Effect of Acute and Chronic Grapefruit, Orange and Pineapple Juice Consumption upon Blood Lipoprotein Synthesis and Metabolism in the Rat

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*Jamil Abou-Khalil*
The effects of acute and chronic juice (grapefruit, orange and pineapple juices) intake on plasma lipid profile and lipoprotein metabolism have been investigated in normolipidemic Sprague Dawely rats. Acute juice intake effects were studied after three hours of a single juice-lipid load instilled intragastrically. Both pineapple and grapefruit groups had significantly lower plasma and chylomicron (CM) triacylglycerol (TAG) concentrations than the control group. This effect may be attributed to delayed gastric emptying since all juice groups significantly inhibited gastric emptying and resulted in higher intragastric TAG retention 3h postprandially. None of the juice types had an effect on plasma cholesterol. The acute study does not support the possibility of a direct effect of juices on liver during the period studied since very low-density lipoprotein (VLDL) TAG and VLDL cholesterol concentrations of all groups were similar. For a period of six month, animals of the chronic study received either water (control group) or half of their daily liquid intake from one of the following juices: grapefruit, orange or pineapple juice. Blood samples from the different groups were collected following 18 h of fast and subjected to different analyses. Results have shown that when compared with the control group none of the juice types used had a significant effect on blood glucose, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol and the ratios total cholesterol/HDL-cholesterol and LDL-cholesterol/HDL-cholesterol. Only animals of the grapefruit group showed a drastic decrease in plasma TAG concentrations with respect to all other groups. This decrease in mean plasma TAG concentration appeared to be the result of a direct effect of grapefruit juice on liver TAG secretion since VLDL particles of the grapefruit group carried by far the least amount of TAG. Unlike grapefruit group, both pineapple and orange groups
significantly increased VLDL TAG secretion with respect to control and grapefruits groups, indicating different impacts of juice types on liver TAG secretion. LDL TAG concentrations of all groups were similar. A significant increase in plasma VLDL apolipoprotein B (apo B) concentration, hence VLDL secretion, has been observed irrespective of the juice type. However, plasma total apo B concentration of the grapefruit group was the highest among all groups, while both orange and pineapple groups had the lowest plasma total apo B concentrations, indicating that either pineapple or orange juice consumption, but not grapefruit, increase the rate of metabolism of lipoprotein particles from the blood. The reduced metabolism and clearance of VLDL particles observed in the grapefruit group may be attributed to the large number and small sized VLDL particles secreted in this group. In conclusion a six-month period of chronic juice intake in normolipidemic rat showed a modest effect on blood lipid profile, and the possible cardioprotective benefit may be through mechanisms independent of a direct effect on blood lipid levels. Orange and pineapple juices but not grapefruit appeared to increase somehow the metabolism and clearance of the lipoprotein particles from the blood. In the acute study, mainly grapefruit and pineapple juices moderate sharp increases in postprandial plasma TAG concentrations accompanying peak digestion and absorption as a result of delayed gastric emptying.
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I'm very honored to accomplish my thesis in the light of professional and efficient contribution by Dr. Costantine Daher who has provided exceptional guidance and support during this project.

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GLOSSARY

ACAT: Acetyl-CoA-cholesterol acyltransferase
Apo: Apolipoprotein
CETP: Cholesteryl ester transfer protein
CHD: Coronary heart disease
CHE: Cholesterol esterase
CHOD: Cholesterol oxidase
CM: Chylomicron
DTT: Dithiothreitol
EDTA: Ethylene-diamine-tetraacetic acid
FA: Fatty acid
GK: Glycerol kinase
GPO: Glycerol-3-phosphate oxidase
HDL: High-density lipoprotein
HL: Hepatic lipase
IDL: Intermediate-density lipoprotein
LCAT: Lecithin cholesterol acyltransferase
LRP: Low-density lipoprotein receptor-related protein
MG: Monoacylglycerol
NaN₃: Sodium azide
LDL: Low-density lipoprotein
LPL: Lipoprotein lipase
PMSF: Phenylmethylsulfonylfluoride
POD: Phenol oxidase
VLDL: Very low-density lipoprotein
RDA: Recommended dietary allowance
SDS-PAGE: Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SEM: Standard error from the mean
Sf: Svedberg flotation rate
T₃: Tri-iodothyronine
TAG: Triacylglycerol
TEMED: Tetramethylethylenediamine
1.1 Beneficial role of juices and aim of the project

Nowadays, people are shifting more and more towards fresh juice consumption instead of carbonated beverages. The most common juices consumed in the middle east are, orange, pineapple, and to a lesser extent grapefruit. These types of juices are well known for their high contents in vitamin C, pectin fiber, and mainly flavonoids (Peckenaugh and Poleman, 1999). Studies have shown the role of vitamin C in diminishing the risk of heart disease, the development of certain cancers, preventing the buildup of "bad" cholesterol, reducing the risk of cataracts and aiding in the body's overall natural healing process (Halliwell, 1994). Pectin fiber on the other hand, has been shown to increase the excretion of lipids, cholesterol and bile acids, and reduce serum cholesterol levels. Pectins operate by binding with bile acids, thereby decreasing cholesterol and fat absorption (Martinez et al., 1981). Pectin is also effective in causing regressions in, and preventing, gallstones (Buhler and Miranda, 1998). There is also evidence that the regular use of pectin may lessen the severity of diabetes (Zeman and Ney, 1996). Flavonoids, found in abundance in orange, grapefruit and pineapple juices, have aroused considerable interest recently because of their potential beneficial effects on human health. They have been reported to have antiviral, anti-allergic, antiplatelet, anti-inflammatory, antitumor and antioxidant activities (Hertog et al, 1995). Flavonoids are polyphenolic compounds. They are categorized, according to chemical structure, into flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones (Middleton and Kandaswami, 1992).
Possessing antioxidant activities, flavonoids are compounds that protect cells against the damaging effects of reactive oxygen species, such as superoxide, peroxyl radicals (Morel et al., 1993; Longeril et al., 1994). An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage. Oxidative stress has been linked to cancer, aging, atherosclerosis, ischemic injury, inflammation and neurodegenerative diseases (Partiff et al., 1994). Flavonoids may help provide protection against these diseases by contributing to the total antioxidant defense system of the human body (Halliwell, 1994). The oxidation of low-density lipoproteins (LDL) has been recognized to play an important role in atherosclerosis. Immune system cells called macrophages recognize and engulf oxidized LDL, a process that leads to the formation of atherosclerotic plaques in the arterial wall (Gorinstein et al., 1994; Bocco et al., 1998). Several studies have shown that flavonoids can protect LDL from being oxidized, thus reducing the risk of coronary heart disease (CHD) (Whalley et al., 1990). On the other hand, other studies have suggested that there is no association between flavonoid intake and CHD (Rimm et al, 1996). In addition, up to our knowledge, no reports have dealt with juice effect upon chylomicrons (CM), very low-density lipoproteins (VLDL), and apolipoproteins apo B100 and apo B48 secretion. In fact, apo B was shown to be a better predictor of CHD than other markers, such as LDL and high-density lipoproteins (HDL), especially in individuals with low or normal LDL-cholesterol (Sniderman et al., 2001). Since the literature covers mainly the benefit of antioxidant effect of juice on CHD, the present work extends the limit to cover the acute and chronic effect of juice intake upon blood lipid profile, focusing on apo B100 and apo B48 as excellent markers of atherogenicity. Therefore the purpose of the study was as follows:

1- To determine the effect of acute juice intake upon:
   a- Plasma cholesterol and Triacylglycerol (TAG).
   b- CM cholesterol and TAG.
   c- VLDL cholesterol and TAG.
   d- LDL cholesterol and TAG.
   e- Total gastric content
   f- Gastric TAG content
2- To study the effect of long chronic juice consumption on:
   a- Plasma cholesterol, HDL cholesterol, TAG and glucose.
   b- VLDL cholesterol, TAG, phospholipids, apo B100 and apo B48.
   c- LDL cholesterol, TAG and phospholipids.
   d- Total apo B100 and total apo B48.
   e- Fecal cholesterol and TAG.
   f- Body weight.
   g- VLDL particle size.

1.2 Lipid digestion and absorption

Fat is the most concentrated source of energy in the diet. Each gram of fat, from any source, releases 9 kcal of energy when completely oxidized to carbon dioxide and water. This is 2.25 times as much energy as is released by an equal amount of carbohydrate or protein (Guthrie and Picciano, 1995). Fat is also the main form in which animals, including humans, store excess energy supplies. The body cells, with the exception of the cells of the nervous system and erythrocytes, can use fatty acids directly as a source of energy. In addition, fats perform several functions, such as furnishing essential fatty acids, sparing burning of protein for energy, promoting absorption of fat-soluble vitamins, providing a structural component of cell membranes, digestive secretions, and hormones, insulating and controlling body temperature in the form of body fat, and protecting body organs (Peckenpaugh and Poleman, 1999). TAG, constituting about 90% of dietary lipids, are digested at lipid-water interfaces. The rate of TAG digestion therefore depends on the surface area of the interface, combined with the emulsifying effect of bile acids which emulsify the lipid drops into tiny emulsion droplets, hence providing better access for pancreatic lipase (Williams, 1999). These bile acids are synthesized from cholesterol in the liver, stored in the gallbladder and released into the small intestine to emulsify the dietary lipids, mostly constituted of TAG. The emulsification of dietary lipids renders them
accessible to pancreatic lipases, primarily lipase and phospholipase A$_2$ (Zeman and Ney, 1996) as indicated by the following reactions:

\[
\begin{align*}
\text{Triglyceride} & \quad 2\text{-MG} & \quad \text{Fatty Acids} \\
\text{Activated by Bile Salts} & \quad & + 2 \\
\text{Pancreatic Lipase} & \quad & \text{Phospholipids} \\
\rightarrow & \quad & \text{Pancreatic Phospholipase A$_2$} \\
& \quad & \text{free fatty acids + lysophospholipids}
\end{align*}
\]

The enzymatic activity of pancreatic lipase, requiring colipase for binding, greatly increases when it contacts the lipid-water interface. Other lipases, such as phospholipase A$_2$ also preferentially catalyze reactions at interfaces. Phospholipase A$_2$ containing a hydrophobic channel, favors the degradation of phospholipids at the 2 position releasing a free fatty acid and a lysophospholipid (Mathews, 1996). Lipids are absorbed by a mechanism distinctly different from that of monosaccharides and amino acids. Fatty acids having fewer than 10 to 12 carbon atoms and 2-monoglycerides pass into the epithelial cells by simple diffusion (Guthrie and Picciano, 1995). A considerable fraction of the fatty acids, which are long-chain fatty acids, reach the blood stream by a different route and require bile for adequate absorption. Bile acids are amphipathic molecules, with both polar and non polar portions. They form micelles which take up the non polar degraded lipid molecules, so as to permit their transport into the enterocytes, leaving the micelles behind (Peckenpaugh and Poleman, 1999). The fatty acids and monoglyceride are transported into the endoplasmic reticulum, where they are used to synthesize TAG. Beginning in the endoplasmic reticulum and continuing in the Golgi apparatus, TAG are packaged with cholesterol, apolipoproteins and other lipids into particles called CM. CM are extruded from the Golgi into exocytotic
vesicles, which are transported to the basolateral aspect of the enterocyte. The vesicles fuse with the plasma membrane and undergo exocytosis, dumping the CM into the space outside the cells (Davidson et al., 1991). Transport of lipids into the circulation is also different from what occurs with sugars and amino acids. Instead of being absorbed directly into capillary blood, CM are transported first into the lymphatic vessels that penetrate into each villus. CM-rich lymph then drains into the lymphatic system, which rapidly flows into blood. Blood-borne CM are rapidly disassembled and their constituent lipids utilized throughout the body (Gunstone, 2001). An overview of lipid absorption and transport into blood is shown in figure 1.1.

Figure 1.1 Overview of lipid absorption and transport into blood (Guthrie and Picciano, 1995).
1.3 Lipid transport

1.3.1 Introduction

Absorption and transport of dietary lipids in the blood represents the classic problem of oil and water not mixing well as the lipids are very hydrophobic and the plasma is an aqueous environment. The problem is solved by associating very insoluble lipids with more polar ones like cholesterol and phospholipids and adding protein to form lipoprotein complexes that are hydrophilic (Mangiapane and Salter, 2001). The protein portions of lipoproteins, called apoproteins, constitute some 60% of some complexes and only 1% of others. The apoproteins are noncovalently bound to the surface of lipoproteins and act as binding sites and enzyme cofactors in the metabolism of the various particles. This fat-transport system is divided into two pathways: an endogenous one for lipids entering the bloodstream from the liver and nonintestinal tissues and an exogenous one for lipids absorbed from the intestine. Lipoproteins secreted into plasma, undergo enzymatic hydrolysis releasing fatty acids to tissues. In plasma, exchange of lipoprotein components, result in changes in their size and shape (Williams, 1999).

1.3.2 Lipoprotein molecular structure

Lipoproteins complexes are soluble aggregates of lipids and proteins that transport lipids through the blood and lymph. Despite their differences in lipid and protein composition, all lipoproteins share common structural features, notably a spherical shape that can be detected by electron microscopy. The lipoproteins help maintain in solubilized form some 500 mg of total lipid per 100 ml of human blood in the postabsorptive state, after the contents of a meal have been digested and absorbed into the bloodstream (Peckenpaugh and Poleman, 1999). The core of the lipoprotein particles, contains the
hydrophobic cholesterol ester and TAG molecules. A coat of more hydrophylic lipids, phospholipids and free cholesterol, provide a protection for cholesteryl ester and TAG from the aqueous environment. Components of this coat are arranged with their hydrophilic regions pointing outwards towards the plasma, and their hydrophobic regions pointed inwards towards the core of the particles. Other major constituents of the lipoprotein particle are the proteins known as apolipoproteins (Zeman and Ney, 1996). The general structure of a plasma lipoprotein is shown in figure 1.2.

**General Structure of a Plasma Lipoprotein**

"peripheral" apoprotein
(e.g., apo A-I, apo C-III, apo E

cholesterol
phospholipid

"integral" apoprotein
(apo B-100 or apo B-48

core of nonpolar lipids

cholesteryl ester
monolayer of membrane lipids

**Figure 1.2** General structure of a plasma lipoprotein (Guthrie and Picciano, 1995).
1.3.3 Lipoprotein classes, characteristics and composition

Lipoproteins are classified by the type and ratio of protein and fats they contain which determines their size and density. The largest lipoprotein complexes are CM and VLDL. The other two classes are HDL and LDL. Different subclasses are also included such as a lipoprotein of density composition between VLDL and LDL, known as intermediate-density lipoprotein (IDL), and other classes like HDL₂ and HDL₃ (Mangiapan and Salter, 2001). Moreover, lipoproteins are characterized by their electrophoretic mobility. When running electrophoresis, HDL migrate furthest, followed by VLDL, LDL, and finally CM that remain at the origin of the electrophoretic strip (Fisher et al., 1983). The characteristics and composition of the major lipoprotein classes are represented in Table 1.1.

1.3.4 Enzymes of lipoprotein metabolism

Lipoprotein metabolism depends on three different enzymes; hepatic lipase (HL), lipoprotein lipase (LPL) and lecithin cholesterol acyltransferase (LCAT). Besides, a specific transfer protein known as cholesteryl ester transfer protein (CETP), facilitates the exchange of cholesteryl esters between TAG-rich lipoproteins and HDL (Fuhler and Klisters, 1997).

1.3.4.1 Hepatic lipase (HL)

HL is synthesized in hepatocytes. It hydrolyses TAG molecules present on VLDL particles, thus enhancing their conversion to LDL, without the need of an apolipoprotein activator. On the other hand, an increase in the concentration of HDL₂, and small TAG-rich VLDL particles in plasma has been associated with HL deficiency (Breckenridge et al., 1982).
Table 1.1 Characteristics and composition of the major classes of plasma lipoproteins (Zeman and Ney, 1996).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>CM</th>
<th>VLDL</th>
<th>LDL</th>
<th>HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density (g/ml)</td>
<td>&lt; 0.95</td>
<td>0.95 - 1.006</td>
<td>1.019 - 1.063</td>
<td>1.063 - 1.210</td>
</tr>
<tr>
<td>Size (nm)</td>
<td>&gt; 200</td>
<td>30 - 200</td>
<td>10 - 30</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>Diameter (nm)</td>
<td>75 - 1200</td>
<td>30 - 200</td>
<td>18 - 25</td>
<td>7.5 - 20</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>$400 \times 10^6$</td>
<td>$10 - 80 \times 10^6$</td>
<td>$2.3 \times 10^6$</td>
<td>$1.7 - 3.6 \times 10^6$</td>
</tr>
<tr>
<td>Flotation (Sf)</td>
<td>&gt; 400</td>
<td>20 - 400</td>
<td>0 - 20</td>
<td>0 - 9</td>
</tr>
<tr>
<td>Electrophoretic Mobility</td>
<td>None</td>
<td>Pre-beta</td>
<td>Beta</td>
<td>alpha</td>
</tr>
<tr>
<td>Source</td>
<td>Intestine</td>
<td>Liver</td>
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<td>7</td>
<td>8</td>
<td>4</td>
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<td>Cholesterol ester (%)</td>
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<td>18</td>
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<td>64-80</td>
<td>10</td>
<td>2-5</td>
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<td>Phospholipids (%)</td>
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<td>6-15</td>
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<td>30</td>
</tr>
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<td>8-10</td>
<td>20</td>
<td>48</td>
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<td>B100, C-I, C-II, C-III,E</td>
<td>B100</td>
<td>A-I,A-II</td>
</tr>
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</table>
1.3.4.2 Lipoprotein lipase (LPL)

LPL is present primarily on endothelial cells in tissue capillary beds. It hydrolyzes TAG molecules present in VLDL and CM, resulting in release of free fatty acids and monoglycerides, with the apoprotein apo CII acting as a specific activator (Eckel, 1989).

1.3.4.3 Lecithin cholesterol acyltransferase (LCAT)

LCAT which is present in a number of tissues, including muscle and adipose tissue, acts on Phosphatidylcholine and free cholesterol present on HDL, by catalysing the transfer of fatty acid from the 2 position of phosphatidylcholine to cholesterol, resulting in the formation of lysophosphatidyl choline and cholesteryl ester, with both apoproteins of HDL, apo AI and apo CII, acting as specific activators (Norom et al., 1989).

1.3.4.4 Cholesterol ester transfer protein (CETP)

CETP acts by transferring TAG molecules from VLDL to HDL particles, and by transferring reciprocally cholesterol esters, from HDL to VLDL particles. On the other hand, significant increases in plasma HDL cholesterol concentrations has been associated with CETP deficiency (Inazu et al., 1990).
1.3.5 Lipoprotein metabolism and importance of apolipoproteins

Three major pathways contribute to the transport of lipids in lipoproteins: the endogenous pathway, the exogenous pathway, and the reverse cholesterol transport pathway. The distribution of lipids of hepatic origin corresponds to the endogenous pathway. The transport of lipids of dietary origin from the intestine to other tissues corresponds to the exogenous pathway. Whereas distribution of cholesterol from peripheral tissue to the liver corresponds to reverse cholesterol transport pathway (Mangiapane and Salter, 2001). An overview of lipoprotein transport pathways and fates is shown in figure 1.3.

Figure 1.3 An overview of lipoprotein transport pathways and fates (Guthrie and Picciano, 1995).
1.3.5.1 Chylomicrons (CM)

CM are the largest of the lipoprotein molecules. They are assembled in the intestinal mucosa as a means to transport dietary cholesterol and TAG to the rest of the body. CM are therefore, the molecules formed to mobilize dietary (exogenous) lipids. The predominant lipids of CM are TAG, and the major protein component is apolipoprotein B48 (apo B48), along with other proteins like apolipoprotein A (apo A) and apolipoprotein C (apo C) (Young, 1990). CM vary in size from 80 to 1000 nm. The larger particles contain relatively more TAG and less polar phospholipids and free cholesterol than the smaller ones. Upon entering the blood stream, CM undergo significant changes in their apolipoprotein composition by gaining apo E and apo Cs which are very important for the further metabolism of the CM particles (Zannis et al., 1991). Hydrolysis of TAG molecules cause the shrinking of CM particles, thus increasing the proportion of the surface material (phospholipid, free cholesterol and protein), which is consequently taken up by other lipoproteins, particularly HDL. The resulting particles, being much reduced in size, are called CM remnants. These particles are relatively enriched in cholesteryl ester although they still contain some TAG. Eventually CM Remnants are removed from the circulation by the liver. The uptake is mediated by apolipoprotein E (apo E) and the low-density lipoprotein receptor-related protein (LRP), which plays an essential role in mediating this uptake (Kowal et al., 1989).

1.3.5.2 Very low-density lipoproteins (VLDL)

The dietary intake of both fat and carbohydrate, in excess of the needs of the body, leads to their conversion into TAG in the liver. These TAG are packaged into VLDL, varying in size from 30 to 80 nm, and released into the circulation for delivery to the various tissues, mainly muscle and adipose tissue, for storage or production of energy through oxidation (Gunstone, 2001). VLDL are therefore, the molecules formed to transport endogenously
derived TAG to extra-hepatic tissues, as well as being the major precursor of LDL. In addition to TAG, VLDL contain some cholesterol and cholesteryl esters and the apoproteins, apo B100, apo CI, apo CII, apo CIII and apo E (Zannis et al., 1991). After secretion by the liver, VLDL acquire apo C and apo E from plasma HDL. On the other hand, hydrolysis of VLDL TAG and phospholipids by LPL and HL results in an increasingly smaller VLDL particles, converting VLDL to IDL, then to LDL, but with a constant content of apo B100. However, VLDL apo C and apo E, are transferred back to HDL, in the process of VLDL metabolism. The catabolism of VLDL remnant particles by the liver is mediated by LDL receptors and LRP (Brown et al., 1991).

1.3.5.3 Low-density lipoproteins (LDL)

The cellular requirement for cholesterol as a membrane component is satisfied in one of two ways: either it is synthesized de novo within the cell, or it is supplied from extra-cellular sources, namely, CM and LDL (Bilheimer et al, 1983). Cholesterol synthesized by the liver can be transported to extra-hepatic tissues if packaged in VLDL. In the circulation VLDL are converted to LDL through the action of lipoprotein lipase. LDL are the primary plasma carriers of cholesterol for delivery to all tissues (Mangiapan and Salter, 2001). Apo B100 is the exclusive apolipoprotein of LDL. Uptake of LDL occurs mostly in liver, and to a lesser extent in adrenals and adipose tissue, via LDL receptor-mediated endocytosis. Binding of LDL to liver cells is enhanced by Insulin and tri-iodothyronine (T3), whereas it is decreased by glucocorticoids (Utermann, 1989). The endocytosed membrane vesicles fuse with lysosomes, in which the apolipoproteins are degraded and the cholesterol esters are hydrolyzed resulting in free cholesterol which is then incorporated into the plasma membranes. Excess intracellular cholesterol is re-esterified by acyl-CoA-cholesterol acyltransferase (ACAT), for intracellular storage (Carr and Simpson, 1991).
1.3.5.4 High-density lipoproteins (HDL)

HDL particles are the smallest of the human lipoproteins, ranging from 7.5 to 20 nm, and contain almost equal proportions of lipids and proteins (Ginsberg, 1990). HDL are synthesized in the liver as disk-shaped particles, containing predominantly proteins, phospholipids and free cholesterol, and to a lesser extent, cholesteryl esters (Guthrie and Picciano, 1995). HDL major apolipoproteins are apo AI, apo CI, apo CII and apo E. In fact, HDL particles provide a reservoir for C apolipoproteins which are transferred to CM and VLDL during alimentary lipemia, and later shifted back to HDL when the larger fat-rich particles are cleared from plasma (Zannis et al., 1991). Free cholesterol found in CM remnants and VLDL remnants can be esterified by lecithin cholesterol acyltransferase (LCAT), which is activated by apo AI found in HDL, thus converting HDL particles to spherical particles, HDL2 and HDL3, that are enriched in cholesteryl esters (McNamara, 1995). Cholesterol-rich HDL undergo endocytosis in the liver. Hepatic uptake of HDL is mediated by an HDL-specific apo AI receptor. Macrophages also take up HDL through apo AI receptor interaction (Mangiapane and Salter, 2001). Aquiring cholesterol and apo E from the macrophages, HDL particles are then secreted into the circulation. Apo E enhances HDL uptake and catabolism by liver cells. Besides, HDL are able to obtain cholesterol by extracting it from cell surface membranes, hence, lowering the level of intracellular cholesterol (Tall, 1990).

1.3.6 Atherogenicity of lipoprotein particles

Plasma lipoprotein particles have been related to atherosclerosis. For instance, numerous studies have indicated that increased plasma concentrations of small LDL particles and cholesterol-rich remnant particles, are accompanied by increased risk of coronary heart disease (CHD) (Mahley and Rao, 1989; Scanu et al., 1991; Rader and Brewer, 1992). whereas in other studies, increased levels of HDL cholesterol, provided a significant protection from atherosclerosis (Avogaro et al., 1979; Gordon and Rifkind,
1989). Other reports on the other hand, have indicated that LDL apo B is a preferred predictor of CHD than is the LDL cholesterol level, where high concentrations of LDL apo B were associated with increased risk of CHD (Fredrickson et al., 1978; Sniderman et al., 2001). Besides, HDL apo A was shown to be a better indicator for CHD than are measurements of HDL cholesterol (Reinhart et al., 1990). Furthermore, hyperchylomicronemia does not appear to be associated with an increased risk of CHD, but concomitant elevations of VLDL do appear to be moderately atherogenic (Santamarina and Brewer, 1991).

1.3.7 Structure and function of apolipoproteins

Lipoprotein particles contain polypeptide chains within them, referred to as apoproteins (apolipoproteins). Eleven of them- AI, AII, AIIV, B48, B100, CI, CII, CIII, D, E, Lp(a)- have been sequenced so far (Schonfeld, 1990; Breslow, 1991). Apolipoproteins, have one common structural feature, a domain containing an amphipathic helix, where hydrophobic amino acids are arranged on one side of the helix and hydrophilic amino acids form the other side (Karpe et al., 1993; Schneeman et al., 1993). The liver and the intestinal mucosal cells are the major sites of synthesis of apolipoproteins (Segrest, 1974). On the other hand, all of the apolipoproteins are capable of shifting from one lipoprotein particle to the other, with the exception of apo B48, apo B100, and apo A (Kane et al., 1980). In addition to their structural role in maintaining lipoprotein stability, several apolipoproteins interact with cellular receptors, thus playing an essential role in the intravascular metabolism and cellular uptake of lipoproteins (Zannis et al., 1991). Besides, apolipoproteins act as activators and inhibitors of enzymes involved in lipoprotein metabolism (Young, 1990). The metabolic roles of plasma apolipoproteins are illustrated in Table 1.2.
Table 1.2 Metabolic functions of plasma apolipoproteins (Guthrie and Picciano, 1995).

<table>
<thead>
<tr>
<th>Apolipoprotein</th>
<th>Molecular Weight</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>AI</td>
<td>28,300</td>
<td>Major protein in HDL; activates LCAT</td>
</tr>
<tr>
<td>AII</td>
<td>17,400</td>
<td>Major protein in HDL</td>
</tr>
<tr>
<td>B48</td>
<td>241,000</td>
<td>Found exclusively in CM</td>
</tr>
<tr>
<td>B100</td>
<td>513,000</td>
<td>Major protein in LDL</td>
</tr>
<tr>
<td>CI</td>
<td>7,000</td>
<td>Found in CM; activates LCAT and LPL</td>
</tr>
<tr>
<td>CII</td>
<td>10,000</td>
<td>Found primarily in VLDL; activates LPL</td>
</tr>
<tr>
<td>CIII</td>
<td>9,300</td>
<td>Found in CM, VLDL, and HDL; inhibits LPL</td>
</tr>
<tr>
<td>D</td>
<td>35,000</td>
<td>HDL protein, also called cholesterol ester transfer protein</td>
</tr>
<tr>
<td>E</td>
<td>33,000</td>
<td>Found in VLDL, LDL, and HDL</td>
</tr>
</tbody>
</table>

Apo B is a hydrophobic apolipoprotein, which is synthesized in the liver and in the intestine, in two molecular forms, apo B100 and apo B48 respectively, as a surface component of VLDL, LDL and CM (Powell et al., 1987; Elovson et al., 1988). Increased fat intake is possibly behind the evolvement and adaptation of intestinal cells to synthesize apo B48, with a greater and more rapid synthetic capacity than would be observed for apo B100 (Bell Quint et al., 1981). Also, apo B48 containing CM can accommodate more apo E
molecules per particle, which may enhance their subsequent uptake by the liver (Zannis et al., 1991). The normal formation of CM and VLDL-LDL particles appears to be directly related to the synthesis of apo B48 within intestinal mucosal cells and of apo B100 within the liver respectively (Karpe et al., 1996).

1.4 Grapefruit

**Name:** Grapefruit  
**Scientific Name:** Citrus paradisi  
**Family:** Rutaceae

Grapefruits are round, with a diameter of between 10 and 15 cm. Their thin skin may be either completely yellow or yellow with a pinkish hue. The pulp of the fruit may be yellow, pinkish, or reddish. It can be more or less sharp-tasting, acidic, sweet, and fragrant (Williams, 1999).

1.4.1 *Nutrition facts and benefits*

**Table 1.3** Grapefruit's nutritional information (Peckenpaugh and Poleman, 1999)

<table>
<thead>
<tr>
<th>Nutritional information per 100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Water</strong></td>
</tr>
<tr>
<td><strong>Proteins</strong></td>
</tr>
<tr>
<td><strong>Fat</strong></td>
</tr>
<tr>
<td><strong>Carbohydrates</strong></td>
</tr>
<tr>
<td><strong>Fibers</strong></td>
</tr>
<tr>
<td><strong>Calories</strong></td>
</tr>
</tbody>
</table>
Grapefruit is high in vitamin C, potassium and pectin fibers. It's also a good source of folate and minerals like iron and calcium. Besides, it contains bioflavonoids and other plant chemicals known to protect against cancer and heart diseases. The major grapefruit flavonoid is naringenin. Grapefruit stimulates the appetite and is used for its digestive, antiseptic, tonic, and diuretic qualities (Ameer et al., 1996). The nutritional value of grapefruit varies with color (white, pink, or red). Red and pink grapefruits have a higher amount of vitamin A, also, they are high in beta-carotene, an antioxidant that the body converts to vitamin A. In general, half a grapefruit provides more than 50 percent of the adult Recommended Dietary Allowance (RDA) of vitamin C; it also has 325mg of potassium, 25μg of folate, 40mg of calcium, and 1mg of iron (Peckenpaugh and Poleman, 1999).

1.4.2 Several uses of grapefruit

Grapefruit and Cholesterol Control: Grapefruits are high in pectin and naringenin, a soluble fiber that is structurally similar to genistein. Studies have shown that the grapefruit pectin has an effect on cholesterol levels and atherogenesis with established hypercholesterolemia (Cerda et al., 1994; Buhler and Miranda, 1998).

Grapefruit for Cancer Control: Several studies have indicated that grapefruits contain substances that are useful in preventing several diseases. Naringenin the major grapefruit flavonoid was proved to inhibit mammary tumorigenesis induced in female Sprague–Dawley rats (Hertog et al., 1993).

Grapefruit and antioxidant activity: Grapefruit extract showed an antioxidant activity on vegetable oils and Freeze-dried grapefruit peel showed remarkable antioxidant activity, due to the flavonoids naringenin and hesperetin (Hertog et al., 1990).
1.4.3 Interactions with drugs and medicines

Grapefruit has serious interactions with many commonly prescribed medications like for instance Viagra (Sildenafil), Valium (Diazepam), Zocor (Simvastatin), and Lipitor (Atorvastatin) (Watkins, 1990). Grapefruit juice inhibits a special enzyme in the intestines that is responsible for the natural breakdown and absorption of many medications (Guthrie and Picciano, 1995).

1.4.4 Cardioprotective effect of grapefruit

Atherosclerosis is still one of the most dangerous diseases in industrial countries and the principal cause of death in Western civilization (Hennekens and Gaziano, 1993). Evidence have suggested that one of the important mechanisms predisposing to development of atherosclerosis is the oxidation of cholesterol-rich LDL particles (Steinberg et al., 1989; Leake, 1991). Oxidation of LDL enhances its atherogenicity and facilitates penetration of lipids into the arterial wall (Rimm et al., 1994; Bartnikowska, 1999). A considerable amount of epidemiological and clinical evidence has demonstrated a significant decrease in morbidity and mortality from cardiovascular and other diseases among fruit and vegetable consumers (Gey et al., 1993; Hertog et al., 1995). The positive influence of such diet is attributed to their polyphenolic compounds, and particularly flavonoids which are known to possess antioxidant effect (Morel et al., 1993; Longeril et al., 1994; Partliff et al., 1994). Studies conducted on the components of grapefruits (Citrus paradisi), oranges (Citrus sinensis) and lemons (Citrus limons), showed the presence of high amounts of polyphenols, dietary fibre, ascorbic acid and trace elements (Peleg et al., 1991; Marlett, 1992). These components possess antioxidant properties (Gorinstein et al., 1994; Bocco et al., 1998) and are known to be effective in prevention and treatment of atherosclerosis and its complications (Whalley et al., 1990).
1.5 Orange

Name: Orange
Scientific Name: *Citrus sinensis*
Family: Rutaceae

Native to tropical Asia, orange is now grown throughout the tropics, subtropics and along the Mediterranean coast. Orange is high in vitamin C, folate and bioflavonoids. It also contains, coumarins, triterpenes, carotene, and pectin. The orange flavonoids are well known for their several useful properties, especially the flavonoid hesperidin (Zeman and Ney, 1999).

1.5.1 Nutrition facts and benefits

Table 1.4 Orange’s nutritional information (Peckenpaugh and Poleman, 1999)

<table>
<thead>
<tr>
<th>Nutritional information per 100 g</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>87 %</td>
</tr>
<tr>
<td>Proteins</td>
<td>0.94 g</td>
</tr>
<tr>
<td>Fat</td>
<td>0.12 g</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>11.75 g</td>
</tr>
<tr>
<td>Fibers</td>
<td>2.4 g</td>
</tr>
<tr>
<td>Calories</td>
<td>47 Kcal</td>
</tr>
</tbody>
</table>
Orange fruit is well known for its properties in stimulating digestion and relieving flatulence and abdominal bloating. The juice helps the body eliminate waste products, and, being rich in vitamin C, helps the immune system ward off infection (Peckenpaugh and Poleman, 1999). Hesperidin is the predominant flavonoid in oranges. It has demonstrated the ability to favorably affect lipids and to treat some vascular disorders in humans. Besides, anticancer, antiallergenic and antiinflammatory effects have been seen with the use of hesperidin in numerous animal studies (Williams, 1999).

1.5.2 Orange juice reduces risk of coronary heart disease

Epidemiologic studies indicate that a high consumption of fruits and vegetables reduces the risk of coronary heart disease. It is thought that this benefit may be due to the minor components, falvonoids, which have been proposed to inhibit LDL oxidation and platelet aggregation as well as to vitamins C, E, and Beta-carotene, which act as antioxidants (Hertog et al., 1995). Orange juice is rich in flavonoids, folate, and vitamin C, leading it to be potentially beneficial in reducing the risk of heart disease (Peckenpaugh and Poleman, 1999). It was found that frequent intake of orange juice increased HDL-cholesterol concentrations and decreased the LDL-HDL cholesterol ratio (Hertog et al., 1995). Moreover, a recent study showed that hesperidin, the predominant flavonoid in orange, has a direct increasing effect on HDL cholesterol (Kurowska et al., 2000). On the other hand, folate was shown to be effective in lowering homocysteine levels in blood, thus decreasing risk of CHD (Guthrie and Picciano, 1995).
1.6 Pineapple

**Name:** Pineapple, ananas  
**Scientific Name:** *Ananas Comosus*  
**Family:** Bromeliaceae

Native to Central and South America, pineapples also grow in other tropical regions. Fresh pineapples weigh from 1 to 2.5 Kg and contain the enzyme bromelain, which has many beneficial properties. The unpeeled fruit is brownish with a hard, spiky covering and a large green sprout from its crown (Taussig and Batkin, 1988).

### 1.6.1 Nutrition facts and benefits

**Table 1.5** Pineapple’s nutritional information (Peckenpaugh and Poleman, 1999)

<table>
<thead>
<tr>
<th>Nutritional information per 100 g</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>85 %</td>
</tr>
<tr>
<td>Proteins</td>
<td>0.5 g</td>
</tr>
<tr>
<td>Fat</td>
<td>0.1 g</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>12.6 g</td>
</tr>
<tr>
<td>Fibers</td>
<td>1.4 g</td>
</tr>
<tr>
<td>Calories</td>
<td>48 Kcal</td>
</tr>
</tbody>
</table>

Pineapple is an excellent source of vitamins A, C and to a lesser extent, B. It is rich in manganese which is an essential part of certain enzymes needed for metabolizing proteins and carbohydrates (Williams, 1999). It was also shown to reduce acidity of urine, normalize menstruation and improve high blood
pressure (Guthrie and Picciano, 1995). Its enzyme Bromelain which is used for digesting proteins is also an effective anti-inflammatory for treatment of minor injuries such as bruising and sprains. In addition, preliminary studies have shown that it may be useful in the treatment of sinusitis, arthritis. It is also known to enhance the effectiveness of medical antibiotics and to contain antibiotic potential itself (Hale et al., 2002).

1.6.2 Cardiovascular effect of bromelain

Several studies have indicated that bromelain prevents or minimizes the severity of angina pectoris. A drastic reduction in the incidence of coronary infarct after administration of bromelain has been reported. It was also shown that bromelain is effective in prevention of certain cardiovascular diseases (Nieper, 1987).
Chapter 2

MATERIALS AND METHODS

2.1 Animal treatment

Animals were maintained and experimental protocols compiled with the Guide for the Care and Use of Laboratory Animals (National Research Council of the United States 1985). All animals were sacrificed using diethyl ether, at the end of the procedures described, without recovery from anaesthesia.

2.1.1 Acute juice study

Male Sprague-Dawley rats (n=32) weighing 200-250 g (Lebanese American University stock) were maintained at an ambient temperature of 20-22°C and were fed the same standard rat chow diet until 18 hours prior to experimentation where they had free access to water only. The fasted rats were divided into four groups of 8 rats each: control, grapefruit, orange and pineapple. The rats received intragastrically, using stomach tubes, 5ml emulsion of either 20% (w/v) olive oil, 4.5% (w/v) sucrose in water (control) or 20% (w/v) olive oil in either grapefruit, orange, or pineapple juice for the three remaining groups. The juices were taken from SANTAL® bottles, since this brand is consumed by a large number of the population. All emulsions were prepared using the ultrasonic homogenizer where each mixture was sonicated just before administration, using the microtip, intermittently at 40% amplitude for a total period of 1 minute. Three hours after intragastric instillation, blood samples were collected from the inferior vena cava, and plasma concentrations of TAG, total cholesterol, HDL cholesterol and glucose were determined. Similarly, TAG, cholesterol and phospholipid concentrations were
measured for the lipoprotein fractions: CM (Sf > 400), VLDL (Sf 20- 400) and LDL (Sf 0-20) after density gradient ultracentrifugation of the serum samples. To determine the effect of juice on gastric emptying in animal receiving the intragastric load, the stomach of each animal was ligated at the distal esophagus and proximal duodenum just after blood withdrawal, removed, opened and the liquid content was estimated after blotting with a pre-weighed filter paper. The TAG adsorbed to the filter paper was extracted according to the method of Folch et al. (1957). Briefly, the filter paper TAG was extracted with chloroform:methanol (2:1) to a final dilution 20 times the mass of the filter paper. The solvent was filtered through a filter paper into a glass-stoppered bottle. The crude extract was washed with 0.2 volume of a salt solution (0.9 % NaCl) in an extraction funnel, and then allowed to separate into two phases. The lower phase was drained and solvent was removed by rotary evaporation to yield a dry extract for weighing.

2.1.2 Long-chronic juice study

In the aim of studying the effects of Long-chronic juice intake on plasma lipoproteins in the fasted state, male Sprague-Dawley rats (n=64) weighing 200-250g (Lebanese American University stock) were used and fed the same rat chow diet 7g/100g (body weight). Three experimental groups received, on a daily basis, drinking water containing 12ml/100g (BW) juice, either from grapefruit, orange or pineapple in a ratio of 1 : 1 juice to water, for a period of 6 months, whereas the control group received 12ml/100g (BW) water in addition to sucrose 5.5g/100ml (water) in order to maintain an isocaloric diet relatively to the other three experimental groups. All rats in the four groups were weighed on a weekly basis, and modifications of their diets and juice intake were done according to changes in body weights. Animals were maintained on the same daily juice intake until 18 hours prior to experimentation where they had free access to water only. Blood samples were then collected from the inferior vena cava without any previous intragastric instillation.
2.2 Determination of rats body weight

All rats in the four different groups were weighed using a balance (Denver Instrument XL-410, U.S.A.) every monday on a weekly basis at noon time for a period of 6 months in order to follow the changes in their body weights. Food, juice and water quantities were changed relatively to changes in rats body weights.

2.3 Fecal study

2.3.1 Determination of fecal triacylglycerol (TAG) and cholesterol

Principle of TAG assay
The TAG assay kit (Spinreact, S.A, Spain) used was based upon a colorimetric method. The TAG are enzymatically hydrolyzed to glycerol and free fatty acids. The glycerol liberated reacts with Glycerol Kinase (GK) and Glycerol-3-Phosphate Oxidase (GPO) yielding H$_2$O$_2$. The H$_2$O$_2$ concentration is determined through the Trinder's reaction. The enzymatic reaction sequence employed in the assay was as follows:

\[
\begin{align*}
\text{Triacylglycerol} + H_2O & \xrightarrow{\text{LPL}} \text{Glycerol} + \text{Fatty Acids} \\
\text{Glycerol} + \text{ATP} & \xrightarrow{\text{GK}} \text{Glycerol-3-phosphate} + \text{ADP} \\
\text{Glycerol-3-phosphate} + O_2 & \xrightarrow{\text{GPO}} \text{Dihydroxyacetone-P} + H_2O_2 \\
H_2O_2 + 4-\text{AP} + p-\text{Chlorophenol} & \xrightarrow{\text{POD}} \text{Quinonimine(red)} + H_2O
\end{align*}
\]
**Principle of cholesterol assay**

The cholesterol assay kit (Spinreact, S.A, Spain) used was based upon a colorimetric method. Cholesterol and its esters are released from lipoproteins by detergents. Cholesterol esterase hydrolyses the esters and H$_2$O$_2$ is formed in the subsequent enzymatic oxidation of cholesterol by cholesterol-oxidase according to the following equations:

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Catalyst</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol Esters + H$_2$O $\xrightarrow{\text{CHE}}$ Cholesterol + Fatty Acids</td>
<td></td>
</tr>
<tr>
<td>Cholesterol + O$_2$ $\xrightarrow{\text{CHOD}}$ Cholest-4-en-one + H$_2$O$_2$</td>
<td></td>
</tr>
<tr>
<td>H$_2$O$_2$ + 4 AP + Phenol $\xrightarrow{\text{POD}}$ Quinonimine (Red) + H$_2$O</td>
<td></td>
</tr>
</tbody>
</table>

**Procedure**

Fresh stools were collected from the rats of each of the four groups, weighed immediately then incubated in the oven (Imperial V, # 3476-1) at 60°C for 24 hours. The dried stools were then ground using a mortar and a pestle and samples weighing 0.2 g were used for TAG and cholesterol extraction. Extraction was performed for 4 hours using 3 ml of $n$-hexane at 50°C with constant agitation. All samples were then centrifuged at 5000 g for 20 minutes. Supernatants containing the TAG and cholesterol were isolated and incubated at 80°C until complete evaporation of $n$-hexane. Following that, TAG and cholesterol contents were calculated using TAG and cholesterol assay kits (Spinreact, S.A, Espana), (Dafer et al., 2003). Samples, along with standards, were run in duplicates, and were mixed with the working reagent, and incubated 10 minutes at room temperature. The absorbances (Abs.) of the unknown and the standard samples were measured against Blank reagent at $\lambda = 505$ nm (Helios-γ spectrophotometer, UVG 101103). Color of the mixtures was varying from light pink to dark red, and it was stable for about 30 mn.
Calculation
Fecal TAG/Cholesterol concentration (conc.) was calculated using a TAG/cholesterol standard of 200 mg/dl concentration according to the following equation:
Sample conc. (mg/dl) = (Abs. of unknown x conc. of standard) / Abs. of standard

2.4 Blood study

Three hours after intragastric instillation of the corresponding loads, animals were rapidly anaesthetized using diethyl ether and placed supine on a homeothermic table. Anesthesia was maintained by the use of a nose cone. A midline abdominal incision was made for about two-thirds of the length of the abdomen and the inferior vena cava was exposed by exteriorizing the intestine. Using 10ml syringes containing disodium ethylene-diamine-tetraacetic acid (Na₂EDTA, 1 mg/ml), about 8 ml of blood were withdrawn from the inferior vena cava and transferred into 10 ml tubes. Centrifugation was carried at a speed of 2000g for 20 minutes at a temperature of 4°C. After centrifugation, the plasma was collected and divided into two aliquots: one sample (V=2 ml) was used to isolate lipoprotein fractions as described in the following section and the remaining volume of plasma was collected in 1.5 ml Eppendorf tubes, stored at -20°C for later blood lipid analysis.

2.5 Isolation of lipoprotein fractions

Each 2 ml of collected plasma was placed in the bottom of a 10 ml polycarbonate ultracentrifuge tube (Sorvall, Kendro, Laboratory Products) put on ice. Proteolytic degradation was minimized by adding a preservative solution containing aprotonin, 2 mg/liter, and phenylmethylsulfonylfluoride
(PMSF), 10 mM dissolved in 2-propanol, at concentrations of 5 μl/ml plasma and 10 μl/ml plasma respectively. Solid KBr (0.34 g/ml) was added to the plasma samples and vortex mixed in order to increase the density to 1.21 g/ml. A 2 ml NaCl solution (1.006 g/ml NaCl was prepared, containing 0.01% (w/v) Na₂EDTA and 0.02% sodium azide (NaN₃) with the pH adjusted to 7.4) was layered on top of the plasma in the ultracentrifuge tube using the peristaltic pump machine (Watson-Marlow Limited, England).

The gradients were then centrifuged for 33 minutes (CM Sf > 400) and 24 hours (VLDL Sf 20-400) and (LDL Sf 0-20) at 15°C in the F-28/13 Supraspeed fixed-angle rotor of the Sorvall RC 28S centrifuge at 28000 rpm. After the first centrifugation, the top TAG-rich lipoprotein (CM) fraction of the gradient was carefully aspirated and replaced with an equal volume of the NaCl solution of d = 1.006 g/ml using the peristaltic pump machine before proceeding with the second centrifugation, after which the upper layer (VLDL) (V = 0.5 ml) and the lower layer (LDL) (V=1.5ml) were respectively aspirated. The lipoprotein fractions collected after each centrifugation were divided into two parts: one sample (V = 300 μl) was used for apo B48 and apo B100 analysis and the remaining volume was placed in 1.5 ml Eppendorf tubes stored at -20°C for subsequent analysis.

Total apo B (apo B48 and apo B100) content in the plasma of fasted animal was calculated in the lipoprotein fraction (d<1.063 g/ml) that includes CM, VLDL, IDL and LDL. Briefly, 2 ml of plasma were put in a 10 ml polycarbonate ultracentrifuge tube (Sorvall, Kendro Laboratory Products) and 140 mg/ml of solid NaCl was added to increase the density to 1.1 g/ml. The plasma sample was overlaid with 5 ml of NaCl solution (d=1.063 g/ml) containing 0.01 % w/v Na₂EDTA and 0.02% w/v NaN₃ (pH = 7.4). The top 0.5 ml lipoprotein layer was collected after 48h of centrifugation at 28,000 rpm at 15 °C (Sorvall RC 28S centrifuge; Supraspeed F-28/13 fixed angle rotor).
2.6 Preparation of samples for apo B48 and apo B100 analysis

Samples containing the different lipoprotein fractions were delipidated according to Karpe et al. (1996) in a methanol-diethyl ether solvent system. A volume of 300 μl of the lipoprotein fraction sample was injected into 4 ml ice cold methanol in a 10-ml glass tube with a syringe (1ml) to ensure efficient delipidation.

A volume of 4 ml of ice-cold diethyl ether was then added to the methanol and the delipidation mixture was centrifuged for 30 min at 4000g at -4°C. After removal of the solvent, another 4 ml of ice-cold diethyl ether was immediately added, and the sample was vortexed and centrifuged for 20 min under the same conditions. After centrifugation, the diethyl ether was removed and the protein material was dissolved in 50-150 μl of sample buffer at room temperature for 30 min: Sample buffer is a solution containing 0.15M sodium phosphate, 12.5% (v/v) glycerol, 2% (w/v) sodium dodecyl sulfate (SDS), 5% (v/v) mercaptoethanol, 0.001% (w/v) bromomophenol blue, adjusted to pH=6.8. The dissolved protein mixture was then transferred to an Eppendorf tube, denatured at 90°C for 3 min after which it was centrifuged for 3 min at 13 000g. Samples were then frozen at -20°C for later analytical SDS-PAGE.
2.7 Sodium dodecyl-sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

2.7.1 Principle

Similarly to many other important biological molecules, proteins as amphoteric compounds have their net charge determined by the pH of the medium in which they are suspended. In a solution with a pH above its isoelectric point, a protein has a net negative charge and migrates towards the anode in an electric field. Below its isoelectric point, the protein is positively charged and migrates towards the cathode. The net charge carried by a protein is in addition dependent on its size, the charge carried per unit mass of molecule differing from a protein to another. Consequently, at any given pH other than their isoelectric point, and under non-denaturing conditions, the electrophoretic separation of proteins is determined by both size and charge of the molecules. Separation of proteins by SDS-PAGE electrophoresis is based on coating all proteins with SDS so that all proteins have a uniform charge. Separation is therefore dependent on the size of the protein-sodium dodecyl sulfate complex. Hence, in SDS-PAGE separations, migration is determined not by intrinsic electric charge of polypeptides but by molecular weight (Laemmli, 1970; Horton et al., 2002). In SDS PAGE, the polyacrylamide gel may be prepared so as to provide a wide variety of electrophoretic conditions. The pore size of the gel may be varied to produce different molecular sieving effects for separating proteins of different sizes. In this way, the percentage of polyacrylamide can be controlled in a given gel. In polyacrylamide gels, the effective pore size is inversely related to acrylamide concentration in the polymerization mixture (Hames, 1990). SDS, an anionic detergent with a long hydrophobic tail, binds to the hydrophobic side chains of amino acids at a constant ratio of 1.4 g of SDS to 1 g of polypeptide. This confers a net negative charge to the polypeptide in proportion to its length and to the molecular weight of the protein (Hames, 1998). This ratio ensures that all SDS-protein complexes have a similar mass:charge ratio; hence,
eliminating the intrinsic charge of the protein as a factor affecting migration (Rybicki and Purves, 2001). Moreover, SDS blocks hydrophobic interactions, and substantially unfolds the protein molecules, minimizing differences in molecular form by eliminating the tertiary and secondary structures (Lodish et al., 2000). When proteins are treated with 2-mercaptoethanol or dithiothreitol (DTT) (in addition to SDS), all disulphide bonds between cysteine residues are broken, producing subunits of proteins (Hames, 1998). Urea may also be employed to disrupt hydrogen bonds. Following SDS-PAGE separation, the protein bands are visualized directly on the polyacrylamide gel by staining with Coomassie Blue. A concentration of 0.2-0.5 μg of any protein can be detected in a sharp band using this dye. Staining is quantitative up to 15-20 μg for at least some proteins (Hames, 1990). Not all proteins have the same chromogenicity with this dye, therefore, standards of the same proteins are usually included in the same gel for appropriate quantification.

2.7.2 Stock solutions

Protogel: 30 g acrylamide and 0.8 g N, N'-bis-methylene acrylamide were dissolved in approximately 70 ml of distilled water; the final volume was adjusted to 100 ml.

1.5 % ammonium persulfate solution: 0.15 g of ammonium persulfate was dissolved in 10 ml of distilled water.

0.5 M Tris buffer (pH 6.8): 6.02 g Tris was dissolved in approximately 90 ml distilled water and the pH adjusted to 6.8 with 12 M HCl. Then the solution was diluted with distilled water to a final volume of 100 ml. This solution is also stable for several weeks at 4°C.
**Water-saturated n-butanol:** 90 ml n-butanol + 10 ml water.

**Electrode buffer (pH 8.3):** 42.6 g glycine and 9.18 g Tris were dissolved in 2 L of distilled water, then 3 g of SDS were added to the Tris/Glycine solution and the final volume made up to 3 L with the addition of 1 L distilled water.

**3M Tris buffer (pH 8.8):** 36.6 g Tris was dissolved in about 80 ml distilled water and pH adjusted to 8.8 with 12 M HCl. The solution was then diluted with distilled water to a final volume of 100 ml. This solution is stable for several weeks at 4°C.

**Urea/SDS/dithiothreitol solution:** 19.2 g urea, 12.3 mg dithiothreitol and 0.08 g SDS were dissolved in 30 ml of distilled water and the final volume was made up to 40 ml.

### 2.7.3 SDS-PAGE preparation

#### 2.7.3.1 Preparation and pouring of resolving and stacking gels

Following the method of Hames (1990) according to the original method of Laemmli (1970), linear gradient (5-20%) polyacrylamide gels were prepared to allow an increased protein sieving effects and a better separation of a larger range of protein molecular weights. A 5% acrylamide resolving gel, a 20% acrylamide resolving gel and a stacking gel, to be layered on top of the resolving gels, were prepared as shown in Table 2.1.
Table 2.1 Gel mixtures for 5-20% gradient gels

<table>
<thead>
<tr>
<th></th>
<th>Resolving Gel</th>
<th></th>
<th>Stacking Gel</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5%</td>
<td>20%</td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>-</td>
<td>4.5 g</td>
<td>-</td>
</tr>
<tr>
<td>3M Tris Buffer</td>
<td>3.7 ml</td>
<td>3.7 ml</td>
<td>-</td>
</tr>
<tr>
<td>0.5M Tris Buffer</td>
<td>-</td>
<td>-</td>
<td>3.75 ml</td>
</tr>
<tr>
<td>Protogel</td>
<td>5 ml</td>
<td>20 ml</td>
<td>3.7 ml</td>
</tr>
<tr>
<td>Urea/SDS/DTT</td>
<td>3 ml</td>
<td>300 μl</td>
<td>0.6 ml</td>
</tr>
<tr>
<td>1.5% APS</td>
<td>0.7 ml</td>
<td>0.7 ml</td>
<td>2.3 ml</td>
</tr>
<tr>
<td>Water</td>
<td>20.2 ml</td>
<td>2.7 ml</td>
<td>15 ml</td>
</tr>
<tr>
<td>TEMED</td>
<td>23 μl</td>
<td>23 μl</td>
<td>22.5 μl</td>
</tr>
</tbody>
</table>

The electrophoresis apparatus (Hoefer SE 600, Amersham Pharmacia Biotech) was assembled according to the manufacturer's instructions. Clamped glass plates were tested to ensure they are water tight. Immediately before pouring the resolving gels, 11.5 μl tetramethylethylenediamine (TEMED) was added to each of the gels to initiate polymerization. The 20% gel was placed in the mixing chamber and the 5% gel in the reservoir chamber of a gradient forming apparatus (Coleparmer, USA). Using a
peristaltic pump, the gels were poured into the glass chambers while stirring of the 20% chamber. After the resolving gel has been poured, it was overlaid with water-saturated butanol and left to polymerize. After about 45 min, the water-saturated butanol was discarded and the gel top was rinsed several times with distilled water. The remaining water droplets were removed with a filter paper. A fifteen-well comb was inserted between the glass plates leaving about 0.5 cm between the bottom of the combs and the resolving gel. Before overlaying the polymerized resolving gel with the stacking gel, 11.25 µl of Temed were added to the stacking gel. The poured stacking gel was left for about 30 min to polymerize then the comb was carefully withdrawn. Electrode buffer was added at this stage to the wells of the gel in order to avoid any later disturbance in the wells. Protein solutions were loaded in the wells along with the molecular weight marker and the protein standard, after which the upper and lower electrode chambers were filled with electrode buffer.

2.7.3.2 Preparation of standard apolipoprotein B100

Since rat apo B48 standards are difficult to prepare, human apo B100 was used as a standard for apo B48 quantification. Kotie et al. (1995) and Van Beek et al. (1998) have shown that apo B100 and rat apo B48 have similar chromogenicity when stained with Coomassie Blue R-250, hence validating the use of human apo B100 as a standard. Apo B100 standards prepared from human LDL (1.030<d<1.040 g/ml) isolated from fasting human plasma samples by the density gradient ultracentrifugation procedure described by Karpe et al. (1996). Briefly, apo B100 was derived from LDL isolated from fasting human plasma samples by the density gradient ultracentrifugation procedure. The LDL subfraction was recovered from a 1 ml portion located 5.5 to 6.5 ml below the top of the gradient after 16 hour of ultracentrifugation. Fast and careful desalting was achieved by passing the LDL through a PD-10 column (Pharmacia). The total protein content was then determined using the Lowry assay (1951) with addition of SDS (final concentration 1 %) to the
reagent to reduce turbidity. The mean of several determinations was taken as the final protein value. After delipidation of LDL as previously described, the dissolved protein mixture was denatured at 95°C for 5 minutes. Batches of the delipidated and denatured apo B100 protein standard can be stored at -20°C and thawed immediately before use.

2.7.3.3 Loading of samples and electrophoresis

Molecular weight markers, (Sigma Chemical Co.Ltd) previously dissolved in sample buffer according to the supplier's instructions, were thawed with previously delipidated samples using disposable gel loading tips, 10 µl of molecular weight marker solution was loaded in one of the lanes. 0.2, 0.8, and 3.2 µg of standardized serum samples were loaded in three other lanes. The remaining lanes were loaded with the lipoprotein fractions under examination. All samples were run in duplicate, thus using more than one gel. After loading all samples, electrodes of the upper chamber lid were properly attached, and the water-cooling system and power supply (Consort E455, Belgium) were then switched on. Electrophoresis was carried out at 220V, 120mA, 20W for both gels. Four to five hours later, the power was switched off, the water-cooling system disconnected and the gel carefully removed for the staining-destaining steps.

2.7.4 Staining and quantitation of proteins

Staining solution was prepared by dissolving Coomassie Blue R-250 (1g/L) in water (450 ml/L), methanol (450 ml/L), and glacial acetic acid (100 ml/L). To remove any insoluble material, the solution was filtered using Whatman No.1 filter paper. The protein destaining solution consisted of (800ml/L) water, (100ml/L) methanol and (100ml/L) glacial acetic acid. Staining and destaining were carried out in an automated gel stainer-destainer (Hoefer processor
plus, Amersham Pharmacia Biotech) for about 16 hours. Then, the gel slabs were carefully removed, placed between two transparencies, labelled and scanned. The image saved was used for quantitation of apo B48 and apo B100 bands using 1-D Advanced software (Advanced American Biotechnology, 1166E Valencia Dr, #6C, Fullerton CA. 92831). A standard curve was then constructed for every gel slab and the concentrations of apo B48 and apo B100 were calculated accordingly.

2.8 Plasma Assays

2.8.1 Determination of plasma TAG

Principle
The TAG and cholesterol assay kits (Spinreact, S.A, Spain) used were based upon a colorimetric method previously described in section 2.3.

Procedure
Samples, along with standards, were run in duplicates, and were mixed with the working reagent and incubated 10 minutes at room temperature. The absorbances (Abs.) of the unknown and the standard samples were measured against Blank reagent at $\lambda = 505$ nm (Helios-γ spectrophotometer, UVG 101103). Color of the mixtures was varying from light pink to dark red, and it was stable for about 30 mn.

Calculation
Plasma TAG concentration (conc.) was calculated using a TAG standard of 200 mg/dl concentration according to the following equation:
Sample conc. (mg/dl) = (Abs. of unknown x conc. of standard) / Abs. of standard
2.8.2 Determination of plasma cholesterol

Principle
The cholesterol assay kit (Spinreact, S.A, Spain) used was based upon a colorimetric method previously described in section 2.4.

Procedure
Samples, along with standards, were run in duplicates, and were mixed with the working reagent and incubated for 10 minutes at room temperature. The absorbances of the samples (unknown) and the standard samples were measured against Blank reagent at $\lambda = 505$ nm (Helios-γ spectrophotometer, UVG 101103). Color of the mixtures was varying from light pink to dark red, and it was stable for about 30 mn.

Calculation
Plasma cholesterol was calculated using a cholesterol standard of 200 mg/dl concentration according to the following equation:

Cholesterol conc. (mg/dl) = (Abs. of unknown x conc. of standard) / Abs. of standard

2.8.3 Determination of plasma HDL cholesterol

Principle
LDL and VLDL are specifically precipitated by phosphotungstic acid and magnesium ions and can then be removed by centrifugation. HDL remain in the supernatant. Determination of HDL cholesterol is performed using the clear supernatant.

Procedure
Samples, run in duplicates, were mixed with the precipitating reagent (Phosphotungstic Acid 14 mmol/L, Magnesium chloride 2 mmol/L), allowed to stand for 10 minutes at room temperature and centrifuged at 12000 rpm for 4
minutes. The clear supernatant was used for cholesterol determination using the cholesterol assay kit as described in the previous section.

**Calculation**

Plasma HDL-cholesterol concentration was calculated according to the following equation:

\[
\text{HDL-cholesterol (mg/dl)} = \text{Absorbance of sample} \times 320 \text{ at } \lambda = 505 \text{ nm}
\]

### 2.8.4 Determination of plasma LDL cholesterol

The concentration of LDL cholesterol in plasma was determined according to the Friedewald equation:

\[
\text{LDL cholesterol} = \text{Total cholesterol} - [\text{HDL cholesterol} + (\text{TAG} / 5)]
\]

This formula is only valid in the fasted state.

### 2.8.5 Determination of plasma glucose

**Principle**

The glucose is determined after enzymatic oxidation in the presence of glucose oxidase (GOD). The formed hydrogen peroxide reacts under catalysis of peroxidase (POD) with phenol and 4-aminophenazone to a red-violet quinonimine dye as an indicator. The enzymatic reaction sequence employed in the assay was as follows:

\[
\text{Glucose} + \text{O}_2 + \text{H}_2\text{O} \xrightarrow{\text{GOD}} \text{Gluconic Acid} + \text{H}_2\text{O}_2
\]

\[
2\text{H}_2\text{O}_2 + 4\text{-aminophenazone} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinonimine (red)} + 4\text{H}_2\text{O}
\]
Procedure
Samples, run in duplicates, were mixed with the working reagent, and incubated 10 minutes at room temperature. The absorbances of the unknown and the standard samples were measured against the Blank reagent at $\lambda = 500$ nm (Helios-$\gamma$ spectrophotometer, UVG 101103).

Calculation
Plasma glucose concentration was calculated using glucose standard of 100 mg/dl concentration according to the following equation:

Glucose conc. (mg/dl) = \frac{\text{(Abs. of unknown x conc. of standard)}}{\text{Abs. of standard}}

2.9 Lipoprotein fraction assays

2.9.1 Determination of phospholipids

Principle
Phospholipids are determined after enzymatic reaction in the presence of phospholipase. The formed choline reacts under catalysis of choline oxidase with $O_2$ & $H_2O$ to form hydrogen peroxide ($H_2O_2$) which reacts then with 4-aminophenazone and diclorophenol under catalysis of peroxidase (POD) to a red-violet quinonimine dye as an indicator.
Procedure
Samples, run in duplicates, were mixed with the working reagent, and incubated for 5 minutes at 37 °C. The absorbances of the unknown and the standard samples were measured against the Blank reagent at λ = 505 nm (Helios-γ spectrophotometer, UVG 101103).

Calculation
Lipoprotein phospholipid concentration was calculated using phospholipid standard of 200 mg/dl concentration according to the following equation:
Phospholipid conc. (mg/dl) = (Abs. of unknown x conc. of standard) / Abs. of standard

2.9.2 Determination of TAG
The TAG concentration in the different lipoprotein fractions, CM (Sf>400), VLDL (Sf 20-400), and (LDL Sf 0-20) was measured using the TAG assay kit. This TAG determination is based upon the same principle as the colorimetric method described in section 2.3 for serum samples.

2.9.3 Determination of Cholesterol
The cholesterol concentration in the different lipoprotein fractions, CM (Sf>400), VLDL (Sf 20-400), and (LDL Sf 0-20) was measured using the cholesterol assay kit. This determination is based upon the same principle as the colorimetric method described in section 2.3 for serum samples.
2.9.4 Estimation of relative CM and VLDL size

The method used to estimate the relative size of CM considers the ratio CM TAG / CM apolipoprotein B 48. The method used to estimate the relative size of VLDL considers the ratio VLDL TAG / VLDL (apo B 48 + apo B100).

2.10 Data handling and statistical methods

Values of the different tested parameters within each group are presented as mean ± SEM. Comparison between each two groups was made by independent t-test. A p value of less then 0.05 (p<0.05) was considered significant.
3.1 Effects of acute juice intake

Three hours after intragastric instillation of the corresponding emulsions in the four groups (grapefruit, pineapple, orange and control), animals were anaesthetized and blood samples were collected. Concentrations of plasma TAG, plasma cholesterol, and plasma HDL-cholesterol were determined as shown in Table 3.1. Similarly, concentrations of TAG and cholesterol, were measured in the different lipoprotein fractions; (CM (Sf>400), VLDL (Sf 20-400), and low-density lipoprotein LDL (Sf 0-20)) which are obtained as a result of density gradient ultracentrifugation of plasma samples. Results are shown in Table 3.2.

3.1.1 Plasma TAG

The mean plasma TAG concentrations (Table 3.1) in both grapefruit and pineapple groups showed a significant decrease (p<0.05) as compared with the control group. Although the orange juice group showed a TAG plasma concentration 11% less than that of the control group, no significant difference has been reached. The mean postprandial plasma TAG concentration in the orange group was relatively higher when compared with pineapple and grapefruit groups, however no significant differences were observed among the three different groups.
3.1.2 Plasma cholesterol

The mean plasma cholesterol concentrations (Table 3.1) in the grapefruit, pineapple and orange groups did not show any significant change compared with the control group. The mean cholesterol concentrations were highest in the orange group and lowest in both the pineapple and grapefruit groups.

3.1.3 CM TAG

CM TAG concentrations of the four groups are shown in Table 3.2. The mean CM TAG concentrations in the grapefruit and pineapple groups were significantly lower than the control group. The mean CM TAG concentration in the orange group did not significantly differ from the control group although it was 23% lower.

3.1.4 CM cholesterol

CM cholesterol concentrations of the four groups are shown in Table 3.2. The mean CM cholesterol concentrations in the grapefruit, pineapple and orange groups were relatively lower when compared with the control group, however, significance was not reached.

3.1.5 VLDL TAG

The obtained concentrations of VLDL TAG in the four groups are shown in Table 3.2. The mean VLDL TAG concentrations in all groups were similar and no significant changes were detected.
3.1.6 VLDL cholesterol

VLDL cholesterol concentrations of the four groups are shown in Table 3.2. The mean VLDL cholesterol concentration in the grapefruit group was the lowest among all groups but it did not reach significance.

3.1.7 LDL TAG

The obtained LDL TAG concentrations of the four groups are shown in Table 3.2. The mean LDL TAG concentrations were similar in all groups (no significant changes were detected among the different groups).

3.1.8 LDL cholesterol

The obtained concentrations of LDL cholesterol in the four groups are shown in Table 3.2. Analysis of the data showed no significant changes among the different groups.

3.1.9 Total gastric content

The mean gastric contents retained in the stomach 3 hours after the intragastric load are shown in table 3.1. Data have shown that increased gastric retention was associated with juice intake. Grapefruit group showed the highest gastric retention, while the orange group showed the least gastric retention among the different juice groups. This increase in gastric retention reached significance when comparing grapefruit and pineapple groups with the orange and control groups. Significance was not attained between orange and control groups.
3.1.10 Gastric TAG content

Determination of gastric TAG content (Table 3.1) using the Folch method revealed an increased TAG retention in the stomach of grapefruit, pineapple and orange groups with respect to the control group. However significance was only reached when comparing grapefruit and pineapple groups with orange and control groups. No significant difference was reached when comparing orange with control group.
# Acute juice intake

Table 3.1 Plasma TAG (mg/dl), plasma cholesterol (mg/dl) concentrations, total gastric content (g) and gastric TAG content (g) as determined 3 hours after intragastric instillation of a 5ml emulsion of either 20% (w/v) olive oil, 4.5% (w/v) sucrose in water (control) or 20% (w/v) olive oil in either grapefruit juice or orange juice or pineapple juice. Values denote mean ± SEM (n=8). \(^{a} = p<0.05\) compared with control. \(^{b} = p<0.05\) compared with orange.

<table>
<thead>
<tr>
<th></th>
<th>Plasma TAG (mg/dl)</th>
<th>Plasma cholesterol (mg/dl)</th>
<th>Total gastric content (g)</th>
<th>Gastric TAG content (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>74.4 ((± 3.7))</td>
<td>53 ((±8.4))</td>
<td>1.25 ((±0.10))</td>
<td>0.20 ((±0.02))</td>
</tr>
<tr>
<td>Grapefruit</td>
<td>54 (^{a}) ((±6))</td>
<td>51 ((±2.5))</td>
<td>2.38 (^{ab}) ((±0.20))</td>
<td>0.38 (^{ab}) ((±0.03))</td>
</tr>
<tr>
<td>Pineapple</td>
<td>59.6 (^{a}) ((±4.8))</td>
<td>51.8 ((±4.6))</td>
<td>1.99 (^{ab}) ((±0.11))</td>
<td>0.32 (^{ab}) ((±0.02))</td>
</tr>
<tr>
<td>Orange</td>
<td>66.6 ((±7.8))</td>
<td>57.1 ((±8.2))</td>
<td>1.50 ((±0.09))</td>
<td>0.24 ((±0.01))</td>
</tr>
</tbody>
</table>
# Acute juice intake

**Table 3.2** Chylomicron TAG (mg/dl), Chylomicron cholesterol (mg/dl), VLDL TAG (mg/dl), VLDL cholesterol (mg/dl), LDL TAG (mg/dl), LDL cholesterol (mg/dl) concentrations as determined 3 hours after intragastric instillation of a 5ml emulsion of either 20% (w/v) olive oil, 4.5% (w/v) sucrose in water (control) or 20% (w/v) olive oil in either grapefruit juice or orange juice or pineapple juice. Values denote mean ± SEM (n=8).

a = p<0.05 compared with control.

<table>
<thead>
<tr>
<th></th>
<th>Chylomicron TAG (mg/dl)</th>
<th>Chylomicron cholesterol (mg/dl)</th>
<th>VLDL TAG (mg/dl)</th>
<th>VLDL cholesterol (mg/dl)</th>
<th>LDL TAG (mg/dl)</th>
<th>LDL cholesterol (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>134.5 (±19.5)</td>
<td>6.1 (±0.8)</td>
<td>36.2 (±3.9)</td>
<td>17.1 (±1.2)</td>
<td>7.8 (±1)</td>
<td>8.8 (±0.7)</td>
</tr>
<tr>
<td>Grapefruit</td>
<td>95.4 (±7.5)</td>
<td>5.3 (±0.5)</td>
<td>35.3 (±3.4)</td>
<td>14.7 (±1.3)</td>
<td>7.8 (±1.5)</td>
<td>7.9 (±0.4)</td>
</tr>
<tr>
<td>Pineapple</td>
<td>92.6 (±12.0)</td>
<td>5.3 (±0.4)</td>
<td>38.6 (±4.4)</td>
<td>18.2 (±1.4)</td>
<td>7.8 (±1.1)</td>
<td>8.2 (±1.2)</td>
</tr>
<tr>
<td>Orange</td>
<td>105.5 (±14.8)</td>
<td>5.9 (±0.8)</td>
<td>35.2 (±3.0)</td>
<td>18.1 (±1.6)</td>
<td>7.5 (±0.9)</td>
<td>8.4 (±1.1)</td>
</tr>
</tbody>
</table>
3.2 Effects of long chronic juice intake

The present study was undertaken to assess the effects of long chronic juice intake (grapefruit, pineapple and orange juices) in the fasted state. 16 rats of each experimental group were maintained for a 6 months period on a chronic juice intake in drinking water (50% v/v), while the control group (n=16) received plain water only during that period. After the 18 hours of fasting, blood samples were immediately collected and concentrations of the following were measured: plasma TAG, cholesterol, HDL-cholesterol and glucose; VLDL (Sf 20-400) TAG, cholesterol, phospholipids, apo B100 and apo B48; LDL (Sf 0-20) TAG, cholesterol and phospholipids; Total apoB - apo B100 and apo B48. All experimental procedures used in order to determine the above parameters are described previously in Chapter 2.

3.2.1 Plasma TAG

The circulating concentrations of plasma TAG determined in the four groups (grapefruit, pineapple, orange and control) are shown in Table 3.6. Compared with the grapefruit group, the control, orange and pineapple groups showed a significant increase in the mean TAG concentrations with p-values less than 0.05. The remaining groups showed similar plasma TAG concentrations.

3.2.2 Plasma cholesterol

The circulating concentrations of plasma cholesterol determined in the four groups (grapefruit, pineapple, orange and control) are shown in Table 3.3. The mean plasma cholesterol concentrations in the grapefruit, pineapple and orange groups did not show any significant change compared to the control
group. However grapefruit group showed the lowest plasma cholesterol concentration among all groups.

3.2.3 Plasma HDL-cholesterol

The circulating concentrations of plasma HDL cholesterol determined in the four groups (grapefruit, pineapple, orange and control) are shown in Table 3.3. The mean plasma HDL cholesterol concentrations in the grapefruit, pineapple and orange groups did not show any significant change among the different groups.

3.2.4 Plasma glucose

The obtained concentrations of plasma glucose in the four groups (grapefruit, pineapple, orange and control) are shown in Table 3.6. The mean plasma glucose concentrations in the grapefruit, pineapple and orange groups did not show any significant change among the different groups.

3.2.5 VLDL TAG

The circulating concentrations of VLDL TAG determined in the four groups (grapefruit, pineapple, orange and control) are shown in Table 3.4. The mean VLDL TAG concentrations in both grapefruit and control groups were significantly lower (p<0.05) than the pineapple and orange groups. Also, VLDL TAG concentrations of the grapefruit group showed a significant decrease compared with the control group. No significant difference was observed between the orange and the pineapple group.
3.2.6 VLDL cholesterol

The obtained concentrations of VLDL cholesterol in the four groups (grapefruit, pineapple, orange and control) are shown in Figure 3.4. Compared with the control and grapefruit groups, pineapple group showed a significant increase (p<0.05) in the mean VLDL cholesterol concentrations. No significant differences were observed among the grapefruit, orange and control groups.

3.2.7 VLDL phospholipids

The circulating concentrations of VLDL phospholipids determined in the four groups (grapefruit, pineapple, orange and control) are shown in Table 3.4. The mean VLDL phospholipids concentrations in the pineapple group showed a significant increase (p<0.05) as compared with the control and grapefruit groups. On the other hand, the mean VLDL phospholipids concentrations in the control, grapefruit and orange groups did not show any significant difference.

3.2.8 VLDL apo B100

The obtained concentrations of VLDL apo B100 in the four groups (grapefruit, pineapple, orange and control) are shown in Table 3.5. The mean VLDL apo B100 concentrations in the control group showed a significant decrease (p<0.05) as compared with the grapefruit, pineapple and orange groups. All latter groups showed similar VLDL apo B100 concentrations.
3.2.9 VLDL apo B48

The circulating concentrations of VLDL apo B48 determined in the four groups (grapefruit, pineapple, orange and control) are shown in Table 3.5. Compared with the control group, grapefruit, pineapple and orange groups showed a significant increase (p<0.05) in the mean VLDL apo B48 concentrations. However the mean VLDL apo B48 concentrations in all juice groups were similar. Calculation of the total apo B (apo B48 + apo B100) concentrations (Table 3.5) revealed a significant increase (p<0.05) in VLDL apo B secretion with juice intake irrespective of the juice type when compared with the control group.

3.2.10 LDL TAG

The obtained concentrations of LDL TAG in the four groups (grapefruit, pineapple, orange and control) are shown in Table 3.6. The mean LDL TAG concentrations in the grapefruit, pineapple and orange groups did not show any significant change compared with the control group and among each other.

3.2.11 LDL cholesterol

The circulating concentrations of LDL cholesterol determined in the four groups (grapefruit, pineapple, orange and control) are shown in Table 3.3. Compared with the control group, the mean LDL cholesterol concentrations in the grapefruit, pineapple and orange groups did not show any significant change.
3.2.12 LDL phospholipids

The obtained concentrations of LDL phospholipids in the four groups (grapefruit, pineapple, orange and control) are shown in Table 3.6. The mean LDL phospholipids concentrations in the grapefruit, pineapple and orange groups did not show any significant change compared with the control group. However the orange group had the lowest LDL phospholipids concentrations among all groups.

3.2.13 Total apo B100 (d<1.063 g/ml)

The circulating concentrations of total apo B100 determined in the four groups (grapefruit, pineapple, orange and control) are shown in Table 3.7. The grapefruit group showed the highest apo B100 concentrations among all groups. However a significant difference (p<0.05) was reached between the grapefruit group and the other two juice groups (orange and pineapple). The latter two groups didn’t show any significant difference from the control group.

3.2.14 Total apo B48 (d<1.063 g/ml)

The obtained concentrations of total apoB B48 in the four groups (grapefruit, pineapple, orange and control) are shown in Table 3.7. The mean total apo B48 concentrations in the grapefruit group showed a significant increase (p<0.05) when compared with the control and orange groups. However neither orange nor pineapple groups had any significant difference from the control group.
3.2.15 Total Apo B (Apo B100 + Apo B48)

Calculations of the total apo B concentrations (Apo B100 + apo B48) are shown in Table 3.7. Data revealed that the grapefruit juice had by far the highest apo B concentrations among all other groups. However significance (p<0.05) was only reached when comparing the grapefruit group with the orange and pineapple groups. Although apo B concentrations of the control group were substantially lower than the grapefruit group, significance was not reached (p<0.09).

3.2.16 Total cholesterol / HDL cholesterol and LDL cholesterol / HDL cholesterol

Calculation of total cholesterol / HDL cholesterol and LDL cholesterol / HDL cholesterol (Table 3.3) revealed that both control and grapefruit groups had the lowest total cholesterol / HDL cholesterol ratio, and the control and orange groups had relatively the lowest LDL cholesterol / HDL cholesterol ratio. The pineapple group consistently showed the highest ratios among all groups.

3.2.17 Fecal TAG

The concentrations of fecal TAG determined in the four groups (grapefruit, pineapple, orange and control) are shown in Table 3.8. Comparison of the mean stools TAG concentrations of all groups did not show any significant difference.
3.2.18 Fecal cholesterol

The obtained concentrations of fecal cholesterol in the four groups (grapefruit, pineapple, orange and control) are shown in Table 3.8. No significant differences have been observed among all groups.

3.2.19 Body weights

The obtained body weight differences in the four groups (grapefruit, pineapple, orange and control) are shown in Table 3.8. No significant changes have been detected in the mean body weight changes among the different groups.
Chronic juice intake

Table 3.3 Total cholesterol (mg/dl), HDL cholesterol (mg/dl), LDL cholesterol (mg/dl) concentrations, total cholesterol / HDL cholesterol ratio and LDL cholesterol / HDL cholesterol ratio as determined in fasted rats for a 6 month period on a chronic juice intake (grapefruit, orange and pineapple) in drinking water (50% v/v). Values denote mean ± SEM (n=16).

<table>
<thead>
<tr>
<th></th>
<th>Total Cholesterol</th>
<th>HDL Cholesterol</th>
<th>LDL Cholesterol</th>
<th>Total Cholesterol / HDL Cholesterol Ratio</th>
<th>LDL Cholesterol / HDL Cholesterol Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>64.0 (±2.9)</td>
<td>43.1 (±3.0)</td>
<td>26.7 (±2.4)</td>
<td>1.48</td>
<td>0.62</td>
</tr>
<tr>
<td>Grapefruit</td>
<td>58.7 (±4.4)</td>
<td>41.3 (±2.7)</td>
<td>28.2 (±2.5)</td>
<td>1.42</td>
<td>0.68</td>
</tr>
<tr>
<td>Pineapple</td>
<td>67.9 (±4.2)</td>
<td>39.8 (±2.2)</td>
<td>30.2 (±3.9)</td>
<td>1.70</td>
<td>0.76</td>
</tr>
<tr>
<td>Orange</td>
<td>63.2 (±4.1)</td>
<td>39.4 (±4.2)</td>
<td>25.0 (±3.3)</td>
<td>1.60</td>
<td>0.63</td>
</tr>
</tbody>
</table>
Plasma VLDL lipoprotein fraction Sf 20-400

Table 3.4 TAG (mg/dl), Cholesterol (mg/dl) and Phospholipids (mg/dl) concentrations in plasma VLDL (Sf 20-400) lipoprotein fractions as determined in fasted rats for a 6 month period on a chronic juice intake (grapefruit, orange and pineapple) in drinking water (50% v/v). Values denote mean ± SEM (n=16). \( ^a \) = p<0.05 compared with control. \( ^b \) = p<0.05 compared with grapefruit. \( ^c \) = p<0.05 compared with control, orange and pineapple.

<table>
<thead>
<tr>
<th></th>
<th>TAG</th>
<th>Cholesterol</th>
<th>Phospholipids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>80.9 ( \pm 8.7 )</td>
<td>114.7 ( \pm 6.8 )</td>
<td>86.2 ( \pm 3.1 )</td>
</tr>
<tr>
<td>Grapefruit</td>
<td>58 ( ^c ) ( \pm 5.7 )</td>
<td>108.5 ( \pm 8.3 )</td>
<td>84.3 ( \pm 3.9 )</td>
</tr>
<tr>
<td>Pineapple</td>
<td>135.4 ( ^a^b ) ( \pm 24.4 )</td>
<td>144.9 ( ^a^b ) ( \pm 12.1 )</td>
<td>113.1 ( ^a^b ) ( \pm 11.7 )</td>
</tr>
<tr>
<td>Orange</td>
<td>133.7 ( ^a^b ) ( \pm 22.5 )</td>
<td>125.1 ( \pm 10.8 )</td>
<td>102.2 ( \pm 12.4 )</td>
</tr>
</tbody>
</table>
Plasma VLDL lipoprotein fraction Sf 20-400

Table 3.5 Apo B48 (mg/L), apo B100 (mg/L), apo B100 + apo B48 (mg/L) concentrations and TAG / (apo B100+ apo B48) ratio in plasma VLDL (Sf 20-400) lipoprotein fractions as determined in fasted rats for a 6 month period on a chronic juice intake (grapefruit, orange and pineapple) in drinking water (50% v/v). Values denote mean ± SEM (n=16). ^a = p<0.05 compared with control. ^c = p<0.05 compared with control, orange and pineapple.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Grapefruit</th>
<th>Pineapple</th>
<th>Orange</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9.1 (±0.8)</td>
<td>11.8 (±1)</td>
<td>12.8 (±1.5)</td>
<td>11.6 (±1.1)</td>
</tr>
<tr>
<td></td>
<td>22.5 (±2.5)</td>
<td>36 (±5.9)</td>
<td>31.7 (±3.4)</td>
<td>33.2 (±3.8)</td>
</tr>
<tr>
<td></td>
<td>30.4 (±3.1)</td>
<td>44.5 (±5.9)</td>
<td>44.5 (±4.3)</td>
<td>44.8 (±4.6)</td>
</tr>
<tr>
<td></td>
<td>26.6 (±4.8)</td>
<td>13.1 (±2.4)</td>
<td>30.4 (±4.3)</td>
<td>29.8 (±5.1)</td>
</tr>
</tbody>
</table>
Chronic juice intake

Table 3.6 Plasma TAG (mg/dl), plasma glucose (mg/dl), LDL TAG (mg/dl) and LDL phospholipids (mg/dl) concentrations as determined in fasted rats for a 6 month period on a chronic juice intake (grapefruit, orange and pineapple) in drinking water (50% v/v). Values denote mean ± SEM (n=16). a = p<0.05 compared with control, pineapple and orange.

<table>
<thead>
<tr>
<th></th>
<th>Plasma TAG (mg/dl)</th>
<th>Plasma Glucose (mg/dl)</th>
<th>LDL TAG (mg/dl)</th>
<th>LDL Phospholipids (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>50.8 (± 4.3)</td>
<td>140.5 (± 11.5)</td>
<td>12.8 (± 1.2)</td>
<td>25.6 (± 1.8)</td>
</tr>
<tr>
<td>Grapefruit</td>
<td>33.1 a (± 3.1)</td>
<td>136 (± 11.0)</td>
<td>10.8 (± 1.3)</td>
<td>26.7 (± 1.6)</td>
</tr>
<tr>
<td>Pineapple</td>
<td>54.4 (± 4.1)</td>
<td>123.3 (± 8.6)</td>
<td>12.9 (± 0.9)</td>
<td>26.1 (± 2.3)</td>
</tr>
<tr>
<td>Orange</td>
<td>54.1 (± 4.9)</td>
<td>123.1 (± 8.2)</td>
<td>10.7 (± 0.6)</td>
<td>22.6 (± 2.0)</td>
</tr>
</tbody>
</table>
Chronic juice intake

Table 3.7 Total apo B48 (mg/L), total apo B100 (mg/L) and total apo B (mg/L) concentrations as determined in fasted rats for a 6 month period on a chronic juice intake (grapefruit, orange and pineapple) in drinking water (50% v/v). Values denote mean ± SEM (n=16). $^a$ = p<0.05 compared with control and orange. $^b$ = p<0.05 compared with pineapple and orange.

<table>
<thead>
<tr>
<th></th>
<th>Total apo B48 (mg/L)</th>
<th>Total apo B100 (mg/L)</th>
<th>Total apo B (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>38.4 (± 2.0)</td>
<td>175.8 (± 20.8)</td>
<td>214.1 (± 22.8)</td>
</tr>
<tr>
<td><strong>Grapefruit</strong></td>
<td>49.2 $^a$ (± 3.4)</td>
<td>227.2 $^b$ (± 25.5)</td>
<td>276.4 $^b$ (± 28.9)</td>
</tr>
<tr>
<td><strong>Pineapple</strong></td>
<td>45.6 (± 3.2)</td>
<td>133.7 (± 16.3)</td>
<td>179.3 (± 19.5)</td>
</tr>
<tr>
<td><strong>Orange</strong></td>
<td>37.1 (± 3.4)</td>
<td>141.8 (± 20.1)</td>
<td>178.9 (± 23.5)</td>
</tr>
</tbody>
</table>
Chronic juice intake

Table 3.8 Fecal TAG (mg/g), Fecal cholesterol (mg/g) concentrations and body weight change (g) as determined in fasted rats for a 6 month period on a chronic juice intake (grapefruit, orange and pineapple) in drinking water (50% v/v). Values denote mean ± SEM (n=16).

<table>
<thead>
<tr>
<th></th>
<th>Fecal TAG (mg/g)</th>
<th>Fecal Cholesterol (mg/g)</th>
<th>Body Weight Change (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>0.75 (± 0.09)</td>
<td>1.8 (± 0.2)</td>
<td>69 (± 4)</td>
</tr>
<tr>
<td><strong>Grapefruit</strong></td>
<td>0.88 (± 0.12)</td>
<td>1.9 (± 0.3)</td>
<td>74 (± 6)</td>
</tr>
<tr>
<td><strong>Pineapple</strong></td>
<td>0.73 (± 0.07)</td>
<td>1.9 (± 0.1)</td>
<td>81 (± 5)</td>
</tr>
<tr>
<td><strong>Orange</strong></td>
<td>0.97 (± 0.12)</td>
<td>2.1 (± 0.2)</td>
<td>76 (± 3)</td>
</tr>
</tbody>
</table>
Previous epidemiologic studies suggested that a high intake of fruit and vegetables is associated with reduced risk of coronary heart disease (Bors et al., 1990). Most of the beneficial effects were related to flavonoids and vitamins C, E and β-carotene, which are thought to act mainly as antioxidants (Charleux, 1996). Fruit juices role in cardiovascular health, especially citrus (orange and grapefruit) and tropical fruits (pineapple), has not been investigated thoroughly. Their potential cardioprotective effects in terms of lipoprotein secretion and metabolism have rarely been investigated.

The effects of a six-month period of juice intake have been investigated in normolipidemic rat model. The results have shown that when compared with the control group none of the juice types used had a significant effect on blood glucose, total cholesterol, HDL-cholesterol, LDL-cholesterol and the ratios total cholesterol/HDL-cholesterol and LDL-cholesterol/HDL-cholesterol.

Surprisingly, only animals of the grapefruit group showed a drastic decrease in plasma TAG concentrations with respect to all other groups. This decrease in mean plasma TAG concentration appeared to be the result of a direct effect of grapefruit juice on liver TAG secretion since VLDL particles of the grapefruit group carried by far the least amount of TAG. Unlike grapefruit group, both pineapple and orange groups significantly increased VLDL TAG secretion with respect to control and grapefruit groups, indicating different impacts of juice types on liver TAG secretion. The similar LDL TAG concentrations observed in all groups may suggest a possible difference in the metabolism of these lipoprotein particles among the different groups.
To better understand the mechanism involved, the concentrations of total apo B and VLDL apo B were measured. In the human model, small chylomicrons and VLDL particles are differentiated by their apo B48 and apo B100 markers respectively. However, a liver secretion of VLDL with either apo B48 or apo B100 as a surface marker distinctively characterizes the rat species (Tennyson, 1989). Therefore, in the present study, it is considered that apo B48 of the Sf > 20 lipoprotein fraction is a VLDL constituent. Knowing that each VLDL particle contains one apo B molecule (Elovson et al., 1988, Martin et al., 1997), measuring plasma apo B, as apo B100 or apo B48 reflects exactly the total number of newly secreted VLDL. The present data have shown that all juices increased significantly total VLDL apo B secretion indicating that irrespective of the juice type an increase in VLDL secretion would be observed. However, determination of total plasma apo B (representing VLDL, IDL and LDL) reveals that the grapefruit group had the highest apo B concentrations among all groups while both orange and pineapple groups had the lowest apo B concentrations. This indicates that either pineapple or orange juice consumption, on a chronic basis, increases the rate of metabolism of lipoprotein particles (from VLDL to LDL and then clearance) from the blood; whereas chronic consumption of grapefruit juice did not improve the clearance rate and metabolism of these lipoprotein particles. Recent studies have revealed that apolipoprotein B is a better indicator of potential myocardial infarction than total cholesterol or LDL cholesterol (Walldius, 2001).

Also, apolipoprotein B was shown to be an extremely useful predictor mainly in individuals with low or normal LDL-cholesterol (Sniderman, 2001). Thus, chronic orange and pineapple juice consumption but not grapefruit may be recommended as a mean to somehow improve (about 15%) total plasma apo B concentration in normolipidemic subjects. Why chronic intake of grapefruit juice did not speed the metabolism and clearance of the lipoprotein particles remains the subject for further studies.

However, a possible explanation may reside in the size of the VLDL particles originally secreted by the liver. Estimation of the rough VLDL particle size as VLDL-TAG / apoB ratio revealed that animal on chronic grapefruit juice intake had by far the smallest VLDL particle size whereas those put on chronic
orange and pineapple juice had VLDL particle size slightly larger than that of the control group. Early studies have shown that lipoprotein particle size (Chajek-Shaul et al. 1983; Martins et al. 1994) and number (Martins et al. 1996) are both important in affecting the clearance rate of lipoprotein particles.

It is established that large particles are cleared more rapidly than smaller ones. Consequently, VLDL particles of the grapefruit group having the smallest particle size are expected to be cleared the slowest from the blood. Previous in vitro studies in HepG2 human hepatoma cells (Borradaile et al., 1999) have shown that, unlike the present study, the flavonoids hesperetin and naringenin, present in orange and grapefruit juices respectively, reduced drastically net apo B secretion. HepG2 human hepatoma cells (Borradaile et al., 1999), a commonly used model of human hepatocytes, have a defect in the second step of lipoprotein assembly, which prevents the addition of substantial quantities of TAG to the primordial particle (Gibbons et al., 1994). As a consequence, these cells secrete small TAG- and apo B-containing particles with a density similar to that of LDL (Ellsworth et al., 1986, Thritt et al., 1986).

On the other hand it is reported in the same in vitro study (Borradaile et al., 1999) that both naringenin and hesperidin failed to inhibit net apo B secretion after 4 h in the presence of oleate. In vivo, hepatocytes are constantly exposed to TAG present in the blood even in the fasted. Consequently, the inhibition observed in the in vitro study (Borradaile et al., 1999) in HepG2 cells in the absence of TAG is not applicable to our in vivo study.

Further in vivo studies on the effects of naringenin and hesperidin are necessary for a better understanding of the mechanism of action of these compounds upon lipoprotein synthesis and metabolism. Prior animal studies on miniature swine have demonstrated that pectin, inhibits diet-induced hypercholesterolemia including increases in LDL and VLDL, while not altering HDL (Backey et al., 1988).

In these studies, pectin was fed concurrently with the atherogenic diet. Other studies revealed that grapefruit pectin did not significantly lower cholesterol levels or lipoprotein fractions of similar animals with long-standing hypercholesterolemia and continued to ingest a lipid rich diet (Cerda et al.,
1994). Surprisingly, the same study (Cerda et al., 1994) showed that pectin either regressed atherosclerosis induced by 1 year of sustained hypercholesterolemia or interfered with lesion progression. This indicates that the ability of pectin to inhibit atherosclerosis may be independent of a direct effect on lipid levels. Prior studies in human with hypercholesterolemia concluded that pectin supplementation had modest cholesterol lowering effect (Cerda et al., 1988), lowering total cholesterol by 7.6% and LDL by 10.8%. However, later human studies by Kurowska et al. (2000a) conducted on individuals with mild-to-moderate hypercholesterolemia showed that consumption of 750 ml but not 250 or 500 ml orange juice per day for 4 weeks improved the plasma lipoprotein profile by significantly increasing HDL-cholesterol and by reducing the LDL-HDL cholesterol ratio, an effect entirely due to changes in HDL cholesterol concentrations.

This observation also contrasts with a substantial reduction in LDL cholesterol observed in hypercholesterolemic rabbits receiving orange juice (Kurowska et al., 2000b), with a cholesterol-lowering effect of citrus flavonoids observed in rats (Bok et al., 1999) and with the lack of changes in plasma lipids in normocholesterolemic young men consuming unspecified doses of fresh orange juice for 2 moths (Harats et al., 1998). This disagreement in the data among the different studies can be attributed to several possible reasons such as species differences, doses of juice and length of the study, and last but not least the state of being normo- or hypercholesterolemic.

Although there are lots of controversies among the different studies, it appears that the ability of chronic juice intake to improve blood lipid profile is mainly correlated with the degree of hypercholesterolemia where subject with the highest blood cholesterol levels would benefit the most while those with normal blood cholesterol may not benefit at all. This is supported by the present study and several other studies in human (Harats et al., 1998, Kurowska et al., 2000b). Similar to the present study, it has been shown (Harats et al., 1998) that chronic juice intake has no affect on cholesterol levels in normocholesterolemic subjects while intake (Kurowska et al., 2000a) of 750 ml of orange juice per day showed a positive correlation between the
baseline LDL-HDL cholesterol ratio and the improvement of this ratio at the end of the study.

The present study reveals some of the important impacts of chronic juice intake upon lipoprotein secretion and metabolism in the rat model. Although, the rat might not be an ideal subject to study, chronic human studies over a six months period are complicated by differences such as gender, liver function, type and quantity of juice ingested, nutritional factors, smoking, physical activity and other parameters. To avoid these confounding factors animal studies of chronic juice effect are easier to control. In order to avoid exaggerated doses of juice intake that might not be applicable to regular daily life, animals of the chronic study received half of their daily liquid intake from the respective juices (grapefruit, orange and pineapple).

Unlike most previous studies (Cerda et al., 1988, Cerda et al., 1994, Kurowska et al., 2000a, Kurowska et al., 2000b) where juice effects covered hypercholesterolemic subjects, animals of the present study were normocholesterolemic, which is the case of the majority of the population. Accordingly our model aims at investigating whether replacing half of our liquid intake with fruit juices (grapefruit, orange and pineapple) can affect lipoprotein synthesis and metabolism and consequently the lipid profile in the greatest majority of the population. In other words, should we recommend juice intake as lipid moderator in all subjects irrespective of the original lipid profile or this applies only to those with hypercholesterolemia. However, one should always keep in mind that atherosclerosis is a multifaceted problem, and lipid status is only one of several markers of atherogenicity. For instance, it has been shown (Cerda et al., 1994) that grapefruit pectin had a direct beneficial effect on atherosclerosis by a mechanism independent of cholesterol and lipoprotein levels. Thus, a lack of effect on blood lipid profile does not necessarily mean that juice intake is discouraged.
Dietary pectins have previously been shown to increase the excretion of fecal neutral steroids in rabbits (Martinez et al., 1981), and thus contribute indirectly to reduction of blood cholesterol levels. Other studies in hypercholesterolemic rabbits (Kurowska et al., 2000b) however showed that both orange and grapefruit juices, both rich in pectin, did not increase fecal cholesterol excretion but on the contrary they significantly decreased its excretion in the feces.

In the present study we have shown that chronic juice (orange, grapefruit and pineapple) intake did not significantly affect fecal excretion of cholesterol and TAG in the rat model. Fecal cholesterol and TAG were only slightly higher than the control group. Differences in results might be attributed to species differences (rat versus rabbit) and to different amount of pectin received by the animals. Thus, the role of the soluble dietary fiber pectin in reducing plasma cholesterol levels via increasing fecal cholesterol excretion needs further studies and characterizations.

Based on possible differences between the impact of chronic daily drinking and acute drinking, this study was designed to cover both chronic and acute states of juice consumption. Acute juice intake effects were studied after three hours of a single juice-lipid load instilled intragastrically. It has been shown (Daher et al., 2003) that after three hours of a similar lipid load a steady state of intestinal lipid absorption and secretion was observed. Determination of the plasma TAG during the steady state period revealed that both pineapple and grapefruit groups had a significantly lower plasma TAG concentrations with respect to the control group. This effect may be attributed to a reduced rate of gastric emptying in all juice groups, and especially in the grapefruit and pineapple groups. Extraction of the intragastric lipid retained in the stomach 3h postprandially showed that much more lipid is still present in the stomach of grapefruit and pineapple groups. Thus, the observed low plasma TAG observed in the latter groups is due to a reduced TAG secretion by the intestine accompanying the reduced gastric emptying. Because of delayed gastric emptying in juice groups one should also expect that this result in a longer but milder postprandial triglyceridemia. Unlike the chronic study, the acute study does not support the possibility of a direct effect of juices on liver
during this short period (3h) since VLDL TAG concentrations of all groups were similar. Thus, intake of juice with the diet is expected to reduce tremendous increases in plasma TAG concentrations accompanying digestion and absorption of food in the small intestine, but at the same time it lengthens the postprandial state by reducing the rate of gastric emptying.

People that may benefit most from taking juice with the diet are those who have a problem in clearing rapidly postprandial TAG from the blood, like the case with lipoprotein lipase deficiency. The increased TAG gastric retention was observed with both grapefruit and pineapple juice intake. Consequently, these juices will help moderating the sharp increases of postprandial plasma TAG concentrations, especially in people with hyperlipidemia. On the other hand, grapefruit juice is well know to interact with a large number of prescribed drugs by inhibiting a special enzyme in the intestine responsible for the natural breakdown and absorption of many medications (Guthrie and Picciano, 1995). Among these drugs are Zocor (Simvastatin) and Lipitor (Atorvastatin) (Watkins, 1990), the most commonly used drugs by hyperlipidemic subjects. Consequently, pineapple juice would be the juice of choice rather than grapefruit in order to avoid drug interaction complications.

Historically, grapefruit juice intake has been associated with weight loss. However, grapefruit itself has no direct effect on weight loss as observed in the present and previous studies (Kurowska et al., 2000b). Grapefruit juice might possibly help people on diet by delaying gastric emptying, extending satiety and reducing the feeling of empty stomach. Then, it might indirectly help people on diet to stick to the limited caloric intake, therefore indirectly helping in the reduction of their body weight.
In conclusion a six-month period of chronic juice intake in normolipidemic rat showed a modest effect on blood lipid profile. One of the interesting findings was the drastic reduction in plasma TAG concentration of the grapefruit group, an effect related to a direct impact of the juice on liver TAG synthesis and secretion. However, this reduction in liver TAG secretion was accompanied by a large number of small VLDL particles, a possible cause of the reduced metabolism and clearance of the particles as appeared in total plasma apoB concentration, and thus increased atherogenic profile. On the contrary, both orange and pineapple juices appeared to increase somehow the metabolism and clearance of the lipoprotein particles from the blood.

In general, the present study suggests that normolipidemic individuals put on chronic juice intake do not benefit much in term of blood lipid profile. When the subject is normolipidemic, the possible cardioprotective benefit may be through mechanisms independent of a direct effect on blood lipid levels. In the acute study, it is shown that especially grapefruit and pineapple juices moderate sharp increases in postprandial plasma TAG concentrations accompanying peak digestion and absorption. This effect, which can be important in lipoprotein lipase deficient subject, is the result of delayed gastric emptying observed when these juices are consumed with food.

Finally, because of the present findings and the fact that grapefruit juice has lots of drug interaction complications, pineapple or orange juice rather than grapefruit juice are to be recommended for consumption.
REFERENCES


