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The Relationship between Meat Consumption and the Biological Determinants of Cardiovascular Disease Risk

Fred E. Kollwitz

Abstract

THE RELATIONSHIP BETWEEN MEAT CONSUMPTION AND THE BIOLOGICAL DETERMINANTS OF CARDIOVASCULAR DISEASE RISK

by

Fred E. Kollwitz, M.D.

Fasting blood samples were collected from 26 omnivore and 27 vegetarian men (35-60 years of age), who were not taking any medications and by self-assessment were in good health. The following analyses were done: glucose (Glu), glycohemoglobin (HBA1), total serum cholesterol (chole), high density lipoprotein-cholesterol (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), platelet factor four (PF4), and beta-thromboglobulin (BTG). In addition, the blood pressure (BP) was determined.

The systolic ($p<0.001$) and diastolic BP ($p=0.016$) were lower in the vegetarian subjects than in the omnivores. The serum total cholesterol levels were also lower in the vegetarian subjects compared to the omnivores ($p<0.05$). There was no significant difference in Glu, HBA1, LDL, VLDL, HDL, PF4, and BTG levels between the two groups.

LOMA LINDA UNIVERSITY

Graduate School

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Each person whose signature appears below certifies that this thesis in his opinion is adequate, in scope and quality, as a thesis for the degree Master of Science.

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INTRODUCTION

The fact that coronary heart disease is a major health problem in the United States is no longer in question. The grim sad evidence of athersclerosis, seen in the autopsies performed on U.S. combat casualties in Korea and Vietnam, revealed the presence of athersclerotic lesions in coronary arteries of 70% of those formerly healthy, active young men (with a mean age of 21.1 years). About 40% had moderate to severe occlusive lesions.[1] Coronary heart disease is responsible for 550,000 deaths per year and more than all forms of cancer combined.

It has been estimated that there are in the U.S. 5,400,000 Americans with symptomatic heart disease, exclusive of a large number who have not been diagnosed. The dollar costs of this illness is approximately $60 billion per year directly and indrectly. Males are at a higher risk than females, however, recent findings show females are closing the gap. Increasing age, obesity, diabetes mellitus, inactivity, and hypertension are also risk factors. These diseases are usually directly related to hyperlipidemia. Genetics has a strong influence on families who have a history of any of the aforementioned pathology. These diseases should lead to a screening of the children for plasma levels of cholesterol, LDL, HDL cholesterol, and triglycerides.[2] It probably behooves a pediatrician to be the "watchman on the wall" for future generations and to make a sincere effort to control the cardiovascular diease death rate.

The threat of cardiovascular disease, and what may be done to alter these impressive mortality statistics has scientists searching for methods to make an early diagnosis. If an early diagnosis can be made,

can anything be done to alter the present trend of morbidity and mortality? With this question in mind, we chose to investigate whether diet could be an important factor regarding the diagnosis and further progression of the pathology.

REVIEW OF THE LITERATURE

Platelets (Anucleated Cells)

The progenitors of platelets are the megakaryocytes which usually comprise a small portion (one per several hundred) of the marrow cells. The megakaryocytes mature through the blast cell to the intermediate stage, then to a mature megakaryocyte. It is at this stage that a percentage of the cells will release or shed platelets. It is estimated that one human maegakaryocyte can release several thousand platelets.[3]

A number of proteins apparently unique to platelets have been identified (PF4, BTG, and a platelet derived smooth muscle growth factor). Some effects of these secretory proteins include stimulation of smooth muscle cells, and cell proliferation, which may be important in the repair of vessel injury. Platelets have also been found to contain other proteins that are present in plasma, that is, fibrinogen, factor V, factor VIII, fibronectin, and factor XIII. In most instances it is not known whether these proteins are absorbed or imbibed from plasma by mature platelets, or are synthesized de novo. Being anucleate, the platelet has only vestigial remains of protein synthetic apparatus, which implies protein synthesis in the megakaryocyte.[3]

The average lifespan of platelets is ten days. The younger platelets are more active than the mature ones, and will promote hemostasis at the site where microvascularization leakage occurs. The spleen sequestrates approximately 30%, the liver 45%, the marrow and lymph 5-15% of the toal number of platelets. Alcohol abuse can lead to decreased platelets, and eventually to thrombocytopenia. This condition can also be acquired in the case of megaloblastic anemia, secondary to a

deficiency of either B12 or folic acid.[4,5]

The platelet count may fall as low as 10,000, to be followed by a rebound phenomenon, when the count may rise to 600,000 to 800,000 for 1-4 weeks of duration. Thrombopoietin is a hormone that stimulates platelet production in response to thrombocytopenic stress. In iron deficiency the platelets can be normal, moderately elevated or strikingly elevated, to a level of one to two million per cubic millimeter.[3] When this deficiency is corrected the platelet count returns to normal within one to two weeks. Hirsch and Dancie [6] reported the development of thrombotic complications in 5 of 15 patients whose platelet count was persistently high.

Platelet stimulation results in the activation of phosphorylases that hydrolyze the membrane phospholipid molecule, with the liberation of Arachidonic acid (AA). The AA is converted mainly to thromboxane A2 (TXA2) in the platelets by the AA cascade.[7]

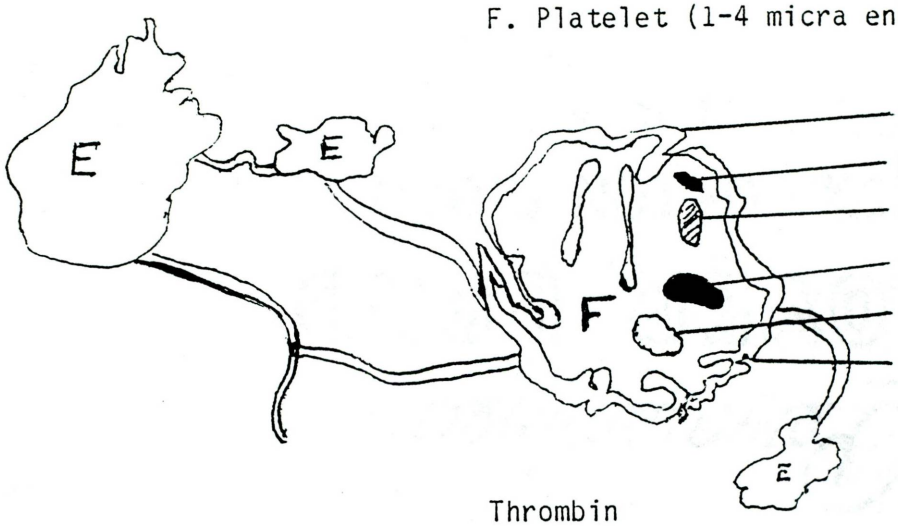
Platelet Secretions

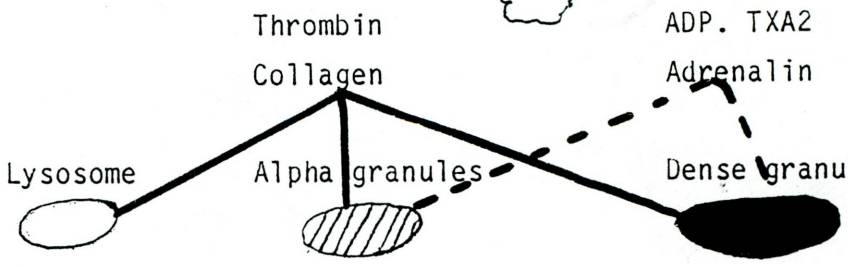
There are two types of storage granules that are distinguishable in the platelets. These are the dense granules which are homogenous and electron dense. The alpha granules are heterogenous, differing from one another and from the dense granules in size and shape.[1] Figure 1 will show this clearly.

The alpha granules may include acid hydrolases containing organelles, whose content when stimulated is extruded into the cell's environment. This specific extrusion is called "platelet secretion." Many of these substances have direct action on cells and on the

Figure 1: The stages of maturation through to the release of the platelet. In actuality the platelet is much smaller than the meta megakaryocyte. As diagrammed, the platelet is connected at the metamegakaryocyte via filamentous threads which also provide a physical structural network to stabilize the position of the platelet. The diagram shows some activating substances of the platelet and their products.

A. Megakaryoblast
B. Promegakaryocyte
C. Megakaryocyte
D. Metamegakaryocyte
E. Metamegakaryocyte with attached platelet
F. Platelet (1-4 micra enlarged)





Acid hydrolases		
B-N-acetylglucosaminadase	factor V	ATP
B-N-acetylgalactaminidase	fibrinogen	ADP
B-N-Glucurnidase	BTG	PPi
B-N-galactocidase	PF4	Ca
	mitogenic factor	Serotonin
	permeability factor	

platelets themselves and are converted to physiologically active substances in the plasma or on the cell surface. The result of "in vivo" platelet activation may vary from a "simple" change in the structure of platelet membrane to the complete release reaction. Stimuli unable to promote a complete release reaction may, however, be sufficient to determine a partial activation resulting in the release of alpha granules.[3] Serotonin is absorbed and secreted by platelets. In addition, they contain substantial actomyosin and this release is promoted by extracellular calcium ions that can enter intercellularly under the influence of thrombin. Once inside the cell, they cause the organelles to contract.

It is important to know that some of the plasma protein analogs, such as fibrinogen, come from the alpha granules. However, they are probably not a product of the granules. Therefore, megakaryocytes or platelets do not synthesize fibrinogen, but accumulate it by some unknown method.[7]

Platelet Factors 1, 2, 3, and 4

Although there are specific substances that will selectively stimulate platelets, one experimental product is a cationophore A-23187.[3] This, as well as other substances, will stimulate the secretory processes of the alpha dense granules and a general platelet response. There is a change in shape, aggregation and AA liberation, Arachidonic is subsequently oxidized to a platelet activating substance, TXA_2. Some strong platelet aggregation inducers are ADP [8,9,10] collagen, epinephrine, TXA_2, and thrombin; however, these are dependent upon the avail-

ability of calcium, therefore, calcium channel blockers are inhibitors of platelet secretion to a limited degree.

Another secretory platelet factor is the platelet growth factor, which is capable of stimulating the proliferation of smooth muscle cells. This is of great importance in the formation of atheromas.

Platelet factor 1 refers to coagulation factor V in the platelets. Platelet factor 2 accelerates the clotting of fibrinogen by thrombin; and these terms are no longer used. Platelet factor 3 has a phospholipid like activity, and platelet factor 4 has an anti-heparin like activity. Phospholipids make up 76% of the total lipids and are approximately constituted as follows: phophatidyl choline (which is a surfactant), phophatidyl ethanolamine and sphingomylin. Neutral lipids constitute 20%, the remaining 4% are lipoproteins. The lipids cement the membranes and at the same time serve a biological function.[2]

There are two types of PF4; one protein is regular, or normal PF4, and is a high affinity protein; the other is a low affinity PF4 (LA-PF4) at times also referred to as platelet factor 3 (PF3).[3,5]

Some characteristics of PF4 are as follows: it is a low molecular weight protein, glycoprotein found in the alpha granules, released from the platelets by releasing factor followed by stimulation and aggregation by ADP, thrombin, and epinephrine.[10] Platelets in a quiescent state have a convex surface with random indentations representing sites of communications between channels on the surface, either connected or are open canalicular systems to the exterior of the cell (Figure 1). This system provides for the orderly access to the interior of the cell for plasma-born substances, and the egress for products of the release

reaction. Two proteins, a high affinity PF4 and a low affinity PF4 are secreted by the platelets. The hydrolitic cleavage product of the latter, produced by platelet proteases, is called beta thromboglobulin, both PF4 and BTG bind heparin. Their levels in circulating plasma are regarded as a measure of platelet activation in vitro. PF4 is a low molecular weight protein that is immunologically specific for platelets, and all experimental evidence suggests that most of the proteins are stored in the alpha granules.[3]

The dense granules contain the minerals and vitamins, namely Ca, Mg, Zn, folic acid, ascorbic acid, and cyanocobalamine. Platelets of themselves do not produce serotonin, but store it as a smooth muscle vasoconstrictor. Consequently, serotonin is absent in the plasma and other cells. There is no reliable method of measuring serotonin except biopsy of the sites of production, namely brain cells and argentofil cells of the small intestines. Serotonin has not been found to be important in hemostasis in humans, but may be so in other species.[7]

The two types of platelet granules are known as storage granules. The reference "dense granules" implies that they are homogeneous and electron dense. If there is a deficiency of these, it is called a "storage pool deficiency," with the resultant impaired homeostasis. The alpha granules are heterogeneous and differ from one another an from the dense granules both in size and shape. All platelets have complete cellular elements. As mentioned earlier they are anucleated, therefore, they do not have DNA.[5]

Functions of PF4 and LA-PF4

1. PF4 shortens the cascade of thrombin acting on fibrinogen to fibrin, thereby reducing the time for clot formation.

2. In the presence of heparin PF4 will potentiate ADP induced aggregation n vitro to induce precipitation of fibrinogen, which is normally soluble in plasma.

3. PF4 will non-enzymatically clot or precipitate soluble fibrinogen in plasma. PF4 will neutralize certain fibrinogen breakdown products; may be a potent and specific agent triggering platelet aggregation, thereby shortening the time for blood clotting.

4. The levels of PF4 in plasma are 3-16 ng/ml. These values are the range of what is commonly accepted as normal. French, et al[11], as a result of their studies of thrombospondin BTG and PF4, suggest 34 ng/ml for BTG and 6.1 ng/ml for PF4. PF4 binds very tightly to heparin to neutralize its anticoagulation activity. LA-PF4 binds to low affinity heparin, and is immunologically identical with BTG.

5. LA-PF4 is acted upon in the circulation to convert to BTG by splitting off the tetrapeptide from the end terminal of LA-PF4.[8]

6. Both LA-PF4 and PF4 are secreted simultaneously and have anti-heparin effect. Then the LA-PF4 is converted to BTG in the plasma. The PF4 is cleared more rapidly due to the substance adhering immediately to the endothelial cell surface, whereas BTG, a derivative of LA-PF4 with low heparin inhibiting effect, remains in the plasma longer.[8,12]

Beta Thromboglobulin (BTG)

The name was given because it migrates in cellulose acetate in the beta-globulin region. The complete primary structure of human BTG is

known and consists of 81 amino acids, with a molecular weight of 8,851. The amino-acid sequence of BTG shows a marked homology to PF4 in that 42 of their residues are identical.[8] The only difference between LA-PF4 and BTG are four amino acids. Experimental evidence suggests that La-PF4 is originally secreted by the platelets during the platelet release reaction, and subsequently converted to BTG by a heat-labile platelet derived neutral protease or by plasmin.[5] Plasmin, fibrinolysin, or neutral protease split off a tetrapeptide from the end terminal of LA-PF4, to yield BTG.[8] Some researchers feel that BTG binds to endothelial cells and thereby inhibits production of prostacyclin formation.[13] BTG, LA-PF4, and PF4 are immunologically specific for platelets, since they do not occur in any other cells or tissues. In platelet free plasma levels are 500 times less than in platelet rich plasma. This is the basis for the test to measure their quantity in circulating blood as an indicator for thrombogenesis.[14]

One of the most crucial steps in the preparation of PF4 and BTG tests is to make a careful preparation of the specimen by avoiding any leakage of the coagulating proteins into the platelet free preparation. Apparently the half-life of PF4 is so short that is cannot be measured according to Daws[12] and others.[15] The half-life of BTG is estimated to be 100 minutes. Other researchers[15] find the half-life of these products to be divided into two periods; namely, an inital rapid loss, followed by a slower loss.

BTG may act locally at high concentration to favor platelet aggregation by inhibiting PGI_2 production. The binding sites of BTG are specific and will not accept PF4 on their sites. LA-PF4 and LA-BTG

accordingly regulate PGI_2, although they do not have similar biological effects.[13]

BTG and PF4 acting in concert, yet independent of one another, are involved in thrombin formation. Forbes[16] in his experiment, measuring the levels of BTG and PF4 in patients with transient ischemic attacks (TIA) has shown that both these levels continue to remain high as long as two months after an attack. This is in agreement with the results of Fisher[17], Stewart[16], and it has been concluded that the highest values carried a poor prognosis. The results of these authors are completely opposite to those of de Boer, et al[18], who in 1982 reported no change in BTG, PF4 plasma levels within 48 hours of a stroke. A plausible explanation of the difference between these authors is offered by O'Brien and Etherington[19], who suggest measuring intra platelet levels of PF4 and BTG to yield more reliable information. The plasma level of any one moment will depend upon the balance between the rate of release of the protein from the platelets and the rate of its removal from the blood, which is fast, the half lives of PF4 and BTG being about 20 minutes and 100 minutes respectively.[15]

Fabris[20] has shown a high specificity for PF4, BTG to antiplatelet drug therapy. When patients used combinations of dipyramidole (antiplatelet aggregator) with aspirin there was no effect on PF4 or BTG levels; both remained at pathological levels. This is also true for the patient with coronary artery disease. Either dipyridamole or aspirin alone are effective.[21]

Increased cardiovascular morbidity and mortality has been established to be higher among smokers than among non-smokers. Previous data

has shown normal individuals could prevent an incrase in PF4, BTG levels if the individual took aspirin prior to smoking. The study of Davis, et al[22], was of men who were habitual smokers and who had coronary artery disease. Their data indicated that smoking stimulated platelet aggregate formation and the release of the content of alpha granules were unaffected by the previous administration of aspirin.

Distribution and Function of Lipoproteins and Aproproteins

In the previous pages of this thesis we have referred to the various lipoproteins, and the accompanying diagram (Figure 2) will explain the relationship of the lipoproteins to the apoproteins. This relationship is important for futher understanding of the cardiovascular risk factors and the cardiovascular protective factors.

Factors HDL, LDL

While we speak in generalities about HDL and LDL, we must be aware that there are subfractions. To illustrate HDL, there are three subfractions: HDL-1, HDL-2, HDL-3. HLD-2 is a beneficial subfraction for human beings, and may be raised with exercise and dietary intervention.

The LDL fraction has been divided into LDL1 and LDL2. In this thesis I shall consider both LDL1 and LDL2 as LDL and avoid mentioning the subfractions.

Analysis for possible correlations between HDL and total cholesterol and other lipids and lipoprotein fractions demonstrate a moderately strong negative correlation between HDL cholesterol and triglycerides which was consistent when measured in the general population,

Figure 2: Summary of percentage distribution of lipids in the human plasma. Adapted from Kwiterovich (2)

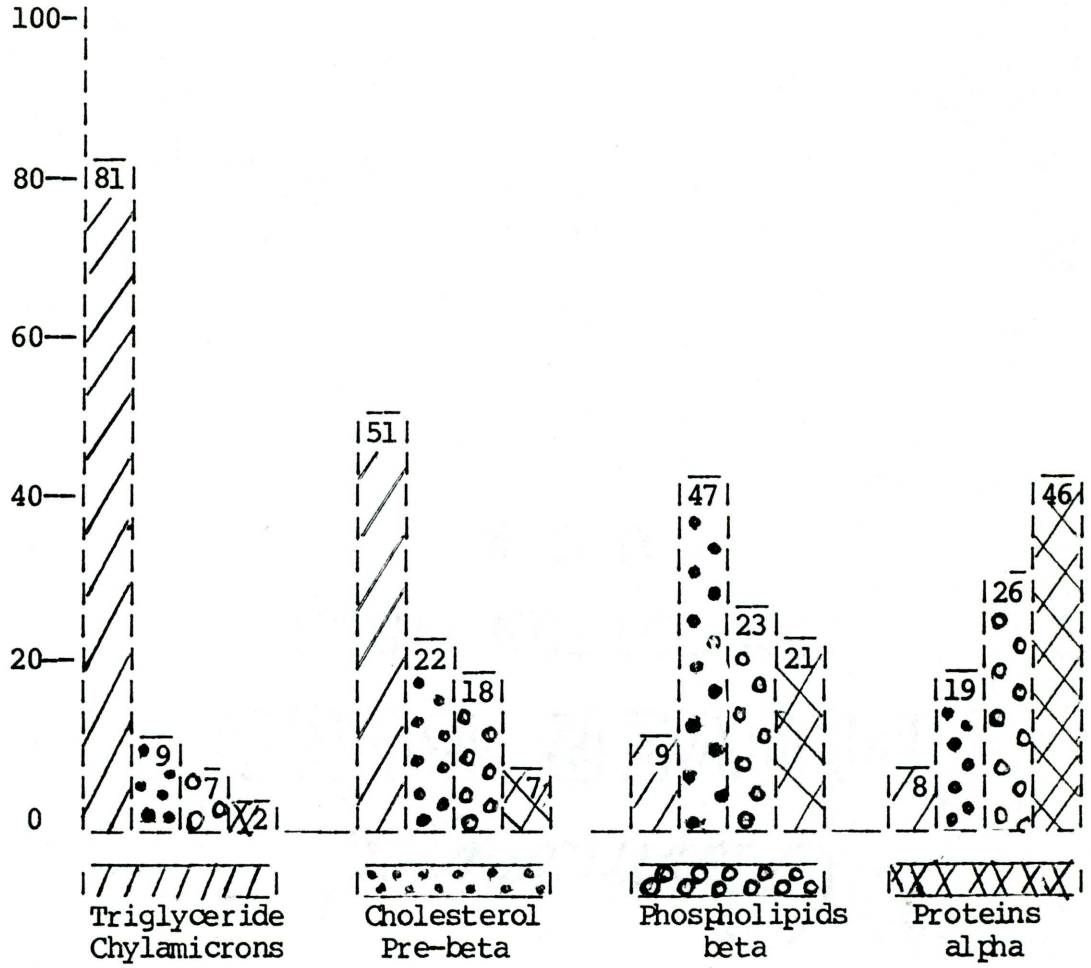

100
80
60
40
20
0
81
9
7
2
51
22
18
7
9
47
23
21
8
19
26
46
Triglyceride
Chylamicrons
Cholesterol
Pre-beta
Phospholipids
beta
Proteins
alpha

Table 1: Composition and Function of Human Lipoproteins

Apoprotein		Lipoprotein	Function
Apo A-1	associated with	Chylomicron, HDL TGC	for enzyme lecithin, cholesterol acyltransferace (LCAT)
Apo B-48	associated with	Chylomicron	transports fat out of intestinal cell
Apo B-100	associated with	VLDL, LDL	Uptake of cholesterol rich LDL
Apo C-II	associated with	VLDL, HDL	cofactor for lipoprotein lipase
Apo D	associated with	HDL	associated with cholesterol esters transfer complex
Apo E	associated with	Chylomicron & remnants	responsible for HDL, VLDL uptake of chylomicrons by liver

except for females. There are indications that increased serum cholesterol is the most important risk factor before the age of 50 years.[11] Triglycerides have small negative correlation with LDL, except in females, and a moderate correlation with total cholesterol. As was expected, there was a strong correlation between LDL and total cholesterol. Statistical analysis indicates that HDL and LDL cholesterol were independent and significant factors in coronary heart disease prevalence. Because the inverse correlation between HDL, cholesterol and triglyceride levels, the role of triglycerides as an independent risk-factor was not found to be signficant on multiverate analysis, except for females.[23]

Freinkel[24] states that weight loss, nicotinic acid and clofibrate treatment lower VLDL and raise HDL levels. He states that diets high in carbohydrates have the opposite effect on the levels of these two lipoproteins. In all these conditions, reciprocity appears to be present between HDL and VLDL levels. The only known exception to this generalization are alcohol consumption and estrogen treatment, both of which raise HDL and VLDL levels.

HDL

With a balanced diet, the mean synthetic rate for HDL protein was 8.5 mg/kg/day.[24] Carbohydrate feedings resulted in a smaller mean particle size, a lower ratio of apoA-1 to apoA-II in HDL, and a larger portion of the total plasma HDL present in the smaller HDL-3 fraction. In all of these repects, reciprocal changes were seen with nicotinic acid treatment. Both perturbations had very little or no effect on the

synthetic rate of HDL protin. Major effects, however, were seen on the HDL catabolic pathway. Thus the high-carbohydrate diet significantly increased the rate of HDL protin catabolism from the plasma compartment (and lowered plasma HDL level), whereas nicotinic acid had reciprocal effects.[17,24] Triglycerides rise during a low fat diet because carbohydrates induce the synthesis of VLDL triglycerides. however, the claim has been made that the rise ini triglyceride concentration accompanying a high carbohydrate diet abates when such a diet is prolonged.

Some reflections on HDL are as follows: lowering of HDL can be caused by reduced levels of the enzyme lecithin cholesterol acyltransferase (LCAT) or reduced levels of apoA-1 and HDL levels can be very low, a finding that is associated with premature atherosclerosis.

Shephard, et al,[25] who studied a tenfold increase of the P/S ratio (from 0.4-4.0) in his subjects showed a decrease in HDL cholesterol and apo protein A-1. This has been confirmed in other studies[2] of infants and formula manipulations to affect the outcome. From this and several other reports[2,25] some consistency is emerging with regards to the effect that diets high in polyunsaturated fat (P/S ratio from 1.6 to 4.0) are associated with lower concentrations of apoprotein A-1 and HDL cholesterol than a low intake of polyunsaturated fats (with a P/S ratio from 0.25-0.45). It also appears, however, that a wide range of dietary cholesterol (20-5,000 mg/dl) has little effect on HDL cholesterol and apo protein A-1, although LDL and total cholesterol rose.[26,27] The Oslo Study[28] and Multiple Risk Factor Intervention Trial[29] have been cited as evidence that polyunsaturated fat does not alter HDL cholesterol concentration in humans. In neither study did the P/S ratio exceed 1.0, and

since the populations were free living, even this ratio cannot be documented. The apparent lack of reduction in HDL concentration might be attributable to the populations studied.

LDL Cholesterol

Earlier in the thesis it was stated that we would consider LDL as one entity; however, there are two LDL cholesterols. It is a point of interest that there is a normal LDL cholesterol and what might be considered an abnormal LDL cholesterol, the latter having as its characteristics a low molecular weight and abnormal chemical composition.[2] It is this latter one that is elevated with a hyper apoB, and is characteristic of those people with an endogenous hypotriglyceridemia. This is associated with the atherosclerosis and those who would appear at greater risk for coronary artery disease.[2]

Biological and Hematological Variables in Vegetarians

Some literature states that vegetarians have lower serum cholesterol than non-vegetarians, and a lower prevalence of ischemic heart disease than the general population. Earlier studies (West and Hays, 1968; Sacks, et al, 1975; Burslem, et al, 1978) confirm previous observations that vegetarians have a lower blood cholesterol than non-vegetarians. Although the HLD cholesterol was the same for both groups, the vegetarians had a higher HDL as a percentage of the total cholesterol. This was in accord with Gears'[30] report. Some variables to consider are the vegetarians' higher fiber intake, difference in life styles, general interest in healthful living and family, and often non-smokers. Vege-

tarians are usually leaner than non-vegetarians, yet in subjects that were matched this did not account for a difference in the lipid level which could be demonstrated in pairs of subjects matched for weight. The study done by Gear[30] stated that the difference in cholesterol concentration might be because of a difference in saturated fat intake, rather than because vegetarians have a higher intake of dietary fiber. Vegetarians with a saturated and a monounsaturated intake of over 50 gm/day had a mean plasma cholesterol concentration of 239 mg ml, which is similar to that of non-vegetarians. No relationship was apparent between cholesterol and dietary fiber. The difference between blood glucose and high density lipoproteins (HDL) expressed as a percentage of total cholesterol was small but consistent and statistically significant.[30]

In the above study, the ratio of cholesterol to HDL in a vegetarian was 3.26 and that of the non-vegetarian was 3.86; small but consistent, and statistically significant (the percentage of HDL in a vegetarian was 30.6 and percentage of HDL in a non-vegetarian was 25.2). It is interesting to note that he reported identical HDL values for both vegetarians and non-vegetarians.[30] Burr, et al[31] in his report, found that 85 vegetarians showed an average of lower cholesterol and a higher HDL than 214 non-vegetarians. The Burr, et al,[3] value of percentage of HDL with vegetarian was 24.35 and non-vegetarians were 19.28. The values represent men of less than 60 years of age.[31]

Blood Pressure in Vegetarians and Omnivores

The mean systolic and diastolic pressure showed no consistent or significant differences between vegetarian and non-vegetarian.[31] This

is in contrast with some of the findings of Sacks, et al[32] (1975) and Armstrong, et al[33] (1979), who reported lower systolic and diastolic pressures in vegetarians, although the results were not large.

A recent study done in London on various disorders among omnivores and vegetarians showed a "significant downward trend in disease prevalence with the adoption of vegetarian-vegan diet." Angina pectoris was diagnosed in omnivores in the fifth and sixth decades of life, and in the lifelong vegetarians not until the seventh and eighth decades of life.[34]

PURPOSE

Blood pressure, glycohemoglobin, total cholesterol, HDL cholesterol, cholesterol HDL ratio, LDL, Platelet Factor 4, and Beta Thromboglobulin were compared between a group of vegetarians and omnivores. The gross pathological anatomy of myocardial infarct has been well appreciated for many years. The micro-pathological anatomy is still in the unfolding stage and for this reason the study of PF4 and BTG was chosen. We limited our patients to vegetarian and omnivore males whose present ages were between 35 and 60 years old. Our source of patients were those who had previously been in the Adventist Health Study of 1978. There were a total of 312 names in this previous study, of which we randomly selected 27 vegetarians and 26 omnivores from the list. All of those who consented and met the criteria were accepted for the study.

METHODS

I personally telephoned each participant and talked directly with him, discussed the tests that were planned and of what value they would be to him regarding his health. An option was offered to the patient, to either mail the results directly to him, or to the physician of his choosing. We made no attempt to discuss the results, other than to point out those results which were out of the normal ranges. We mailed each patient his results and made ourselves available for any further inquiry from their physician. This was done to avoid any potential of malpractice implications to either the University or team members. At the outset of the telephone conversations we did a survey concerning their self-appraisal of their own health. That is, we questioned them as to whether they were, generally speaking, in good health. We also aksed if they had any known diease for which they were being treated. All of our candidates met the above mentioned standards. In addition, none were taking medications on a regular basis.

The patients were mailed a consent form and brought it with them the morning of the phlebotomy. Included in the mailing were instructions to the participant for the drawing of their fasting blood. Each blood sample had a minimum of a 12-hour fasting state.

The majority of patients were physicians, dentists, or allied health professionals who were assumed Seventh-day Adventists living within a radius of 15 miles from the Loma Linda University Graduate School, Department of Nutrition. We assumed that none of these volunteers were using tobacco in any form, since it is prohibited by the Articles of Faith for Seventh-day Adventists.

Each candidate was allowed to be seated for a few minutes in order to relax prior to measuring his blood pressure (BP) in each arm. The antecubital fossa was prepared in a routine manner for the phlebotomy. The BP cuff was kept in place and maintained at a level between the systolic and diastolic levels; this was done to facilitate the ease of phlebotomy. Two separate 20cc disposable syringes were used per patient, and each syringe had blood drawn up to the 15 ml mark. The #20 gauge disposable needle was left in the vein during the syringe changes. Prior to taking blood into the second syringe, we allowed a free flow of blood for approximately 3-5 cc before connecting the syringe. This was done to minimize any platelet disturbance or extraneous clotting material from contaminating the sample. The first syringe of blood was promptly put into respective tubes with anticoagulant material. The second syringe of blood was put into separately labelled PF4, BTG tubes for radioimmunoassay (RIA). The blood was gently allowed to run down the side of the tube to limit any physical disturbances to the cells. When filled, the tube was put into an ice bath until a sufficient number of tubes were collected to continue the next step in the procedure, which was to centrifuge the specimens at a precalculated speed of 3,200 rpm in a temperature controlled centrifuge at 2-4 degrees Centigrade for 30 minutes.

The tests performed for glucose, glycohemoglobin, total cholesterol, and high density lipid analysis were done utilizing Sigma Chemical Company laboratory kits. The procedures outlined in the kits were followed very carefully to ensure good laboratory results.

It was physically impossible to do all of the lab testing in a

single day, therefore, the plasma was frozen when not in use, and stored to be available as needed. Thawing and refreezing was avoided and the samples were used as needed for that day. Several of the fasting blood sugars were run on whole blood and some on plasma. The LDL and VLDL values were done by the Faculty Medical Laboratoy.

The PF4 RIA diagnostic kit was supplied by Abbott Diagnostic Laboratories.

The Amersham Corporation has available a thrombotect kit for the BTG, which was used to measure BTG. Both the PF4 and the BTG are RIA procedures.

RESULTS

Table 1 shows a significant difference in p values in both the systolic and diastolic BP of vegetarians vs. omnivores. The obvious results show the vegetarians are significantly lower. When we recorded the BP of our subjects, we took the pressure in each arm and then recorded the average of the two systolic readings and the average of the diastolic readings.

Tables 2 and 3 dealing with glucose in the fasting state and the glycohemoglobin show no statistical difference between the vegetarian and the omnivore. This is somewhat unexpected since other literature shows significant differences between these two groups, largely as the result of the vegetarian having a higher complex carbohydrate diet and with its associated glucose lowering effect.[32,33,35]

The lipid profile, as seen in Table 4, does show significant difference in the cholesterol and the cholesterol high density lipid ratio betwen the vegetarians, who have lower levels, and the omnivores, who in turn have higher levels. The HDL and LDL showed no significant difference.

However, in Table 5 the p value on the LDL studied showed 0.07, which almost reached a value of significance between the vegetarian and the omnivore groups.

Table 6, the last part of this study, involved PF4 and BTG. These showed no significant difference between either vegetarian or omnivore.

Table 2: Means of right and left arms systolic and diastolic blood pressure of vegetarians (23) and omnivores (25). Five readings are missing separate variance estimates (mmHg ± S.E.)

	Systolic BP	Diastolic BP
Vegetarians (23)	106.2 ± 2.5	74.2 ± 2.2
Omnivores (25)	121.7 ± 3.1	83.5 ± 2.3

Recorded readings of patients in seated and related position.

$p<0.01$ vegetarians vs. omnivores

Table 3: Fasting blood sugar values of vegetarians and omnivores. Fasting levels are an overnight fast of 12 hours (mg/dl ± S.E.)

	Fasting Blood Sugar	P Values
Vegetarians (27)	95.8 ± 6.2	N.S.
Omnivores (26)	98.2 ± 3.4	N.S.

N.S. - Not Significant

Normal fasting glucose levels 60-110 mg/dl

Table 4: Determination of glycohemoglobin in vegetarians and omnivores (mg/dl ± S.E.)

	Glycohemoglobin	P Values
Vegetarians (27)	6.5 ± 0.52	N.S.
Omnivores (26)	6.2 ± 0.42	N.S.

N.S. - Not Significant

Normal Glucohemoglobin levels 4.0 - 9.6 mg/dl

Table 5: Overnight 12-hour fasting levels of plasma cholesterol and HDL of vegetarians and omnivores (mg/dl ± S.E.)

	Cholesterol	HDL-Chol	Cholesterol/HLD-Chol
Vegetarians (27)	200.4 ± 8.8*	47.3 ± 2.6	4.5 ± 0.34*
Omnivores (26)	235.3 ± 11.3	43.7 ± 2.9	5.9 ± 0.44

* $p<0.05$ comparing vegetarian with omnivore

Normal cholesterol levels 125-220 md/dl

Normal cholesterol/HDL levels (male) < 4.97 md/dl

Table 6: Overnight 12-hour fasting levels of plasma LDL for vegetarians and omnivores (mg/dl ± S.E.)

	LDL	
Vegetarians (16)	144.6 ± 7.4	N.S.
Omnivores (12)	181.7 ± 18.1	N.S.

Normal LDL levels 66-178 md/dl

HDL and LDL were performed by Loma Linda University Faculty Medical Lab.

Table 7: Radioimmunoassays for PF4, BTG on vegetarians and omnivores.

	PF4	BTG
Vegetarians (27)	38.2 ± 6.3	67.8 ± 13.0 N.S.
Omnivores (26)	38.1 ± 6.8	87.1 ± 17.1 N.S.

DISCUSSION

The findings in this study concerning the BP were consistent with a great many other authors, as stated earlier in this thesis.[31,33] There was a significant decrease in both systolic and diastolic values when the vegetarian is compared to the omnivore. Yet, Burr, et al,[31] who in 1981, while studying plasma cholesterol and BP in vegetarians, found no consistent or significant difference between vegetarians and non-vegetarians when measuring the mean systolic and diastolic pressures. These findings may be related to some of the health habits of some Seventh-day Adventist vegetarians, in that they do not drink any caffeine, or very limited amounts, whereas the study of Burr, et al,[31] may have not had this variable controlled.

Concerning glucose and glycohemoglobin, it was anticipated that both of these values would be reduced.[9,35] One of the reasons, maybe, that there was no difference between vegetarians and omnivores might be due to no known history of diabetes in either one of the groups studied. The literature is replete with references for the treatment of both type I and type II diabetes by increasing the complex carbohydrates and lowering fat.[9,32,35] This makes a significant change, lowering glucose and glycohemoglobin. This is one of the goals which the American Diabetic Association strongly endorses. The men stuied were of above average intelligence, and for the most part were health professionals; they may have been aware of the value of complex carbohydrates in their diet and consumed more in their diets than non-professionals.

Table 3 showed both cholesterol and cholesterol/HDL ratio indicating signifcant differences between the vegetarian and omnivore. Earl-

ier studies by West, Hayes, and Gear also show significant reduction in these values among vegetarians, compared to omnivores.

The LDL and the VLDL values showed no significant differences. However, as noted earlier, the p value for the LDL was .07 and almost of significant value. Even though the lab results are in a normal range, the vegetarians in this study are somewhat at risk if we would assume that 100 mg/dl or more would put a person at risk.

The RIA as shown in Table 6 showed no significant difference between the PF4 and BTG of the two groups studied. PF4 normal values are 13 ng/ml and BTG 50 ng/ml. Perhaps there is not any significant difference in either PF4 or BTG, in what appears to be normal health patients, and any differences may be relevant when cholesterol levels are higher than what these subjects have.

There was no significant correlation between the following:

total cholesterol and PF4

total cholesterol with BTG

HDL with PF4

HDL with BTG

systolic BP with PF4

diastolic BP with PF4

systolic BP with BTG

diastolic BP with BTG

PF4 with BTG

systolic BP with cholesterol

diastolic BP with cholesterol

systolic BP with HDL

diastolic BP with HDL

systolic BP with glucose

diastolic BP with glucose

systolic BP with HBA1 or

glychohemoglobin or diastolic BP with HBA1.

CONCLUSIONS

If this experiment were repeated again, I now am aware of a number of variables that should be controlled in a future study. Some of the variables are to try to make an estimate of the number of large and small platelets, evaluate if there is a possible iron or vitamin B12 deficiency which could affect the platelet count, and any recent previous platelet count to evaluate with the present platelet count, thereby evaluating if rebound phenomena are present. Some of the antiplatelet factors that were not considered in this study were whether or not the patient had ingested aspirin within five days prior to his phlebotomy, what amount of fish the omnivore is eating (involving omega-3 fatty acid ingestion, thus influencing platelets), what individual donor thrombopoiesis accountability, the matter of platelet separation (the force of centrifugation) and the specific gravity of the centrifuge media, the variation in platelet concentration affecting platelet metabolism due to thrombocytopenia and/or thrombocytoses, mainain the syringes in a cool temperature and be certain that the assistances minimize physicaol disturbance to the samples. Smoking has been overlooked as a risk factor in my study. I would also wish to know how much exercise that person does on a regular basis, and to what extent he drinks coffee. Frederickson's classification would have organized the groupings of those patients who had an elevated cholesterol. If we would have had their classifications, we would have an approximate idea of the concentrations of HDL, VLDL, and LDL.

SUMMARY

The vegetarians (26) versus omnivores (27) showed significant decrease in systolic BP, diastolic BP, chole, RHDL while the LDL, Glu, HDL, VLDL, PF4, and BTG, as well as correlation between these values, were not significant by statisticaly analysis.

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APPENDIX A

Vegetarian

IDENT	BLD PRESS	GLUC	Hb A1C	CHOLES	HDL	CHOLES HDL	LDL	VLDL	PF-4	B-Tg
1	138/86	113	4.5	133	47	2.8			9	208
2	-----	145	10.6	230	66	3.4			36	235
3	120/70	134	4.2	178	66	2.6			92	225
4	118/75	128	5.4	318	47	6.8			14	205
5	146/106	94	5.4	281	44	6.4			62	196
6	122/88	112	5.8	244	31	7.9			52	118
7	112/68	103	5.7	181	23	7.9			14	30
8	128/100	103	5.9	259	23	11.3			10	35
9	144/102	83	8.5	204	38	5.4			4	232
10	120/94	75	7.5	212	41	5.2			36	235
11	120/82	75	9.3	216	20	10.8			88	262
12	118/84	85	8.9	278	31	9.0			26	223
13	160/102	75	10.0	275	47	5.8			49	280
14	100/72	80	10.5	245	41	6.0			38	280
15	148/86	96	6.4	236	38	6.2	198		07	144
16	132/94	96	4.9	177	51	3.5	126		05	280
17	118/88	92	4.6	259	41	6.3	187	31	09	63
18	122/82	99	5.1	226	29	7.8	126	72	09	71
19	108/78	99	5.1	216	51	4.2	155		09	75
20	100/68	92	4.4	239	54	4.4	185		31	60
21	120/80	99	4.6	223	62	3.6	161		4	67
22	110/80	104	3.3	427	82	5.2	345		7	44
23	110/70	95	5.5	161	39	4.1	122		95	42
24	108/86	91	4.1	186	36	5.2	150		88	180
25	110/80	93	4.9	228	50	4.6	178		96	180
26	110/66	93	5.1	285	38	7.5	247		100	170
	X	98	6.0	233	44		176	52	41	159
	X	96	5.5	228	41		161		26	180
	Mode	0	0	0	0		0		9	280
	Midrange	110	7.3	280	51		233		52	205
	Range	70	6.4	294	29		111		48	150

Omnivore

IDENT	BLD PRESS	GLUC	Hb A1C	CHOLES	HDL	CHOLES HDL	LDL	VLDL	PF-4	B-Tg
1	138/86	113	4.5	133	47	2.8			9	208
2	-----	145	10.6	230	66	3.4			36	235
3	120/70	134	4.2	178	66	2.6			92	225
4	118/75	128	5.4	318	47	6.8			14	205
5	146/106	94	5.4	281	44	6.4			62	196
6	122/88	112	5.8	244	31	7.9			52	118
7	112/68	103	5.7	181	23	7.9			14	30
8	128/100	103	5.9	259	23	11.3			10	35
9	144/102	83	8.5	204	38	5.4			4	232
10	120/94	75	7.5	212	41	5.2			36	235
11	120/82	75	9.3	216	20	10.8			88	262
12	118/84	85	8.9	278	31	9.0			26	223
13	160/102	75	10.0	275	47	5.8			49	280
14	100/72	80	10.5	245	41	6.0			38	280
15	148/86	96	6.4	236	38	6.2	198		07	144
16	132/94	96	4.9	177	51	3.5	126		05	280
17	118/88	92	4.6	259	41	6.3	187	31	09	63
18	122/82	99	5.1	226	29	7.8	126	72	09	71
19	108/78	99	5.1	216	51	4.2	155		09	75
20	100/68	92	4.4	239	54	4.4	185		31	60
21	120/80	99	4.6	223	62	3.6	161		4	67
22	110/80	104	3.3	427	82	5.2	345		7	44
23	110/70	95	5.5	161	39	4.1	122		95	42
24	108/86	91	4.1	186	36	5.2	150		88	180
25	110/80	93	4.9	228	50	4.6	178		96	180
26	110/66	93	5.1	285	38	7.5	247		100	170
	X	98	6.0	233	44		176	52	41	159
	X	96	5.5	228	41		161		26	180
	Mode	0	0	0	0		0		9	280
	Midrange	110	7.3	280	51		233		52	205
	Range	70	6.4	294	29		111		48	150

APPENDIX B

Health Questionnaire

Study # _____

Part A

1. Today's Date: / / /
   mo day yr

2. Date of Birth / / /

   mo day yr

3. Sex: Male [ ] Female [ ]

4. Has a doctor ever told you that you had any of these conditions? Mark "yes" only for conditions diagnosed by a doctor, even if the condition is no longer present. Be sure to mark "no" for conditions you have never had.

   4a. Heart attack or coronary (myocardial infarction) No [ ] Yes [ ]

   4b. Stroke or apoplexy No [ ] Yes [ ]

   4c. High blood pressure No [ ] Yes [ ]

   4d. Diabetes No [ ] Yes [ ]

   4e. Cancer or leukemia No [ ] Yes [ ]

5. Please list the prescription drugs you have taken in the last 30 days: (Please print)

   _____

   _____

   _____

   _____

6. Please list the non-prescription drugs (e.g., aspirin, Bufferin, Tylenol, Contact) that you have taken in the last 30 days (please print):

_____

_____

_____

_____

7. What is your best estimate of your present weight and height in normal indoor clothing <u>without</u> <u>shoes</u>?

   a. Current height _____ft _____ in.

   b. Current weight _____lbs

APPENDIX C

Loma Linda University

School of Health
Department of Nutrition
Loma Linda, California 92354
714/824-4598

Consent Form for Diet and Health Study

I, ----------, hereby agree to participate in this study on the relationship between dietary factors and cardiovascular disease risk. Specifically we will be measuring blood pressure, blood cholesterol and other blood fats, blood sugar and blood clotting.

As part of this study, I have agreed to complete a questionnaire about my diet and lifestyle and to spend approximately one hour or less at the Faculty Medical Offices located in Loma Linda. I have agreed to not eat anything after 10 p.m. the evening before my visit to Loma Linda, however no limit for plain water. Furthermore, I will not eat breakfast on the day of my visit to Loma Linda.

During my visit to Loma Linda, I have been told that two samples of blood will be drawn. The blood will be drawn by a physician. The blood samples will be used to detect diabetes, measure the amount of blood cholesterol and other substances related to the clotting action of my blood. Each sample will consist of approximately three tablespoons of blood (i.e., approximately 30 cubic centimeters). It is possible that a small amount of blood may temporarily accumulate at the site on my arm where the blood is drawn. This may cause the site to become slightly discolored like a small bruise. (This is only a temporary condition and is a common condition following blood drawing.) My blood pressure will also be taken during my visit to Loma Linda.

Loma Linda University

School of Health
Department of Nutrition
Loma Linda, California 92354
714/824-4598

I have been told that I can request that my study results on blood chemistries and blood pressure be forwarded to me. These study results may alert me to the presence of diabetes, high blood pressure, and high blood cholesterol.

I have been told that all the information collected in this study will be kept strictly confidential. My name will never be associated with any of the data released from this study.

While the risks involved in this study are minimal, I have been told that emergency treatment is available should a health problem develope during my Loma Linda visit. I should contact Glenn Sharmann (714) 824-4634, Patient Representative, for information and the required forms. I also have been told that I am free to withdraw from this study at any time. I may call Dr. Blankenship at (714) 824-4300, ext. 7172 if I have questions about this study.

After signing this form, I will receive a copy. I have read the contents of this consent form and questions regarding this study have been answered to my satisfaction. I hereby give voluntary consent to participate in this study.

_____ _____

Signature of Subject *Date*

_____ _____

Signature of Witness *Date*

_____ _____

Signature of Investigator *Date*