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Original Article

The Impact of Cholesterol Diet on NPC_1L_1 and ABCG5 Transpoters and Some Biochemical Parameters in Mouse

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Abstract

Cholesterol homeostasis in the body is controlled mainly by endogenous synthesis, intestinal absorption, and hepatic excretion. Changes in cholesterol absorption and hepatic excretion were reflected by changes in major transporters: ATP-binding cassette G5/G8 (Abcg5/8) and Nieman Pick C1 like 1 Protein (Npc1l1).

The aim of our study is to investigate the impact of cholesterol diet on those transporters. Eight (8) weeks male mice have been used, fed during fifteen (15) days and divided into two (2) groups: a control group of (15) mice fed with standard diet and other (15) mice fed with 5%cholesterol diet (second group) .The dosage of excreted cholesterol in feces was determined by gas chromatography coupled with mass spectrometry. Concentration of total cholesterol and triglycerides in the plasma and in the liver is determined by enzyme assay.The expression of mRNA was quantitatively analyzed by RT-PCR. In the jejunum as in the liver of mice subjected to 5% cholesterol diet the RT-PCR revealed the induction of the gene encoding Abcg5 expression. The level of Npc1l1 protein, involved in the intestinal uptake of cholesterol was decreased significantly in mice subjected to 5% cholesterol diet. In those mice, the level of phospholipids has increased, but the rate of triglycerides does not vary. Similarly the masses of livers of mice of that group have increased sharply. The plasma concentration of transaminases, confirmed the development of steatosis. This may explain partially, why mice subjected to 5% cholesterol diet did not develop the syndrome of high cholesterol.

Key words: Mouse, ABCG5/G8, NPC1L1, ALT, AST

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Abbreviations

CHOL	Cholesterol
ABCG5/G8	ATP-binding cassette G5 and G8
NPC1L1	Nieman Pick C1 likes 1 Protein
ALT	Alanine Amino Transferase
AST	Aspartate Amino Transferase.

Introduction

Increased blood cholesterol is an independent risk factor for atherosclerotic cardiovascular disease. Atherosclerosis is characterized by the formation of atherosclerotic plaques which constitute the inflammatory sites in the arterial wall with accumulation of lipids and fibrous elements (Berliner JA et al., 1995; Lusis AJ 2000).

The incidence of coronary events is directly related to plasma level of LDL cholesterol (low density lipoprotein) and inversely related to the level of HDL-cholesterol (high density lipoprotein) (Assmann G, Cullen P, Jossa F, et al., 1999; Braunwald E 1997). Thus, cholesterol's metabolism is a key development of cardiovascular diseases (Ros E 2000; Russell DW 1992). Nowadays, studies of cholesterol absorption have steadily grown and continue with the arrival of new cholesterol-lowering agents (Ezetimibe), which act either by inhibiting the synthesis, of cholesterol and also by inhibiting its absorption. In search of the molecular target of ezetimibe, Altmann et al. (Altmann, S. W., H. R. Davis, Jr., L. J. Zhu et al., 2004) identified a polytopic transmembrane protein named Niemann-Pick C1-Like 1 (NPC1L1), which localizes at the brush border membrane of the small intestine and mediates intestinal absorption of cholesterol.

Physiological, genetic and nutritional factors are involved in cholesterol metabolism. But it is difficult to determine the relation between the cause and effect, because of the complexity of the regulatory mechanisms of cholesterol and the involvement of several factors (Santosa S et al., 2007C). Cholesterol homeostasis in the body is controlled mainly by endogenous synthesis, intestinal absorption, and hepatic excretion. That homeostasis implements complex regulatory mechanisms that involve both the absorption and cholesterol synthesis.

It is known that the metabolism of cholesterol and bile acids are linked. Bile acids are synthesized in the liver from cholesterol. Following this review, approximately 98% of bile acids synthesized are conjugated to glycine or taurine and then excreted in the bile. Bile is stored in the gallbladder until it is released into the duodenum during food intake.

The transport of conjugated bile acid in key organs requires more carriers. The modulation of the excretion of these transporters may be responsible for the excretion of bile acids through cholesterol elimination. The cholesterol carried in LDL is derived principally from de novo synthesis and absorption from the diet (Turley SD and Dietschy JM; 1988). In population, there is a significant and positive correlation between the level of plasma LDL-cholesterol and the efficiency of intestinal cholesterol absorption (Kesäniemi YA and Miettinen T., 1987***)***. Therefore, understanding the genetic regulation of cholesterol absorption may lead to novel approaches to the treatment of cardiovascular diseases that affect millions of people in societies. Thus great efforts have been made to search for molecular, genetic, biochemical, and physical-chemical determinants of intestinal cholesterol absorption (Lammert F and Wang DQH., 2005*)*. Recent studies show that ATP-binding cassette (abc) transporters ABCG5 and ABCG8 may work together as an apical sterol export pump promoting active efflux of cholesterol and plant sterols from the enterocyte back into the intestinal lumen for excretion (Plosch T, Bloks VW, Terasawa Y et al.,2004**;** Wang HH et al.,2005; Yu L et al., 2003).

Also, the newly identified Niemann-Pick C1-like 1 (Npc1l1) protein may play a critical role in the ezetimibe-sensitive cholesterol absorption pathway and might induce active influx of cholesterol from the intestinal lumen into the enterocyte (Altmann SW**,** Davis HR Jr, Zhu LJ et al., 2004; Davis HR, Zhu LJ, Hoos LM et al., 2004).

It functions as a sterol transporter to mediate intestinal cholesterol absorption and counterbalances hepatobiliary cholesterol excretion.These findings strongly support the notion that cholesterol absorption is a multistep process that is regulated by multiple genes at the enterocyte level (Lammert F and Wang DQH, 2005; Wang DQH et al.,2001*)*. Therefore, it is imperative to investigate molecular mechanisms underlying the dominant rate-limiting step/factor in cholesterol absorption. Cholesterol may be directly excreted when the major carriers were identified. In the liver,

the hetero dimer formed by the ATP-binding cassette G5 and G8 (Abcg5/g8) secrete cholesterol in the bile. The two ATP-binding cassette (abc) half-transporters, g5 and g8, reside at the canalicular membrane of hepatocytes and the apical membrane of absorptive enterocytes where they function as heterodimers to transport cholesterol and noncholesterol sterols into the bile canaliculus and the gut lumen for fecal excretion (Yu, L., J. Li-Hawkins et al.,2002; Yu, L., R. E. Hammer et al., 2002; Graf, G. A., W. P. Li et al., 2002; Graf, G. A., L. Yu, W. P. Li et al., 2003). At the ileum level, the protein Nieman Pick C1 like Protein 1 (Npc1l1) could permit intestinal capture of biliary and diet cholesterol.

The aim of this study is not only to evaluate the impact of a high cholesterol diet on the ileal and liver transport system and on some biochemical parameters in mice but also to contribute to a better molecular and physiological knowledge that would be the origin of cholesterol metabolism.

Materials and Methods

Animals

Eight (8) weeks C57BL/6J male mice have been used. Those mice have been fed fifteen (15) days and Divided into Two (2) groups:

- A control group of fifteen (15) mice fed with standard diet: wheat, fish and vitamins
- Other fifteen (15) mice fed with a rich diet of 5% cholesterol called: group of 5% cholesterol then, mice are killed. For Physiological Studies, the fifteen (15) control mice and the fifteen (15) 5% cholesterol mice were placed into two different metallic cages to monitor parameters like the fecal excretion of cholesterol. Finally, the mice were then sacrificed and target organs were removed and frozen at -80 ° C. The experimental protocol was approved by Benin Ethics Committee.

Biochemical assays

Dosage of cholesterol excreted in feces

The dosage of cholesterol excreted in feces was determined by gas chromatography coupled with mass spectrometry.

Dosage of plasma concentrations of free cholesterol, cholesterol ester, total cholesterol, and triglycerides

Enzyme assays were performed to determine plasma concentrations and for dosages following concentrations were used:

- Concentration of free cholesterol: Free Cholesterol C-R1, Wako
- Concentration of cholesterol ester: see Principle
- Concentration of total cholesterol: Cholesterol 100, ABX Diagnostics
- Concentration of triglycerides: Triglycerides 25, ABX Diagnostics

The principle of the assay is as follows:

$$\text{Cholesterol ester} + H_2O_2 \longrightarrow \text{Cholestrol} + \text{Fat acids}$$

$$\text{Cholestrol} + O_2 \xrightarrow{\text{Cholestrol oxydase}} \text{Cholestrol} - 3 - \text{one} + H_2O_2$$

$$2H_2O_2 + 4\text{a min oantipyrine} + \text{Phenol} \xrightarrow{\text{Peroxydase}} \text{Quinone imine} + 4H_2O_2$$

The absorbance is measured at 590nm

$$\text{Triglycerides} + H_2O \xrightarrow{\text{Lipoprotein lipase}} \text{Glycerol} + \text{Fat acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol kinase}} \text{Chycerol} - 3 - \text{phosphate} + \text{ADP}$$

$$\text{Glycerol} - 3 - \text{phosphate} + O_2 \xrightarrow{\text{G}-3-\text{P oxydase}} H_2O_2 + \text{DHAP}$$

It is indeed by the principle of Triglyceride dosage.

Storage of liver phospholipids of triglycerides and total cholesterol

Liver phospholipids were assayed using the enzymatic kit Phospholipids PAP 150 (BioMérieux).

- The principle is based on the release of cholin under hydrlitic action of phospholipase D. Cholin is then oxidized by cholin oxydase. That reaction generates hydrogen peroxide which under the action

of peroxydase gave a colored compound which absorbance is measured at 450nm.

- Then from the liver we proceed to prior extraction of total lipid by Ter-butanol.The principle remains the same as previously.

Transaminases dosage (AST, ALT)

Assays were carried out according to the IFCC enzyme kinetics. The principle is the determination of activity of GOT or GPT according to the following reactions:

AST

L-aspartate + 2-Oxoglutarate → oxaloacetate + Lglutamate oxaloacetate + NADH + H^+ → NAD^+ + Lmalate

ALT

alanine + alpha-ceto pyruvic acid glutamate + glutamate → Pyruvic acid + NADH + H^+ → NAD^+ + lactate

Decreasing in absorbance due to conversion of NADH to NAD^+ and proportional to the activity of GOT (or GTP) was measured at 340 nm.

RT-PCR quantification assay

Total RNA from treated fish Prepared WAS T-cells using Trizol reagent (Invitrogen Life Technologies, Groningen, the Nederland) According to the manufacturer's instructions. The Integrity of RNA electrophoretically checked by ethidium WAS bromide staining and by the absorption ratio OD OD260nm/OD280nm.A microgram of RNA transcribed reversibly with WAS RNAze Superscript II reverse transcriptase using H-oligo (dT) According To the manufacturer's instructions (Invitrogen Life Technology, France).

PCR Real Time was performed I cycler one year, real time detection system (Bio-Rad, Hercules, CA, USA), and amplification WAS done by using SYBR Green I detection (SYBR Green Jumpstart Taq Ready mix for quantitative PCR, Sigma-Aldrich, St Louis, MO USA). Oligonucleotide primers and probes for mouse Abcg5, and Npc1l1 have been described elsewhere (Duan LP et al., 2004; Wang HH et al., 2004) (Tableau 1), used for mRNA analysis, were based on the sequences of mice gene in the GenBank database. Primers, which were designed by Primer Express Software (Applied Biosystems, Foster City, CA).

To obtain a normalized target value, the target amount was divided by the endogenous reference rodent glyceraldehyde-3-phosphate dehydrogenase (Gapdh) as the invariant control (part no. 4308313, Applied Biosystems).

Table 1: Primer and probe sequences used in mRNA quantification by real-time PCR

Abcg5	Forward: 5-CCTGCAGAGCGACGTTTTTC-3; Reverse: 5-GCATCGCTGTGTATCGCAAC-3; Probe: 5-AGCAGCCTCACTGTGCGCGAGA-3 ;
Npc1l1	Forward: 5-CCACAGACCCTGTGGAACTGT-3; Reverse: 5-GCTCGTCATGGAAAGCCTTT-3; Probe: 5-CCCCTAAAAGCCAAGCCCGGAAAG-3.

The amplification WAS carried out in a total volume of 25µl Containing 12.5µl SYBR Green Taq Ready Mix, 0.3µM of each primer and diluted cDNA. Cycling conditions consisted to denaturation step initial year of 95 ° C for hot start has 3minas Followed by 40 cycles of 95 ° C for 30 sec or at 60 ° C for 30sec with a simple fluorescence detection developed at the end of the annealing or extension under segment. At the end of the PCR, the temperature from 60 WAS laundry charges to 90 ° C for 15sec and 2 at 58 ° C for 60sec, and the fluorescence WAS Measured Every 15 seconds to draw the melting curve. The standard curves generated were for each protein or β-actin using serial dilution of positive control template in order to ESTABLISH PCR efficiencies. All determinations were performed at least in duplicates using two dilutions of each assay reproducibility to achieve. Results were evaluated by IQ software I cycler including standard curves, amplification

efficiency (E) and threshold cycle (Ct). Relative mRNA quantization of speech was determined using the ΔΔCt ΔΔCt = ΔCt in which (gene of interest)-Δ Ct (β-actin). ΔCt = Ct (interest group)-Ct (control group). Relative quantity (RQ) was calculated as follow: RQ = (1 + E) (-ΔΔCt)

Statistical Analysis

Results are shown as means clustering ± SEM. The significance of the differences between mean values was determined by two-way ANOVA (STATISTICA, version 4.1, Stat soft, Paris, France), followed by the least significant difference (LSD) test. Were differences regarded significant at $P < 0.05$.

Results and Discussion

Expression of ABCG5 transcript levels involved in the transport of cholesterol into the jejunum and liver

To appreciate the impact of rich cholesterol diet on cholesterol transport, two systems have been studded. Regarding to the two proteins: ABCG5 and ABCG8 associated in heterodimer, only the expression of ABCG5 has been appreciated.

In the jejunum (Fig. 1A) as in the liver (Fig. 1B), the analysis by real time PCR revealed an induction of the gene coding for ABCG5 mice subjected to 5% cholesterol diet.

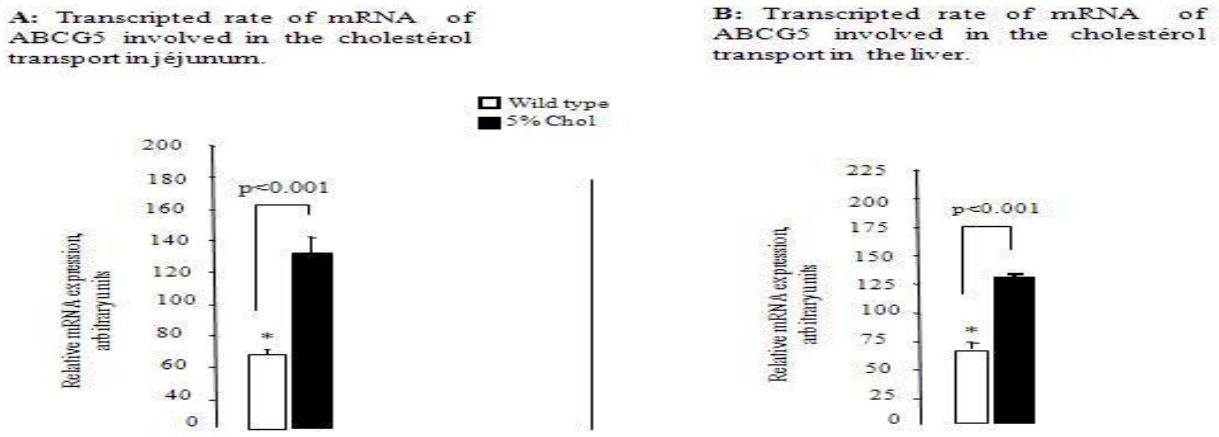

Figure. 1 Expression of transcripted ABCG5 levels involved in the transport of cholesterol into the jejunum and liver.

Eight weeks male mice wild type C57BL/6J have been used. Those mice have been fed during fifteen days and divided into two groups: control mice group fed with standard diet: wheat, fish and vitamins. Other mice second group fed with a rich diet of 5%cholesterol . The parameters were determined as described in materials and methods section. Values are means ± SEM, n = 15 per group of animals. Each value is the mean of six determinations. Data were analyzed by two-way ANOVA. *significant differences ($p<0.001$) between WT and mice subject to the 5% cholesterol regime. NS =insignificant differences. ABCG5: ATP-binding cassette G5.

Expression levels of NPC1L1 transcripts involved in transport of cholesterol into the jejunum

NPC1L1 protein involved in the intestinal uptake of cholesterol and specifically localized in the jejunum, sees its expression decreased significantly in mice subjected to 5% cholesterol diet (Fig. 2).

Cholesterol excretion in feces

Npc1l1 protein involved in intestinal storage of cholesterol at jejeunal level has seen its expression decreased in mice subjected to 5%cholesterol diet so we think to another regulatory mechanism which took over. Hence the idea of quantifying excreted cholesterol in the feces. In mice subjected to 5% cholesterol diet, there is a sharp increase in fecal

leakage of cholesterol compared to the control group (Fig. 3).

Plasma concentrations of free cholesterol, cholesterol ester, total cholesterol and triglycerides

To verify that mice subjected to 5% cholesterol diet developed in the 15th day hypercholesterolemia or not, we proceed to the plasmatic dosage of free cholesterol, cholesterol ester, total cholesterol and triglycerides. The results of the dosage of all those biochemical parameters showed no significant differences between the two groups of mice (Fig.4).

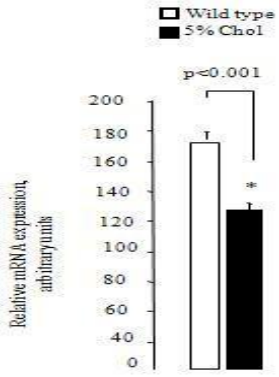

Figure. 2: Expression levels of transcripted NPC1L1 involved in transport of cholesterol in the jejunum.

The expression of mRNA was quantitatively analyzed by employing RT-PCR as described in materials and methods section.. Values are means ± SEM, n = 15 per group of animals. Each value is the mean of six determinations. Data were analyzed by two-way ANOVA. *significant differences (p<0.001) between WT and mice subject to the 5% cholesterol regime. NPC1L1: Nieman Pick C1 like Protein 1.

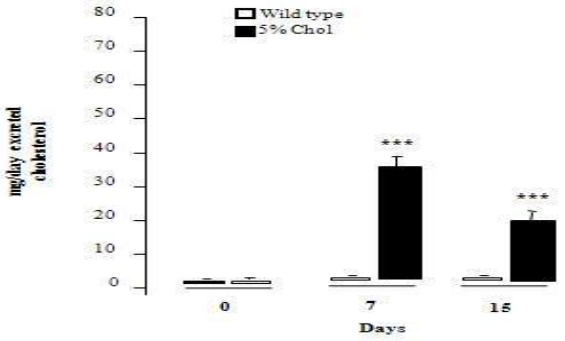

Figure. 3: Quantity of excreted cholesterol in feces

The dosage of cholesterol excreted in feces was determined by gas chromatography coupled with mass spectrometry.). Each value is the mean of six determinations. Data were analyzed by two-way ANOVA. *** significant differences (p<0.001) between different treatments.

Storage of liver phospholipids of triglycerides and total cholesterol

In order to better appreciate cholesterol storage modification with physiological modifications which accompany it, we proceed to the dosage of phospholipids, triglycerides and total cholesterol. In mice subjected to 5%cholesterol diet, the quantity of phospholipids has increased comparing to control mice. The rate of triglycerides didn't change. Endly, we assist to a strong storage of cholesterol in the livers of mice subjected to 5% cholesterol diet compared to the control mice (Fig. 5).

Liver mass of mice

The results of biochemical parameters determination like the concentration of free plasmatic cholesterol, cholesterol ester, total cholesterol and triglycerides which didn't reveal any significative differences between control mice and those subjected to 5% cholesterol diet confirmed that mice subjected to 5% cholesterol diet didn't develop hypercholesterolemia.

Otherwise, despite of the maintain of normal cholesterolomia, cholesterol hemostazia can be disturbed. Hence the importance of weighing of mice livers. Livers mass of mice subjected to 5% of cholesterol have increased significantly. In any case those livers were gray with blacks spots suggesting steatosis (Fig. 6).

Determination of ALT and AST

The increase in liver mass of mice subjected to 5% of cholesterol diet has been significantly then we suggest a liver damage. The plasma level of hepatic transaminases which are non specific markers of liver damage like hepatitis, steatosis including tumors.

We found that the plasmatic concentration of transaminases has increased significantly in mice subjected to 5% cholesterol diet, compared to the control group; it confirmed the development of steatosis.

We also noticed a greater increase of alanin aminotransferaze (ALT) than aspartat amino transferaze (AST) (Fig.7)

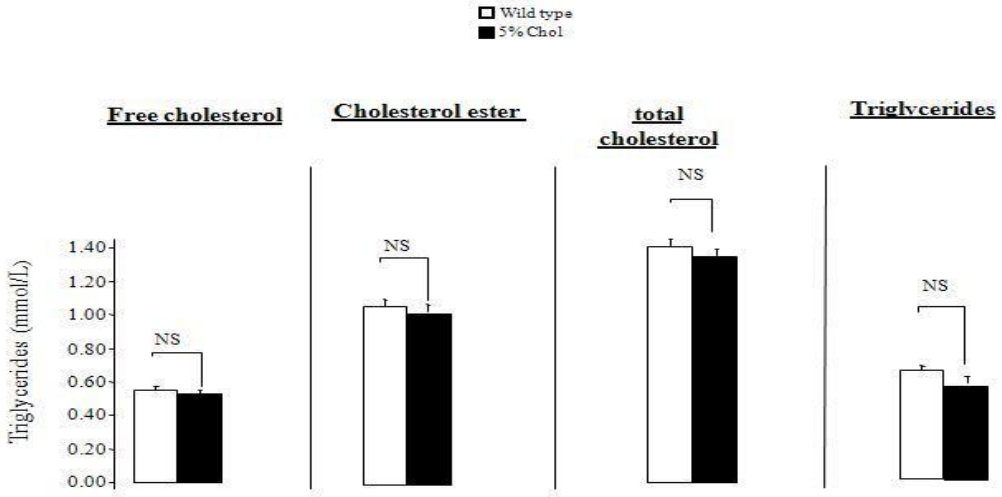

Figure. 4: Plasmatic concentration of free cholesterol, cholesterol ester, total cholesterol and triglycerides

. Plasmatic concentration of different cholesterol was performed as described in materials and methods section. Values are means ± SEM, n = 15 per group of animals. Each value is the mean of six determinations. Data were analyzed by two-way ANOVA. NS: =insignificant differences. ($p<0.001$) between WT and mice subject to the 5% cholesterol regime.

Cholesterol overload (CO) is likely to cause at least two effects on various intestinal genes:

The induction of expression of ileal bile acid-binding protein (*Ibabp*), which increase the reabsorption of bile acids (BA) in the ileum, and the rise, via the liver-X-receptor (*LXR*), of the synthesis of membrane transporters Abcg5 and G8, leaders of the efflux of cell cholesterol.

Recent progress in understanding the molecular basis of intestinal sterol transporters has provided many new insights into the complex physiological mechanisms involved in intestinal cholesterol

absorption (Lammert F et al., 2005). The first effect contributes to maintain the integrity of the ileac mucosa, whereas the coordination of the two effects is likely to increase fecal excretion of cholesterol.

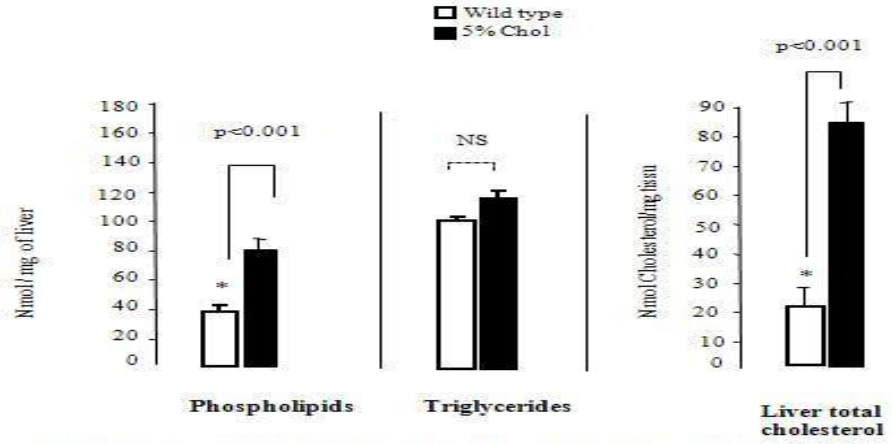

Figure. 5: Storage of liver phospholipids , triglycerides and total cholesterol.

Liver phospholipids were assayed using the enzymatic kit Phospholipids PAP 150 (BioMérieux).
The storage of liver phospholipids, triglycerides and total cholestérol were performed as described in materials and methods section. Values are means ± SEM, n = 15 per group of animals. Each value is the mean of six determinations. Data were analyzed by two-way ANOVA. NS: =insignificant differences. (p<0.001) between WT and mice subject to the 5% cholesterol regime .

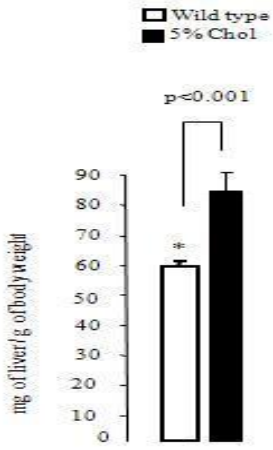

Figure. 6: Liver mass of mice.

The parameters were determined as described in materials and methods section. Values are means ± SEM, n = 15 per group of animals. Each value is the mean of six determinations. Data were analyzed by two-way ANOVA. *significant differences (p<0.001) between different treatments.

Cholesterol induced in the liver the transcribed rate of Cholesterol 7α-hydroxylaze (CYP7A1), limiting enzyme of bile acids synthesis. What's more expression of CYP7A1 encoding gene is induced in mice with 5% diet of cholesterol. Also we noticed an increase of distribution and wealth

expression related to the RNA and CYP7A1 in the liver reported by Northern Blotting. According to ileum role in intestinal re-absorption of bile acids, our interest was focused on the ileac transportation: the standard expression of Ileal Bile Acid Transporter (IBAT) has strongly reduced; in mice subjected to the rich diet of cholesterol the Western Blotting of proteins has quantitatively reduced. IBAT's oppression can be explained by the diminution of the ileac transportation of taurocholic acid (TCA). It results an increase of fecal flight of TCA in mice with a rich diet of cholesterol (Attakpa S. E et al., 2012).

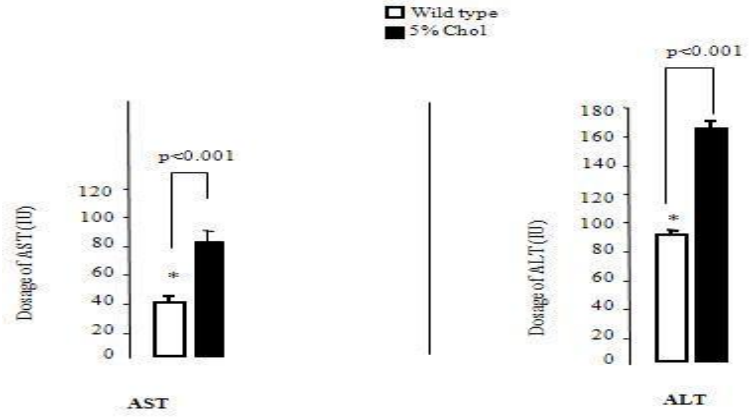

Figure. 7 The plasma level of liver transaminases.

Assays were carried out according to the IFCC enzyme kinetics. The principle is the determination of activity of GOT or GPT . ALT: Alanine amino transferase, AST: aspartate amino transferase.). Each value is the mean of six determinations. Data were analyzed by two-way ANOVA. *significant differences ($p<0.001$) between different treatments.

To appreciate the impact of rich cholesterol diet on cholesterol transport, two systems have been studded. Regarding to the two proteins: ABCG5 and ABCG8 associated in heterodimer, only the expression of ABCG5 has been appreciated. We have shown that, under our experimental conditions, under cholesterol diet: In the jejunum as in the liver, the analysis revealed an induction of the gene encoding ABCG5 for mice subjected to 5% cholesterol diet. The Abcg5 is important for biliary secretion and intestinal excretion of cholesterol. Invalidation of the genes encoding these proteins leads to a significant drop of bile secretion, while their over expression increases it seriously (Yu L, Gupta S et al., 2005).

An indirect role of I-babp in the fecal excretion of cholesterol is also possible. This hypothesis involves the joint involvement of membrane transporters responsible for cellular efflux of cholesterol, the ATP-binding cassette G5 and G8 (Plösch T et al., 2002).

It was recently shown that the expression of genes encoding these proteins is under the control of LXR, and the simultaneous induction of the expression of *I-babp*, *Abcg5* and *Abcg8* resulting from a charge could result in cholesterol level by increasing the ileocyte joint reabsorption of bile acids and cholesterol efflux. This opposing flow cell, causing the progressive imbalance of cholesterol / bile acids in the intestinal lumen, should lead to a drop of micellar solubilization of cholesterol. In addition, genes involved in cholesterol uptake (Ldlr) and bile acid excretion (Abcg5 and Abcg8) were increased in the livers of fat-1 transgenic mice (fat-1) capable of converting n-6 to n-3 PUFAs mice (Kim EH et al., 2012)

NPC1L1 protein involved in intestinal storage of cholesterol at jejeunal level has seen its expression decreased in mice subjected to

5%cholesterol diet so we think to another regulatory mechanism which took over. Biliary and dietary cholesterol is less absorbed. Our results corroborate the observations of Jia L et al. (2010) who have shown that the NPC1L1 deficiency in mice prevents HFD-induced fatty liver by reducing hepatic lipogenesis, at least in part, through attenuating HFD-induced insulin resistance, a state known to drive hepatic lipogenesis through elevated circulating insulin levels.

Hence the idea of quantifying excreted cholesterol in the feces. In mice subjected to 5% cholesterol diet, there is a sharp increase in fecal leakage of cholesterol compared to the control group. We therefore observed that the leakage of fecal cholesterol is correlated to the decrease of intestinal absorption and the increase of intestinal excretion of cholesterol. The final consequence would be an increased fecal excretion of cholesterol, a phenomenon actually observed in mice treated with a pharmacological agonist of LXR (Plösch T et al., 2002). Mice whose gene for Npc1l1 was invalidated, have the intestinal absorption of cholesterol more reduced (Altman SW, Davis HR et al., 2004), illustrating the fundamental role played by this protein in cholesterol transport. Recent studies have identified Npc1l1 protein may play an important role in the hepatic secretion of cholesterol. Hepatic over expression of Npc1l1 in mice significantly decreases biliary cholesterol concentration (Temel RE, Tang W, Ma Y, Rudel LL et al., 2007). In rodents, Npc1l is almost exclusively expressed in the small intestine, and there are no detectable Npc1l1 proteins in the liver (Altmann, S. W., H. R. Davis, Jr., L. J. Zhu et al., 2004; Yu, L., S. Bharadwaj, J. M. Brown et al., 2006; Davies, J. P., C. Scott, K. et al., 2005; Temel, R. E., W. Tang, Y. Ma et al., 2007). The direct effect of hepatic Npc1l1 on biliary cholesterol secretion would be minimal, if any, in rodents.

We proceed to the plasmatic dosage of free cholesterol, cholesterol ester, total cholesterol and triglycerides to verify that mice subjected to 5% cholesterol diet developed in the 15^{th} day hypercholesterolemia or not. The results of the dosage of all those biochemical parameters showed no significant differences between the two groups of mice.

In order to better appreciate cholesterol storage modification with physiological modifications which accompany it, we proceed to the dosage of phospholipids, triglycerides and total cholesterol. In mice subjected to 5%cholesterol diet, the quantity of phospholipids has increased comparing to control mice. The rate of triglycerides didn't change. Endly, we assist to a storage of cholesterol in the livers of mice subjected to 5% cholesterol diet compared to the control mice. Campbell et al. (2002) have explained this phenomenon by demonstrating that there is a marked increase in malonyl-CoA, a potent inhibitor of fatty acid oxidation, in the hearts of mice.

The results of biochemical parameters determination like the concentration of free plasmatic cholesterol, cholesterol ester, total cholesterol and triglycerides which didn't reveal any significative differences between control mice and those subjected to 5% cholesterol diet confirmed that mice subjected to 5% cholesterol diet didn't develop hypercholesterolemia.

Otherwise, despite of the maintain of normal cholesterolomia, cholesterol hemostazia can be disturbed.

Hence the importance of weighing of mice livers. Livers mass of mice subjected to 5% of cholesterol have increased significantly. In any case those livers were gray with blacks' spots suggesting steatosis.

The increase in liver mass of mice subjected to 5% of cholesterol diet has been significantly then we suggest a liver damage.

The plasma level of hepatic transaminases which are non specific markers of liver damage like hepatitis, steatosis including tumors.

We found that the plasmatic concentration of transaminases has increased significantly in mice subjected to 5% cholesterol diet, compared to the control group; it confirmed the development of steatosis. Our observations, in part, corroborate the findings of Kim EH et al., (2012) who have also shown that Wild-type (WT) and fat-1 transgenic mice (fat-1) capable of converting n-6 to n-3 PUFAs mice were maintained on a high-fat diet (HFD) for 5months. HF diet fed mice HFD-induced

weight gain and fatty liver were more prominent in WT mice than fat-1 mice. Histological analysis indicated that WT mice fed the HFD developed moderate-to-severe macro vesicular steatosis, whereas fat-1 mice developed very mild steatosis. In addition, HFD-induced hepatocyte ballooning and fibrosis were ameliorated in fat-1 mice. Serum alanine transaminase (ALT) and aspartate transaminase (AST) levels were within the respective normal ranges in HFD-fed fat-1 mice, whereas both were significantly elevated in HFD-fed WT mice. We also noticed a greater increase of alanin amino transferase (ALT) than aspartat amino transferase (AST).

We conclude that this work therefore may lead to novel approaches to the treatment of hypercholesterolemia.

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