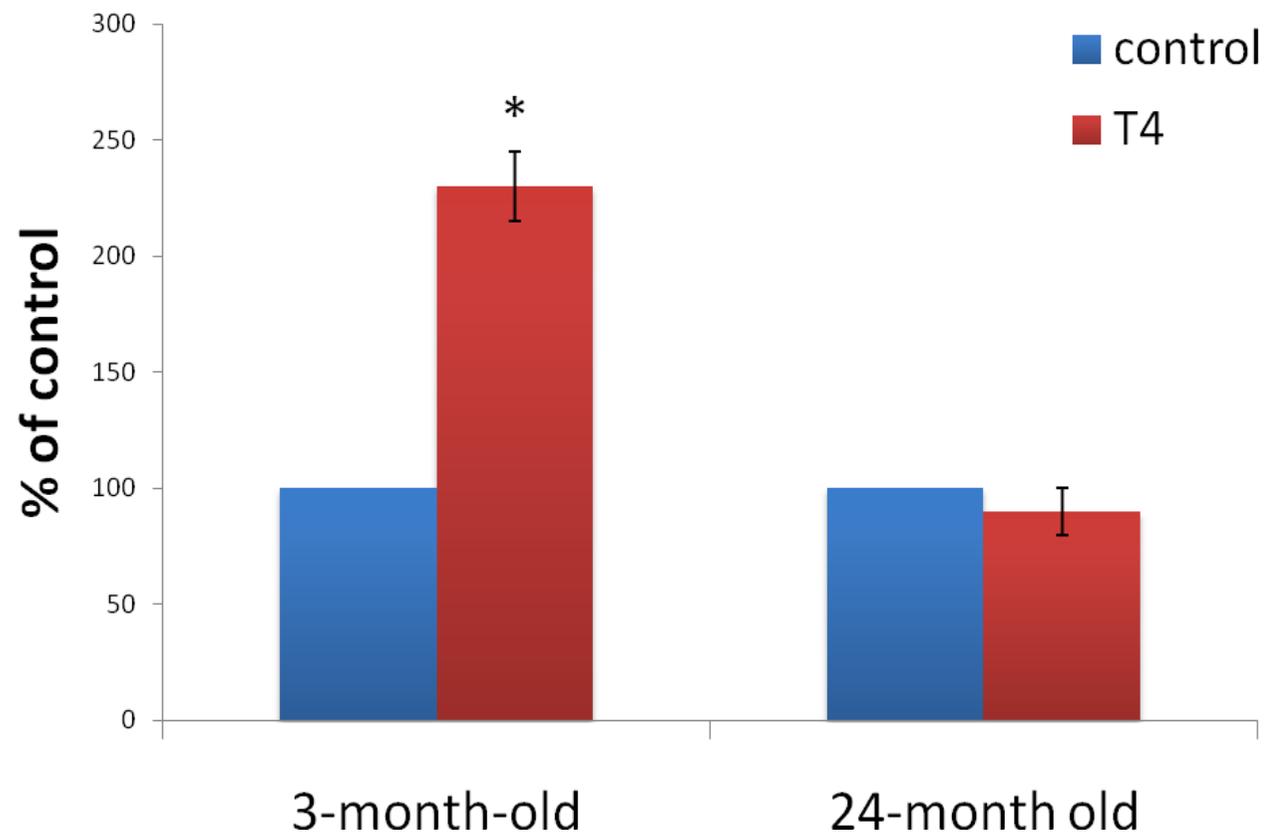
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## Illustrations

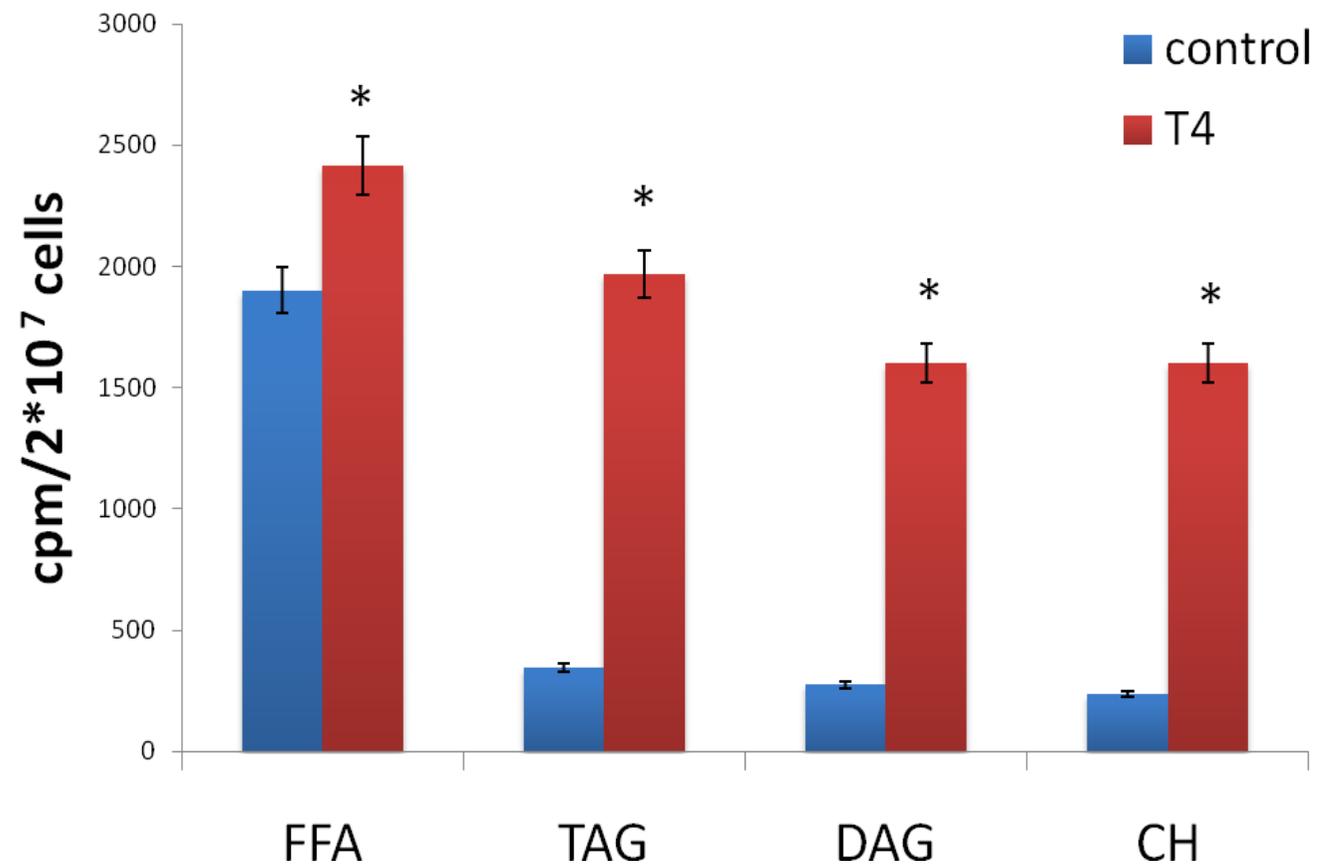
### Illustration 1

Effect of thyroxine on free fatty acids synthesis in the liver of 3- and 24-month-old rats (experiments in vivo). \* $P < 0.05$ , thyroxine-treated vs. control.



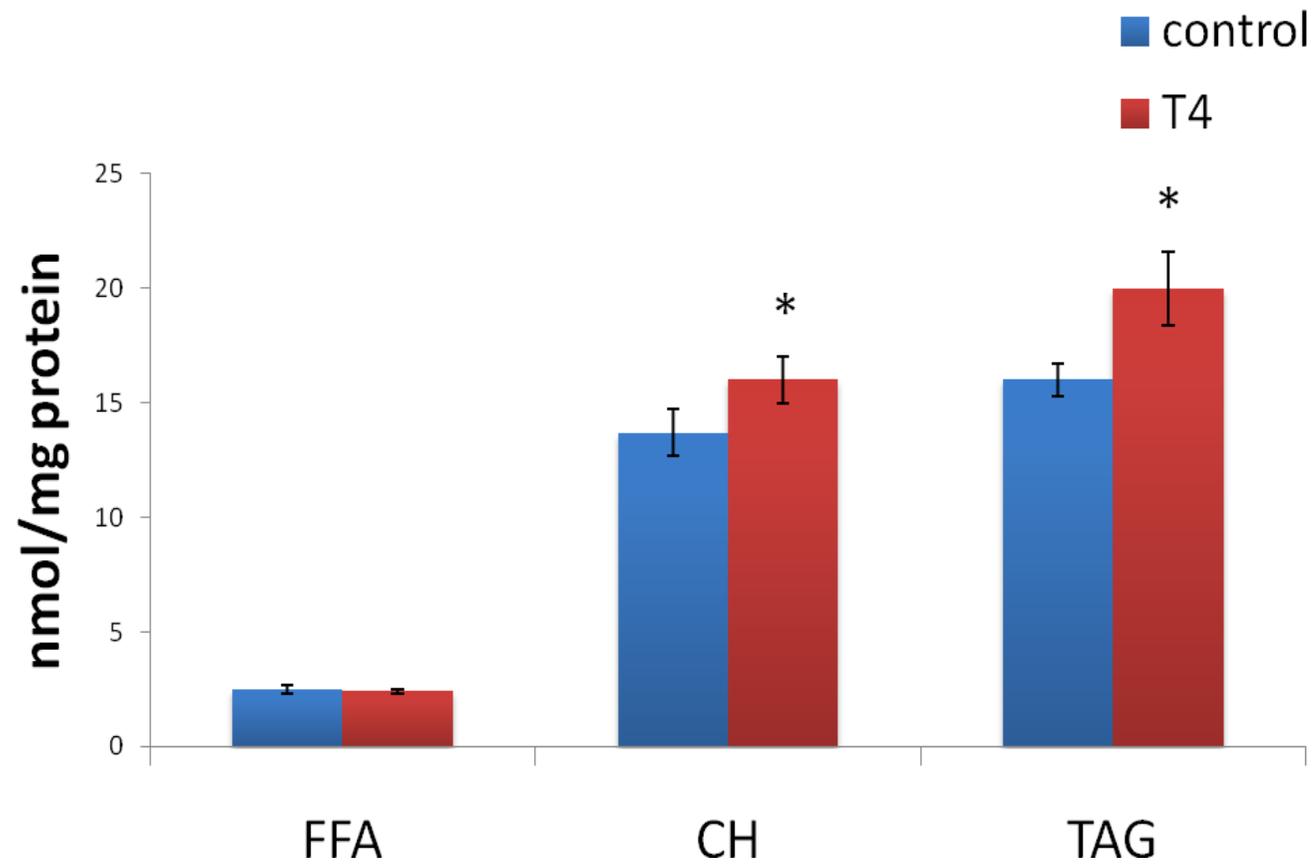
## Illustration 2

Effect of thyroxine on lipids synthesis in the hepatocytes of 3-month-old rats (experiments in vitro). \*P< 0.05, thyroxine-treated vs. control.



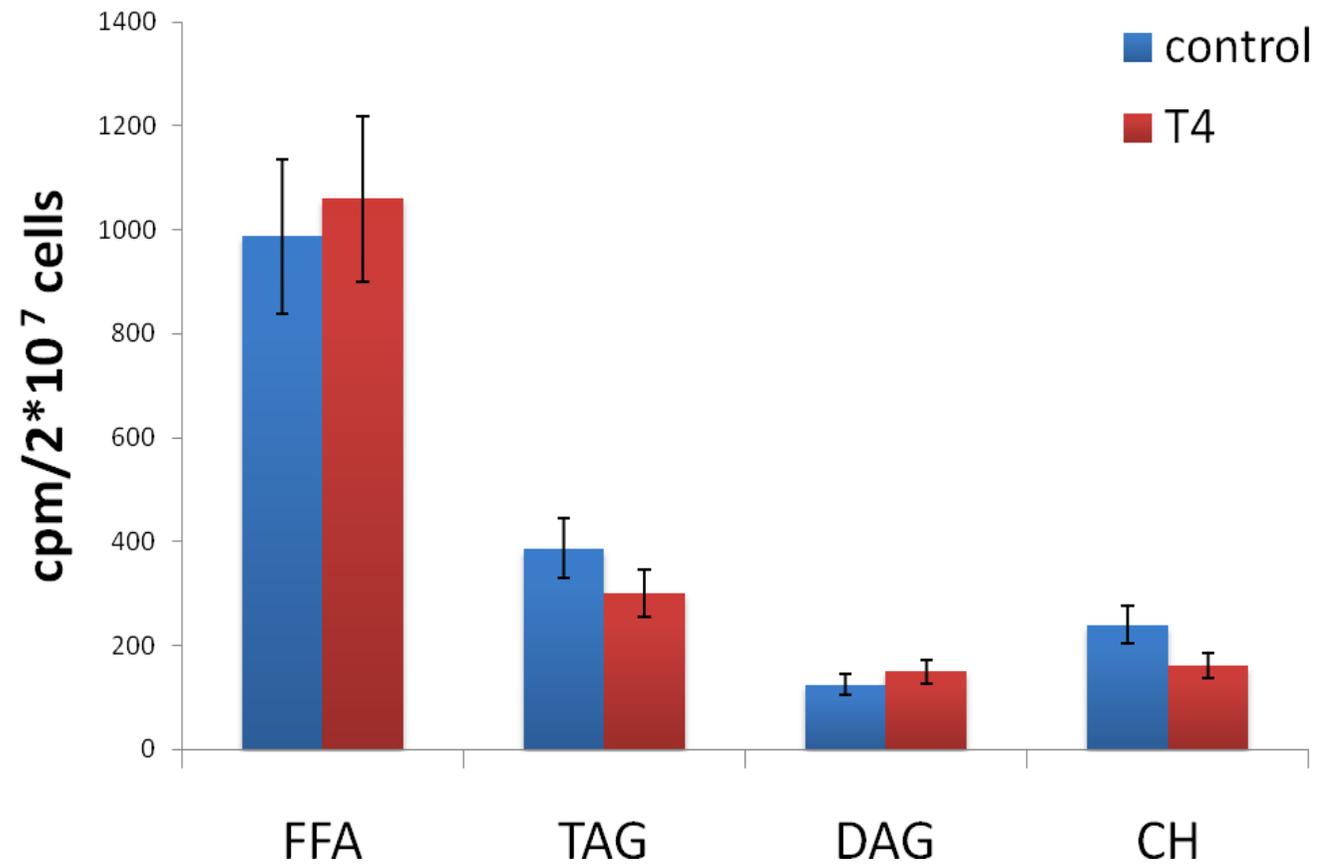
### Illustration 3

Effect of thyroxine on lipids contents in the liver of 3-month-old rats (experiments in vivo). \*P< 0.05, thyroxine-treated vs. control.



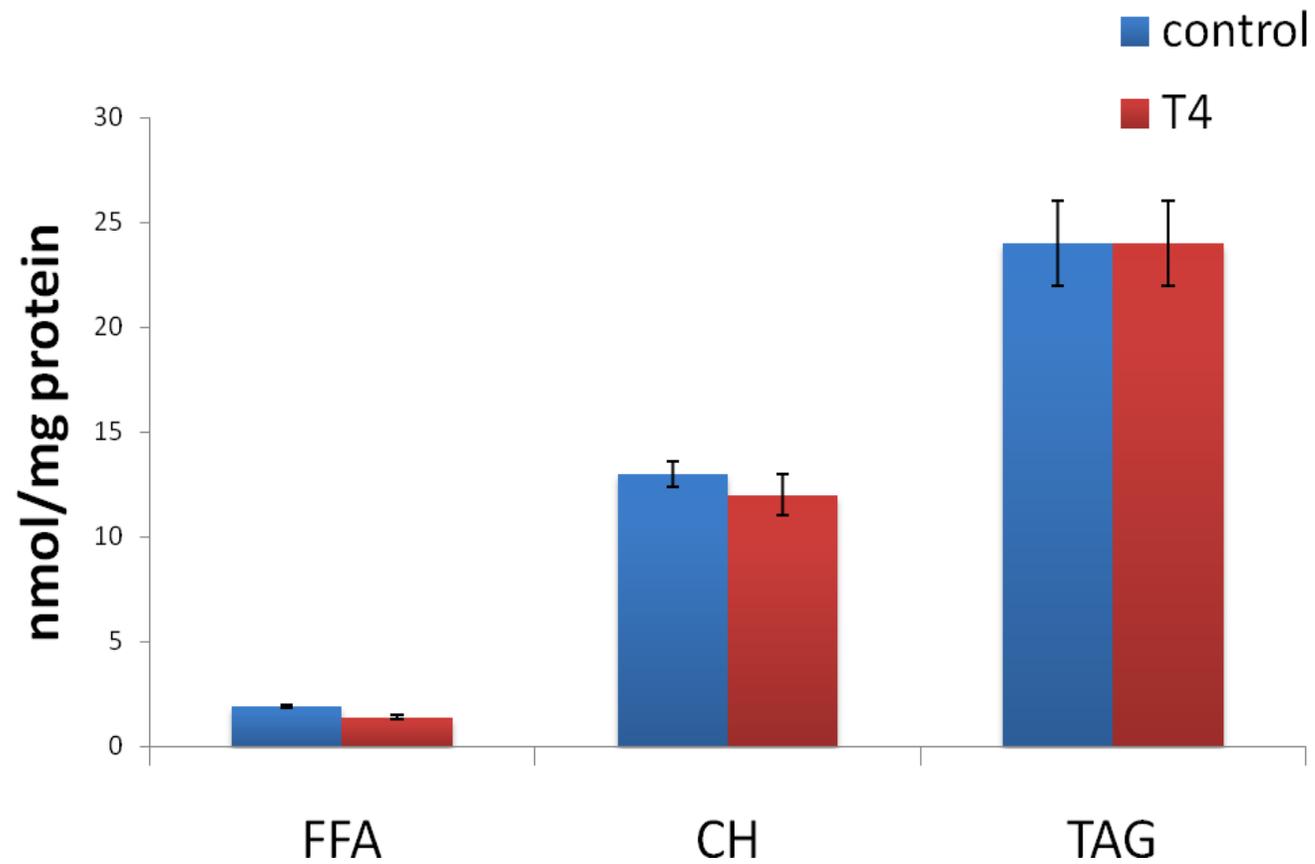
## Illustration 4

Effect of thyroxine on lipids synthesis in the hepatocytes of 24-month-old rats (experiments in vitro).



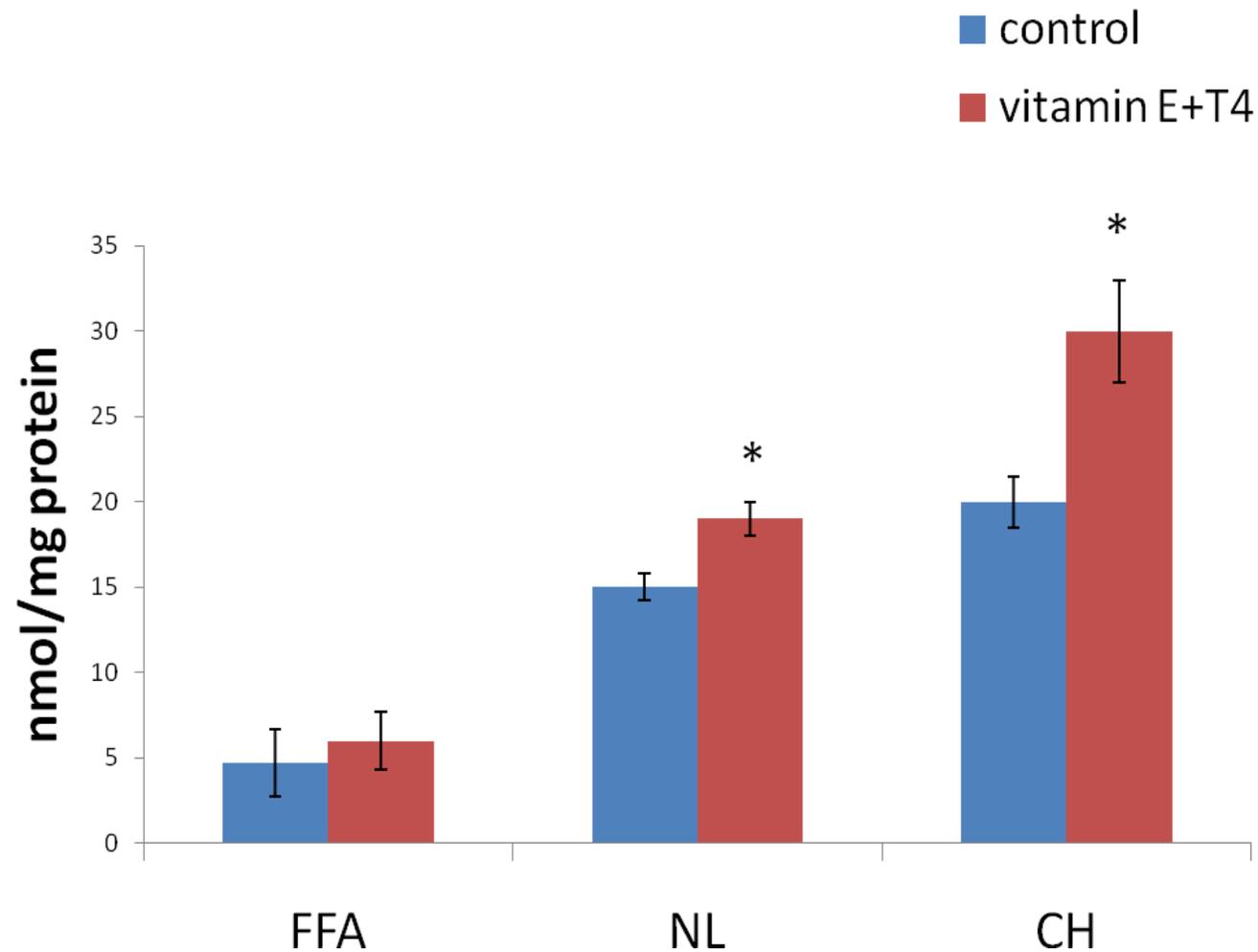
## Illustration 5

Effect of thyroxine on lipids contents in the liver of 24-month-old rats (experiments in vivo).



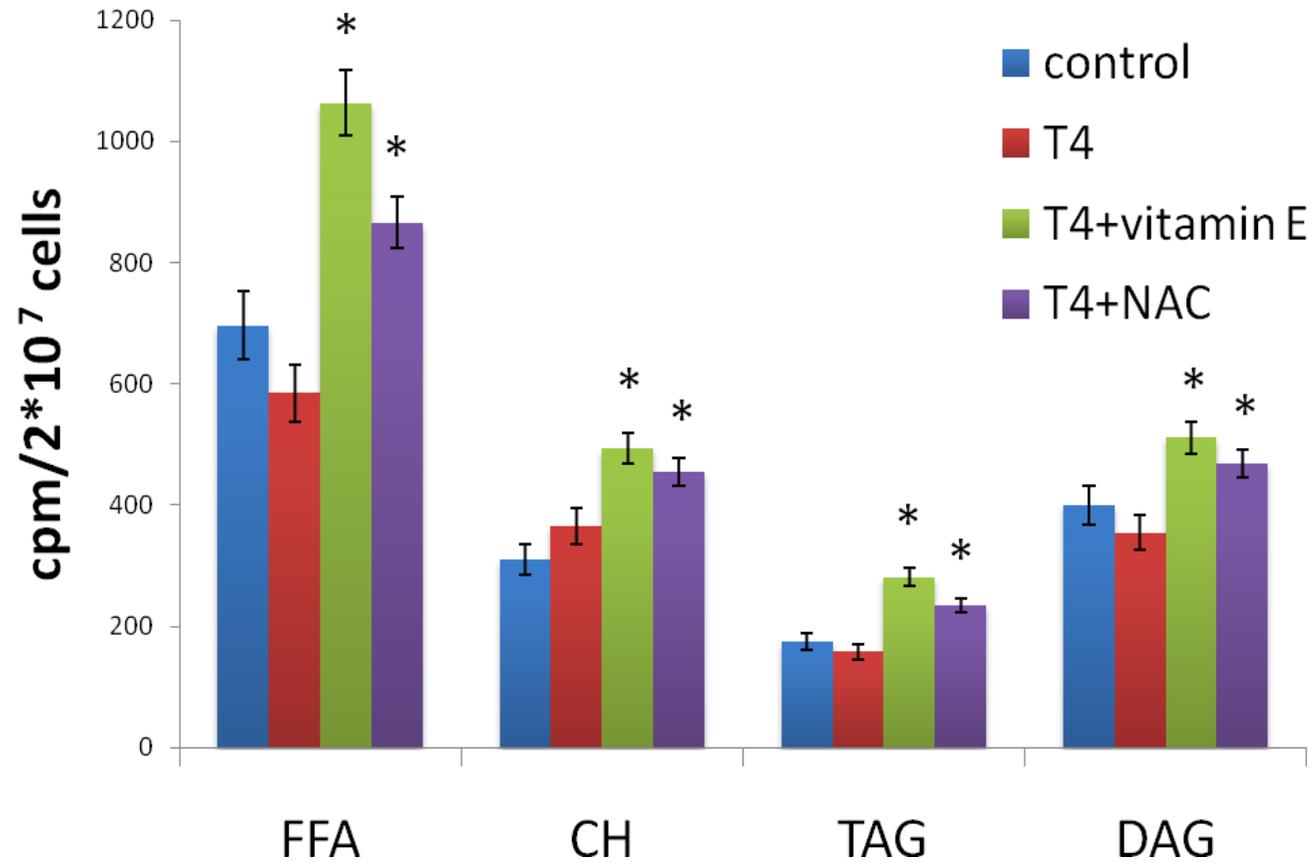
## Illustration 6

Effect of thyroxine plus vitamin E on lipids contents in the liver of 24-month-old rats (experiments in vivo). \*P< 0.05, thyroxine plus vitamin E-treated vs. control.



## Illustration 7

Effect of thyroxine plus vitamin E/N-acetylcystein on lipids synthesis in the hepatocytes of 24-month-old rats (experiments in vitro).  
\*P< 0.05, thyroxine plus vitamin E/N-acetylcystein-treated vs. control.



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