1974

Arterial fine structure and blood chemistry changes induced by periodic starvation and refeeding a high carbohydrate diet

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by

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A Dissertation Submitted to the

Graduate Faculty in Partial Fulfillment of

The Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Department: Veterinary Physiology and Pharmacology
Major: Physiology (Domestic Animals)

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa

1974

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INTRODUCTION

Numerous investigations have shown factors such as lipid concentrations in the plasma, constituency of the diet, blood pressure, smoking, and genetics to influence the development of arterial disease. Coincident with aging, smooth muscle cells and extracellular matrix components accumulate in the intima. Areas of thickening occur which are generally associated with fatty deposits. Commonly affected vessels are the aorta, iliac, femoral, coronary, and cerebral arteries. It is these areas of thickening that can progressively or abruptly interfere with blood flow and cause ensuing physical and/or mental impairment or death to the individual. A task force of the National Heart and Lung Institute (1971) indicated that over 50% of all deaths in the United States can be attributed to heart and blood vessel diseases, of which atherosclerosis is the chief cause.

The sequence of pathological events at the cellular level needs to be clarified, however, to aid fully in the explanation of mechanisms which lead to different cardiovascular diseases. Thus, selected experiments have been designed which induce structural variation within the wall of the blood vessels to allow for a better appreciation of the possible disease processes.

The primary goal of this project was to determine if starvation and the refeeding of a high carbohydrate diet to dogs would result in any fine structural change of the femoral artery and, thus, contribute to a decreased arterial capacity and decreased arterial distensibility. If fine structural change with associated physiological properties occurred, the possibility of

hypertension may be increased.

A second goal of this project was to evaluate various blood chemistries. Specific interest was given to lipoprotein patterns which would indicate how the dog reacts to dietary stresses.

REVIEW OF LITERATURE

Arterial Fine Structure and Pathophysiological Change

Blood vessels in general are conduits for the circulation of blood, and the arteries in particular are regulators of blood distribution and flow. Interpretation of structural change in the pathological state depends on the understanding of normal fine structure. A description of the distributing artery's architecture is presented for a better appreciation of the vessel's physical properties that relate to function.

Fine structure of muscular arteries

A complete description of the ultrastructure of normal large arteries can be found in the literature (Pease and Paule, 1960; Keech, 1960; Paule, 1963; Buck, 1958; Parker, 1958; and Simpson et al., 1962). The walls of normal distributing arteries are composed of three morphologically distinct layers. The inner layer of the artery, or tunica intima, is observed in the newborn and children as a narrow region bounded on one side by a single continuous sheet of endothelial cells which line the vessel lumen and on the other side by the internal elastic lamina, a perforated sheet of elastic tissue. Paule (1963) reported that the endothelium of the adult rat aorta frequently abuts directly on the internal elastic lamina, whereas, the endothelium in newborn rat aorta usually rests on a subendothelial layer or space.

Parker (1958) demonstrated that rabbit coronary arteries possess no evidence of intercellular or intracellular gaps, fenestrations, or pores in

the endothelium. The endothelium of the coronary artery resembles that lining the aorta (Buck, 1958) and the capillaries in the skeletal muscle as pictured by Moore and Ruska (1957) and by Palade (1953). It was shown by Buck (1958) that in the rat, rabbit, puppy, cat, and ferret endothelial cells, particularly in the large muscular arteries such as the femoral, were ballooned into the lumen with their outer margins extending into the accordion-like folds of the internal elastic lamina. Aortic endothelial cells, however, show this to a lesser degree because of the relatively smaller amount of contraction of the aortic wall on removal. The endothelial cell profiles in longitudinal section are longer than in cross-section. The thickness of the endothelial cell ranges from 0.2 to 0.8 μm over the thinner portions of the cytoplasm. These values may reflect shrinkage and distortion imposed by preparative procedures. The thickness can be expected to vary with stretching or constriction of the blood vessels. Close to the nucleus, endothelial cells bulge, achieving a thickness of about 3.5 μm. The endothelial cells lining the vessel have long cytoplasmic processes and a prominent nucleus in the center of the cell. In cross-section the nucleus appears as oval and flattened in the plane of the endothelium. It measures about 2.5 μm in thickness along the radial axis of the vessel. Along the long axis of the nuclei, and parallel to circumferential planes, the diameters range from 3.5 to 7.5 μm.

The plasma membrane is prominent, with a thickness of 50-100 Å. Associated with its surfaces, luminal, outer and intercellular, are small invaginations forming caveolae and vesicles. Palade (1953) reported that

transitional stages could be recognized between open caveolae and oval, thin-walled vesicels about 300-500 Å. Although a few pinocytotic vesicles are to be found in the endothelial lining of muscular arteries, they are not an outstanding feature, as they are in smooth muscle cells of the tunica media. Presumably, if the capillaries utilize the small vesicles for the transport of fluids (pinocytosis) as suggested by Buck (1958), the arterial endothelium may also. He, also, suggested that it seems likely that small indentations develop at the interior or exterior plasma membrane, then pinch off to form vesicles, move across, and join with the opposite plasma membrane to release their contents. Fusion of the vesicles with endoplasmic reticulum and the perinuclear cisternae would serve to supply these systems of the cell with fluid as well as membrane substance. Lipoproteins may, perhaps enter the cells in a molecular form through the small vesicles. However, in the case of cholesterol-fed rabbits, the lipoprotein is reported (Buck, 1958) to induce such a reaction that the endothelial cells no longer can be said to be normal. In endothelial cells one can also find mitochondria and various granules and membrane components corresponding to endoplasmic reticulum and the Golgi complex. The mitochondria are usually spherical with an average diameter of 2.2 μm. The number of mitochondria indicates no highly-energy demanding processes. Rhodin (1962) states that the morphological features of this layer tend to demonstrate that the endothelial cell is engaged in a moderate production of proteins as part of its own metabolism, but not for extracellular discharge.

Also in the cytoplasm are a number of relatively dense bodies, limited by a single membrane and about the size of a mitochondrion, but having no such distinctive internal structure. Buck (1958) demonstrated that upon administering thorium dioxide to an animal 3-24 hours prior to observation these dense bodies were found to contain the compound. The bodies were thus demonstrated to be inclusions and concerned with phagocytosis. The nature of the dense material normally found in them is not known. Endothelial cells also contain glycogen and have alkaline phosphatase activity.

Besides being regenerative (Ham, 1969), the normal endothelial cell displays a state of tone which enables it to maintain a fixed shape and to resist distortion by external forces. However, the cytoplasm may render itself flexible when constantly submitted to stress. This is accomplished by numerous fine filaments in the cytoplasm with an average thickness of 70 Å. The length of these may vary, but usually they are not greater than 0.33 µm. Rhodin (1967) found in his work with mammalian arterioles and precapillary sphincters that these endothelial filaments are ultrastructurally no different than myofilaments of smooth muscle cells. They are probably tonofilaments which strengthen the cell. There are stretches of membrane along the surfaces of the cell where areas of firm attachment exist. These dense areas have been termed as annular attachment plaques corresponding to the intercalated discs of the heart muscle, or desmosomes of stratified squamous epithelium (Parker, 1958).

A thin irregular layer of relatively dense material surrounds the surface of the endothelium; it may be defined as the basement membrane. It

is 900 Å wide and has a characteristic structure which is more prominent in the coronary artery than in the aorta. It tends to be broken up and to disappear in the larger muscular arteries. Some investigators have asked questions as to its existence in the aorta and others have found its virtual absence not too surprising (Pease and Paule, 1960).

Mediating this membrane and the internal elastic lamina is the subendothelium which contains occasional smooth muscle cells that are surrounded with various extracellular components of the connective tissue matrix. These subendothelial smooth muscle cells are arranged longitudinally. Endothelial cells appear to form at least the subendothelial proelastin fibrils, in lesser amount but exactly like the medial cells (Fyfe et al., 1968). Work by Pease and Paule (1960) showed that the endothelial side of the internal elastic membrane of the aorta was seen to contain areas where probably elastin formation was in progress at the time of tissue preservation. A finely divided reticulum was seen there. There is every reason to believe that this is homologous to the reticulum observed in muscular arteries. The reticulum is known to be mucopolysaccharide on the basis of the periodic-acid-Schiff histochemical staining reaction. In this reticulum small aggregates of elastin units could be defined with certainty on the basis of their characteristic unit structure. Morphological patterns of this type have not been found in muscular arteries. The intracellular threads in the associated dark cytoplasmic areas of the endothelial cells, however, do not have such extensive parallel arrays as those in media cells, and they do not persist after elastogenesis is complete.

In ordinary sections the internal elastic lamina of a muscular artery presents a wavy appearance, but this is probably due to the artery contracting after the blood pressure within the vessel becomes almost negligible as would exist in the surgical excision of an artery. Strong (1938), however, indicated that this appearance is due to the intrinsic elastic qualities of the artery and not consequential of post-mortem contraction. It is not unusual to observe what is termed a split internal elastic lamina in the walls of some arteries. Perhaps it would be better to term this as a duplication of the internal elastic lamina, because when the term "split internal elastic lamina" was coined it was not appreciated to the same extent as it is today that smooth muscle cells in the undifferentiated state in this region can keep on forming elastin in postnatal life (Ham, 1969). The internal elastic lamina appears as a thick sheet of moderately dense material consisting of two components. The one appears to be a homogeneous and tenacious looking matrix which often appears folded or as if it had been pulled away from the endothelium and smooth muscle cells (Parker, 1958). This could be due to the action of the knife during sectioning.

A second component, consisting of many fibrils, is incorporated within the matrix. These fibrils appear either as small dense strands approximately 500 Å in diameter or dots depending on the plane of sectioning. Rhodin and Dalhamn (1955) reported that no periodicity is observed in these fibrils. An experiment by Dempsey and Lansing (1954) demonstrated that when elastic fibers are chemically digested by elastase or unpurified trypsin or are mechanically fragmented, there is found an associated fine

fibrous component. With electron microscopy, they observed that partially digested fibers seemed to be composed of small fibrils at the frayed edges. If digestion were prolonged, this process of fraying of larger fibrils would allow only short filaments of 180 Å to remain. These coiled fibrous elements are of great interest because of the role they may play in the elastic properties of elastic tissue.

In still other places the internal elastic lamina may be interrupted for as much as a few microns. In the vicinity of such fenestrations relatively large subendothelial spaces are apt to develop. Endothelial cells occasionally project filiform processes through the openings towards the media (Moore and Ruska, 1957). Also observed were branches of elastic material extending from the internal elastic lamina into the tunica media between smooth muscle cells. Mucopolysaccharide reticulum and even extrusions of muscle cell protoplasm from the media are evidenced. The processes that extend from the highly irregular surface of the elastic mass come very close to the endothelial cell membrane at many points. Between these processes there are less dense areas which may represent extracellular fluid. Parker (1958) reported that his work on coronary arteries showed that no well-organized subendothelial layer of collagenous fibers, fibroblasts, and macrophages could be recognized. Pease and Paule (1960) have seen these things as such a common feature of aortic structure that they do not regard them as in any way pathological. Age is not considered to be a cause for these common fenestrations and subendothelial spaces. Waters (1965) reported that small amounts of fibrous

connective tissue are contained in the arterial intima of a dog. Dog intima is especially thin and contains little more than an endothelial lining. The fine structure of the normal dog aorta endothelium does not differ appreciably from other species (Buck, 1958; Parker, 1958; and Keech, 1960).

The arterial tunica media consists of several layers of smooth muscle cells arranged generally in a circular fashion within the vascular wall. Each cell is surrounded by small amounts of collagen and varying numbers of small elastic fibers and other connective tissue matrix components. No fibroblasts are present in the media of mammalian arteries. Large amounts of elastic tissue are found in the aorta in contrast with the smaller, muscular arteries. The morphology of the media, in contrast to that of the intima, generally does not alter with age.

Whereas, anatomically, dogs' arteries, including the aorta, are largely muscular in type and correspond rather closely to muscular arteries in man (Waters, 1965), only muscular arteries from other species will be discussed. The limitation is on the relationship that elastic and collagen tissues have on the smooth muscle cells in the media.

Cellular organization is not particularly evident in a transverse section of a muscle layer. However, when any one cellular layer is viewed in the horizontal plane (tangential to the artery), it is obvious that the cells of a particular layer are all arranged parallel to one another. Pease and Paule (1960) observed in rat thoracic aorta that the orientation of cells in successive layers are the same in one layer but their direction

changes from one layer to the next. If the aorta is quite exactly aligned for making transverse sections, it is indeed rare that muscle cells of even one layer are cut entirely in one profile (longitudinal in this case). Invariably, profiles are obtained that indicate that smooth muscle is arranged obliquely to the plane of section, proving that muscle actually exists in a helical arrangement around the artery. This helical arrangement was demonstrated in human and mammalian renal and mesenterial arteries by Strong (1938). Its existence has also been found in mouse femoral arteries by Rhodin (1962). The angle between the helical turn and the long axis of the vessel differs some from vessel to vessel and in segments of the same vessel (Rhodin, 1967). The small arteries that were used in Rhodin's investigation (1962) were thought to show angles of 30°. Decreasing the vessel diameter only seems to increase the angle until finally in the arterioles the smooth muscle cells are arranged in a truly circular fashion. It would seem evident that such an architecture would be nearly ideal to counterpoise the internal pressure of the vessel.

Smooth muscle cells are usually spindle shaped. They have a diameter in the central cell region of approximately 1.5 to 2.5 μm and an average length of 60 μm. Keech (1960) and Pease and Paule (1960) indicated the smooth muscle cells of aortas and arteries have irregularly branched processes. This is true, also, in mouse femoral arteries (Rhodin, 1962).

Distinct plasma and basement membranes have been seen separating individual muscle cells in some arteries. This characteristic has not been so obvious in mouse femoral artery and cat and monkey pial vessels.

Where it is not so apparent, it is considered that both membranes constitute the smooth muscle sarcolemma. The plasma membrane has a thickness of 80 Å. The basement membrane varies in thickness. It is conceivable that adjacent smooth muscle cells may be separated by only a single shared basement membrane. It was shown by Pease and Molinari (1960) in their electron microscopy study of cat and monkey pial vessels, that since basement membranes follow the contours of associated cells, they are free to bifurcate or branch complexly wherever intercellular planes diverge. Undoubtedly this type of framework is flexible and yet it binds cells together and makes the whole vessel wall function as a unit.

The smooth muscle plasma membrane shows several characteristics not usually associated with the striated muscle membrane. The presence of many caveolae and vesicles are seen associated with the plasma membrane. The vesicles have often been termed pinocytotic implying a function upon which there is not general agreement. Moore and Ruska (1957), Rhodin (1962), and Palade (1953) claim the vesicles are being created, pinched off, and moving across to the opposite plasma membrane to release their contents. Though this may exist in the endothelium, there is controversy on whether it occurs in the plasma membranes of smooth muscle cells. Caesar et al. (1957) showed that the plasma membrane on occasion was thicker than the basement membrane by approximately 40 nm. The dense thickenings would alternate with regions of pinocytotic activity. The thickenings also would lie at corresponding regions of adjacent cells and where denser regions of the basement membrane would occur. This was not just a consequence of contraction, for these areas would occur in both stretched and

contracted portions of the cell. Pease and Molinari (1960) and Devine et al. (1971) found the same to exist in cat and monkey pial vessels and guinea pig mesenteric arteries. Muggli and Baumgartner (1972) further investigated this problem using rabbit iliac arteries. The membrane invaginations are interpreted by these two investigators to be possibly analogous to the T-system of striated muscle.

Though vascular smooth muscle shows very little smooth-surfaced endoplasmic reticulum, the rough-surfaced type is abundant. Ribosomes may be evident with an average diameter of 150 Å and may be free or attached to reticulum. In that the smooth endoplasmic reticulum, or sarcoplasmic reticulum, of the striated muscle cell is probably involved in the inward propagation of excitation for muscle contraction, its lack of prominence in vascular muscle may suggest that it is not functionally the same.

Nucleus, mitochondria, and some lipid granules are components of the smooth muscle cell. Myofilaments have a length of about 1.0 μm. The shortness in length may be all that can be followed due to possible twisting of myofilaments around one another. The width of myofilaments averages 80 Å in both longitudinal and transverse sections of vascular smooth muscle. Though this average is representative of the femoral artery in the mouse, an average of 30 Å was reported for cat and monkey pial vessel myofilaments. The myofilaments presumably represented actomyosin since extraction with alkaline 0.25 M KCl (pH 8.5) removed the filaments.

The dense thickenings that were aforementioned were thought to be attachment devices for myofilaments (Rhodin, 1962). An investigation by

Devine and Somlyo (1971) gave evidence for 180 Å thick and 84 Å thin myofilaments in vertebrate vascular smooth muscle. A ratio of 12:1 existed for thin to thick myofilaments. Thin filaments, although arranged predominantly along the longitudinal axis of the cell often pursued a wavy course between thick filaments in the dense area. At high magnifications some thick filaments appeared to have densely stained areas or lateral extensions. Wavy thin myofilaments were observed to come close to the extensions. Thick filaments were not always parallel to each other and sometimes V-shaped junctions existed between thick filaments. Somlyo and Somlyo (1968) reported that during contraction, the plasma membrane near the dense areas appeared retracted. This suggested that a considerable amount of tension must be transmitted to and through a sarcolemma. It was reported, too, that the dense areas represented zones of increased contractile material or regions of overlap between the thin actin and thick myosin filaments which would be analagous with the "A" band in striated muscle.

Innervation of smooth muscle is rare, and if so, is of the unmyelinated type. Nervous control is not altogether necessary to mediate stress. Keatinge (1964) reported that the inner muscle layer of a sheep carotid artery which was not innervated was 10 to 100 times more sensitive to norepinephrine than the outer muscle layer or adventitia where nerves were. The tunica adventitia, which adheres closely to the media, shows rarely an elastic fiber. Fibroblasts and collagen are the prominent constituents.

Though a possible mechanism for muscle contraction has been presented, other elements in the artery must exist to provide variable reaction to

different physiological pressures. One element is elastic tissue. Predominant elastic distribution for the muscular artery exists in a circumferential arrangement. The internal elastic lamina is a continuous sheet of elastin, except for fenestrations. Elsewhere in the media, elastin appears in islands which are net-like around muscle cells.

Tangential sections indicate that most if not all small profiles of elastin seen in transverse sections are actually spicules affording smooth muscle cells multiple points of anchorage to laminae. Probably, the tip of spicules contain buried elastin fibrils. Myofilaments which retain their attachment to the spicules maintain their identity by the intracellular intermingled organelles in the smooth muscle cell. Spicular attachments are fewer where a second element, collagen, is abundant. Fyfe et al. (1968) suggested that spicules under tension may be released and retracted into the substance of the elastic laminae. This could provide a source and way for successive amounts of elastin needed in circumferential and longitudinal growth of the artery.

Pease and Molinari (1960) demonstrated from their investigation of the pial vessels, that a close relationship existed between elastic fibers and basement membranes of smooth muscle cells. If phosphotungstic acid staining is not used, a basement membrane may mistakenly be recognized as elastin. There was seemingly a blending and attachment of the elastin to the basement membranes to form an apparent, continuous system. Collagen, however, was not found to be associated with either elastin or basement membranes, but demonstrated a separate framework in the media. This

structural element has an average diameter ranging from 400-700 Å and an axial periodicity of 500-600 Å. Since the authors had seen no fibroblasts in either endothelium or media, they felt that smooth muscle cells are responsible for organizing the medial connective tissue framework.

Regarding the origin of elastin, Fyfe et al. (1968) found groups of fine electron-dense fibrils, considered to be proelastin, projecting from a developing smooth muscle cell, better known as a multifunctional mesenchyme cell. These fibrils were then seen connecting with less electron-dense islets of homogeneous elastin. Apparently, the fine fibrils were originating from dark areas in the mesenchymal cell. The authors have found no evidence that fibrils polymerized as such within the cytoplasm. They think a precursor substance is secreted which is polymerized outside the cell. Many times the fibrils that become polymerized remain attached to the cell that secreted the precursor. These same dark areas are concerned with formation of myofilaments. Collagen fibers and mucopolysaccharides are thought, also, to have an extracellular origin or are fabricated at least extracellularly, though a precursor substance may be secreted from multifunctional mesenchymal cells.

In vessel walls, elastic tissue is considered to be most important for spreading stresses uniformly onto collagenous fibers which provide the true mechanical framework to the wall. Cliff (1970) reported that elastin is probably the main functional component at low and physiological pressures. Wolinsky and Glagov (1964) found in their studies with aortic media that collagen was the effective structural component at and above physiological pressures. It was found that if mechanical stresses were applied

to a vessel, fibroblastic activity was induced and areas of newly formed collagen fibers occurred in the wall of the vessel.

Fine structural alterations in the induced state

The dog is relatively resistant to the production of lesions (Waters, 1965). Extreme measures such as thyroidectomy and intensive cholesterol feeding followed by precautionary administration of ^{131}I are needed before the dog will produce even the most meager of lesions (Jordan et al., 1959). Gresham and Howard (1966) did not rate this animal very high on the list of experimental animals for the production of atherosclerosis. Spontaneously or naturally occurring lesions are found commonly as fibroelastic plaques in the aorta of old dogs (Detweiler et al., 1961). Luginbühl et al. (1965) have observed most arterial lesions to exist and to be most severe in dogs over 7 years of age. Getty (1966) reported that in dogs over 10 years of age most of the major blood vessels demonstrated sclerotic lesions to some degree. Although beginning plaques were microscopically evident in the 7-month old animal, they were devoid of lipids until the animal was 5 years of age. It was Getty's conclusion that morphological changes in the major blood vessels of the older dogs are similar to those in the human that are known as atherosclerosis, arteriosclerosis, and intimal sclerosis.

Certain experiments have been designed, however, to induce structural variation upon blood vessels, so that a possible elucidation could be had on the manner in which a dog and other species react to certain abnormal processes.

Geer (1965) reported on an experiment which involved five female and two male mongrel dogs about 1 year of age. After 1 month on a basal ration, the dogs were fed cholesterol and 2-thiouracil for 4 months. Eight other dogs were fed the same basal ration but supplemented with varying amounts of corn oil or cholesterol. At the termination of the experiment these latter dogs showed no gross lesions, no stainable lipid in the aorta, nor abnormalities at the fine structural level. All seven dogs fed cholesterol and 2-thiouracil had atheromatous arterial lesions in most major arteries. Electron microscopy revealed electron-dense, coarsely granular lipid inclusions that were limited by a single membrane. The Golgi apparatus was not observed to be participating in the process of lipid accumulation. It was thought that agranular endoplasmic reticulum forms the lipid. The real source of these arterial lipids is in question. Lipids carried by the plasma may be assimilated by the artery. Insull (1972) claimed arterial lipids may arise in situ by de novo synthesis, or by elaboration on or modification of lipids obtained from the plasma.

Suzuki et al. (1964) conducted an experiment utilizing two male and three female mongrel dogs, all of which were less than 4 years of age. For a period of 86 days, each of the dogs was fed daily 270 g of a diet consisting of 7.3% cholesterol, 0.5% thiouracil, 8.1% lard, and the remainder in Friskies dog food. The diet was changed on the 87th day to 200 g of high fat diet/day/animal. This diet consisted of 40.0% butter, 5.0% cholesterol, 2.0% sodium cholate, 0.3% thiouracil, 20.0% casein, 20.5% sucrose, 0.2% choline chloride, 2.0% vitamin mix, 4.0% salt mix, and 6.0% alphacel. Only two dogs survived the experiment. Dense granular material,

thought to be calcium salts, was observed along the internal elastic lamina in coronary, iliac, caudal, and aorta vessels.

Hoff and Gottlob (1969) did a study on the uptake of egg lipoproteins that were injected into doubly-ligated rabbit carotid arteries. The vacuoles they observed in both endothelial and smooth muscle cells were presumably lipid as they were Sudan and oil red O positive. The smooth muscle cells were oval rather than spindle-shaped.

Some other work on experimental cholesterol atherosclerosis in rabbits (Buck, 1958) revealed that in the walls of the aorta and femoral artery there were endothelial lesions which contained cells, relatively large extracellular spaces, and fibrils of collagen. It was assumed that the lesions developed as a result of the incorporation of substances from the blood into endothelial cells. A high level of blood lipids was associated with the development of the lesions, and the lipid was shown to be combined with the protein.

Lee et al. (1966) demonstrated that monkeys fed an atherogenic diet showed the presence of large amounts of lipid in mature or nearly mature smooth muscle cells. Some cells had regions of cytoplasm almost devoid of normal components, and containing irregular shaped masses of variable electron density, possibly representing degenerative organelles. Intermingled among these cells were huge rounded cells thought to be mature mononuclear cells of the lymphoid series. These contained numerous lipid droplets and phagocytized materials.

Schaper et al. (1972) reported on the fine structural changes which resulted from inducing ischemia in the precapillary anastomotic network of

the heart for 10 days. Four dogs showed after 3 weeks of constriction of the left circumferential coronary artery that the arterial wall was thinned and had an extremely stretched endothelial lining. Though the internal elastic lamina was often fragmented or absent, an entirely intact elastic membrane generally could be seen in the presence of necrotic smooth muscle cells. The subendothelial space was frequently widened and filled with abundant ground substance, clusters of elastic material and collagen fibers. Increased amounts of endoplasmic reticulum, mitochondria, and Golgi in endothelial and medial cells seemed to indicate signs of metabolic hyperactivity. Some myofilaments were absent and swollen mitochondria were evidenced. Acute vascular lesions were observed in most dogs. Lipid droplets were seen with necrosis and vacuoles.

Four dogs demonstrated after 8 weeks of constriction changes mainly between the endothelium and the internal elastic lamina. Three different types of cells accumulated in the subendothelial space: smooth muscle cells, modified smooth muscle cells, and fibroblast-like cells. After 1 year of constriction, two remaining dogs showed only alterations in the subendothelial space. Muscle cells were arranged concentrically as in the normal animal. There was some subintimal thickening. In conclusion, it can be said that a preponderance of changes occurred at 3 weeks and then gradually there was a return to normal.

Buck (1961) showed in a similar way how arteries have the capacity to adapt to changes in blood flow and to injury. Ten to 14 days subsequent to tying a length of carotid artery, the internal elastic lamina

split. Smooth muscle cells infiltrated the breaks and extracellular material increased in the intima.

Rats have been used by various workers to demonstrate how hypertension may affect the intimal region of the artery. Still (1967) found that by ligating the aorta, the resultant increased intraluminal pressure above the ligature produced localized infiltrations of mononuclear cells, monocytes, and lymphocytes into the intima. The sites of infiltration probably existed where gaps were in the internal elastic lamina. Within hours after arterial constriction, the presence of lipid within such cells (monocytes) demonstrated their ability to become lipophages.

Olsen (1969) reported that the internal elastic lamina was more or less destroyed in walls of dilated arterioles with chronic hypertensive damage. The membrane was thought to be most likely destroyed by the high intraluminal pressure in the arterioles, and possibly somewhat affected by elastase, one of the penetrating components of the plasma.

Gardner and Matthews (1969) have shown that as early as 16 days after unilateral nephrectomy, feeding of 1.0% NaCl and administering 50 mg deoxycorticosterone each day, rats developed systolic blood pressures in excess of 150 mm Hg. Rats with such pressures were classified hypertensive. Vascular injury was typified by distorted medial cells surrounded by vacuolated spaces. Fibronoid necrosis usually followed. Fine structure showed a dispersion of mitochondria throughout the sarcoplasm rather than their normal concentration at the poles of the nuclei. Contractile protein myofibrils tended to lose their normal longitudinal alignment. Nuclear distortion was present. Between injured muscle cells were numerous

collagen fibrils and fluid-containing spaces. Adventitial collagen appeared more abundant than normally. Irregular thickenings in the intima contained amorphous material and particulate osmiophilic deposits. Fenestrae of the internal elastic lamina appeared larger.

Electron microscopy evidence supports the light microscopy findings that in all forms of accelerated rat hypertension a similar if not identical sequence of morphological changes occurs in small visceral arteries. Interestingly, after 7 days of this treatment, there were found significant increases in alkaline phosphatase and glucose-6-dehydrogenase activities in adventitial and medial-endothelial areas, respectively. These changes in enzyme activity are thought to be a direct result of the mechanism used to induce the rise in blood pressure. In deoxycorticosterone hypertension, these increased enzyme activities may be caused by the hormone upon plasma or electrolyte balance.

Effects of inadequate nutrition, starvation, and refeeding on the cardiovascular and related systems

Various dietary influences can act upon the cardiovascular system either in the developmental or adult stages of the organism to modify its intended function.

Coulson and Carnes (1963) reported on the high incidence of arterial lesions in swine that were fed copper-deficient diets since birth. Varying degrees in severity of the lesions ranged from frequent interruptions of the internal elastic lamina to radially oriented fissures penetrating the muscular media and sometimes adventitia. Muscular arteries of many

different parts of the body were observed; hemorrhagic areas were common to all. Coronary arteries had ruptured walls which caused myocardial infarctions. Collagen was often observed replacing muscle fibers, presumably as a reparative process. The association of lesions and copper deficiency was thought by the authors to lie in the defective elastin production for the internal elastic lamina.

Coulson et al. (1965) showed from normal and copper-deficient swine that aortic rings immersed in a buffer solution demonstrate two phases in the stress-strain curve relationship as described by Bergel (1961). Rings subjected to elastase show virtual elimination of the first phase of the curve. The breaking strength of elastase treated rings for both control and copper-deficient swine is less than similar rings in buffer only. The aortic rings (in buffer solution) from copper-deficient swine had breaking strengths almost one-half that of the controls. Elastase digestion of skin from both groups of animals did not show any significant differences in tensile strength.

Apparently, the aortic collagen and elastin are related in a more unique manner than "in parallel," as Burton (1954) suggested. Elastin contributes something to the mechanical properties of the collagenous component, possibly by binding the collagen fibrils together. It is the author's opinion that in copper deficiency the alteration in ultimate tensile strength and the stretch modulus of the second phase of the aortic stress-strain curve, theoretically a function of collagen, is the result of a defect in elastin.

In recent years, starvation has received considerable attention in view of its possible role in treating obesity. However, as early as World War II, starvation has been implicated as having some effect on the cardiovascular system. Brozek et al. (1948) investigated the effects that the war years had upon the health of the Russian population and found that of all the admissions to the Pavlov Fist Medical Institute prior to the war, 10% represented a hypertensive condition. During the period of late 1941 to early 1942, these same people experienced diets of inadequate nutrition. Coincident with this, a general decrease in the percentage of hypertensive patients occurred. However, hypertensive cases increased during 1943, and by the middle of 1943 better than 60% of the population admitted to the Institute were classified hypertensive. The incidence was independent of age and was attributed to the refeeding at the end of the war, after people had experienced hard work and long periods of inadequate nutrition or periods of starvation.

An extensive amount of research on the effects of human starvation was done by Keys et al. (1950). The results of an experiment involving 32 men, who underwent 24 weeks of semi-starvation and 12 weeks of refeeding, showed during the semi-starvation period bradycardia, decreased systolic and diastolic blood pressures, diminished venous pressure and pulse rate, and decreased cardiac output. There were, also, amplitude decreases in all electrocardiogram deflections, and right axis shifts in the QRS and T complexes. Though absolute plasma volume increased during semi-starvation, absolute blood volume was decreased. The variables returned slowly to normal during the refeeding period. Keys found that the heart was severely

deteriorated by starvation. Normal function was regained slowly and the heart was also in danger of failure when overeating occurred in the refeeding period.

Young and Scrimshaw (1971) reported that a 20-year old girl, who weighed 260 pounds, fasted for 30 weeks and lost 128 pounds. Upon resuming eating, her heartbeat became irregular after the seventh day and she died 2 days later from ventricular fibrillation. She had not only lost fatty tissue during the fast but also had consumed half of the lean tissue mass in her body, including part of the fibrous tissue of the heart muscle. These authors also reported that radiographs of chests from persons on starvation diets indicated a decrease in heart size.

Wilhelmj et al. (1951) reported lowered blood pressure and heart rate in dogs that were exposed to periods of 2 to 14 days of starvation. Blood pressure generally declined in the first few days of the fast and subsequently rose to a level above the pre-fasting pressure. Finally, the blood pressure showed an irregular decline to a stable fasting level.

Wilhelmj (1955) showed that type and quantity of diet precedent to and succeeding a fast influenced the stable fasting blood pressure. One experiment involved 6 episodes of fasting and realimentation of fat in a period of 2 years. Repeated fat episodes not only caused intensification of blood pressure elevating effects, but have been shown to cause several deleterious changes in the cardiovascular system. Luxus consumption diets high in carbohydrate caused little or no effect on blood pressure. The author's aforementioned article and this study cite investigators, such as Selye and Keys, to be advocates of the idea that fasting is a typical alarm

reaction. Starvation within the first 48-96 hours produces marked counter shock phenomena to alleviate the tendency of blood pressure to fall (shock phase of alarm reaction). As the fasting state continues the counter shock phase becomes weaker, the shock phase predominates and the pressure finally falls to a stable level. It may be the pituitary adrenal mechanism that was involved at first, and then as the fast continued, a gradual depletion of adrenocorticotropic hormone (ACTH) or cortical hormones caused blood pressure and heart rate to sink to the stable fasting level. Injections of ACTH were found to raise blood pressure and heart rate.

Wilhelmj et al. (1956) reported upon the effect that 6 "fat episodes" during the course of 14 months had on four trained dogs. Each episode consisted of a prolonged preliminary fast followed by refeeding a diet that contained 50% or more of the calories from butter and beef suet. A luxus consumption level of 120 $Cal/m^2/24$ hours was administered to the animals. Blood pressure was high and frequently rose during the fasting period. After the dogs were returned to a normal kennel diet, the blood pressure returned to normal. It was the authors' opinion that the homeostatic mechanisms that tend to prevent the fall in blood pressure during fasting became hypereffective. If such mechanisms could be maintained indefinitely, hypertension might be achieved. These same dogs were fed for 475 days on different dietary regimens without fasting. Three of the four dogs demonstrated a diastolic hypertension resembling that of benign essential hypertension in man.

Wilhelmj et al. (1957) investigated the effects that total sympathectomy would have on blood pressure in fasted and refed dogs. Bilateral

paravertebral ganglionectomy and adrenal denervation produced systolic pressures within normal range and diastolic pressures at the lower limits of the normal range. Blood pressure failed to decline and remained the same or rose above control levels when the dogs were fasted for periods of 3 to 6 weeks. Realimentation of a high carbohydrate diet demonstrated blood pressures at the fasting level. Normal fasted dogs when realimented on a high carbohydrate diet show significant rises in systolic blood pressure and normal or low diastolic pressures. Convulsions and/or hypoglycemia (50-60 mg glucose/100 ml blood) have been observed in these latter animals.

Wilhelmj and McCarthy (1963) indicated that the experiments already done on starvation and refeeding unintentionally resemble dietary habits of many individuals in countries where hypertension is frequent. Overindulgence in high fat-high caloric diets with resultant obesity and followed then by strenuous, erratic, and irregular attempts to reduce are common. The inability to continue the reducing regime and the consequent renewal of the cycle are repeated often by some individuals.

Smith et al. (1964) observed in swine a substantial rise in blood pressure when the animals were subjected to total starvation and refeeding. Control pressures for the swine were 133 $\pm$ 6 mm Hg for systole and 96 $\pm$ 7 mm Hg for diastole. Following a fifth period of starvation and refeeding of a diet high in glucose, the swine demonstrated chronic hypertension, with pressures of 183 $\pm$ 10 mm Hg for systole and 139 $\pm$ 10 mm Hg for diastole. Arrhythmia and T wave inversions were evidenced during periods of starvation. Mild-to-moderate left hypertrophy of the heart ventricles,

particularly the left, existed. There were degenerative changes in the myocardium, loss of striations and some fatty displacement of muscle tissue in hearts of starved-refed swine. Thickening was observed in pulmonary vessel intima and in the arteriole media of one animal's heart. Lumen size was diminished and perivascular edema and fibrin were seen in this heart. Though mild deposition of lipid was seen in aortic intima, it was relatively meager in comparison to changes in cases of human atherosclerosis.

Hembrough and Link (1968) reported that swine which were starved and refed with a normal diet demonstrated a decreased arterial capacitance and elevated blood pressure. Crouch (1968) found in dogs which were starved and refed a normal kennel diet that their thoracic aortas had significantly greater ($P<0.05$) distensibility than those of control animals. A highly significant ($P<0.01$) decrease in femoral artery distensibility existed, however, in the treatment animals. Hembrough and Riedesel (1970) showed that by starving rats for several days and then refeeding them with dextrose there were resultant distensibility changes but no significant elevations in blood pressure.

The actual means by which blood pressure is elevated after starvation is yet undetermined. Changes in vessel distensibility have been observed. It is conjecture as to whether or not the diet consumed during refeeding is the cause for this change in distensibility. Some of the aforementioned investigators, who used carbohydrate in the refeeding diet, seemed to find some enhancement of distensibility and/or blood pressure changes. Interestingly enough, sucrose presently is considered to be a contributory

factor in coronary artery heart disease in man.

Repeated starvation and refeeding may produce a change in the elastin and collagen content of the arterial wall. Isotope labeling studies indicate that after 5 weeks of age there is no detectable turnover of elastin in the rat aorta (Tao et al., 1962). In senescence, elastin fibers fray and smooth muscle cells degenerate. These damaged constituents are replaced by newly synthesized collagen (Fuller et al., 1972). A similar damaging and reparative process may be occurring during the repeated episodes of starvation and refeeding. Decreased distensibility and eventual hypertension possibly will then occur.

In individuals with essential hypertension, the arterioles are narrowed, but the reason for it is not known. Of the 20% in the adult population of the United States which are affected by hypertension and/or hypertensive heart disease, the majority has primary or essential hypertension. Some genetic predisposition can be attributed as the cause as some races suffer higher incidence of it than others. The disease can be characterized generally with diastolic pressures exceeding 90 mm Hg, increased systolic pressure, increased pulse pressure, normal cardiac output, increased cardiac work, and normal plasma volume.

It has been demonstrated both by Folkow (1957) and Conway (1960) that in essential hypertensive patients with abolished arteriolar muscle tone, the arteriolar lumen remains smaller than that of normotensive subjects. Something is decreasing the lumen which is unrelated to smooth muscle contraction. Thiazide drugs reverse the condition by enlarging the vessel lumen and producing an associated drop in blood pressure.

An increased wall thickness/radius ratio has been observed in people with essential hypertension (Sivertsson, 1970). This change in wall thickness to radius potentiates the amount of lumen reduction. This increased ratio could increase the peripheral resistance above normal. Ferguson and Varco (1955) showed that increased pressure in the pulmonary circulation produced thickening of the walls of the pulmonary vessels. Feigl et al. (1963), however, did not find a significant change of the wall thickness/radius ratio in the femoral artery after 4 weeks of experimental renal hypertension in a group of dogs. Possibly, it is first a change in vessel distensibility rather than an effect of pressure that causes an increased and sustained peripheral resistance.

It has been suggested by Pickering (1965) that essential hypertension may be dependent upon a "multifactorial" inheritance. Environment may play a greater role than inheritance as the cause of a rise in blood pressure with increasing age. Family size, physical work, and obesity are environmental factors which may indicate whether a man's blood pressure will be lower or higher than normal.

Though many etiological theories and therapeutical practices could be presented on essential hypertension, only those that relate to the nutritional aspects will be considered here. One such study was administered by Hartroft (1966), wherein rats were fed diets deficient in choline. Refeeding normal concentrations of choline later produced permanent elevations in blood pressure later in life. If a deficiency of choline were maintained, hypertension did not develop. Also, diets high in protein and sodium enhanced the rise in blood pressure in hypertensive rats. Diets with lower

amounts of protein and sodium decreased blood pressure.

Starke (1950) reported that a 2000 Cal rice diet reduced the total cholesterol in patients with hypertensive vascular disease. Watkin et al. (1950) found that of 500 hypertensive patients treated on an unmodified Kempner rice diet (250-300 g rice/day) for 10 1/2 weeks, 322 were benefitted. Blood pressures decreased from a group mean of 199/117 to 145/95. Both metabolic rate and cholesterol decreased. However, patients who were fed the rice diet with 3 g salt/day failed to undergo a reduction in blood pressure.

Schroeder (1965) found in patients dying of "hypertensive complications" larger concentrations of renal cadmium and higher renal ratios of cadmium to zinc. Human hypertensive patients were found to excrete 40 times more cadmium in the urine than did normotensives. If these hypertensive patients do not manifest any other organic renal disturbances, cadmium may be acting as a "biochemical renal lesion."

Certain other systems as well as blood chemistry may be affected by starvation and refeeding. They, in turn, may affect the cardiovascular system and, also, aid in the overall interpretation of the cause of hypertension.

Nitrogen metabolism plays an important role in all fasting and refeeding studies. A change of nitrogen in the body is associated with a change in living tissue. The magnitude and direction of change during partial and complete fasting have been found to be variable in serum and plasma proteins (Keys et al., 1950), blood urea nitrogen (Sunderman, 1947), and non-

protein nitrogen concentrations (Hazelwood and Lorenz, 1959). Blood urea nitrogen levels have been reported to be increased in man when fasted for 45 days (Sunderman, 1947). By comparison, levels were significantly lower in pigs fasted 6, 15, 27, 95, and 167 hours (Kornegay et al., 1964).

Lavéty and Love (1972) reported that cod, during spawning (which is an actual period of starvation for them), demonstrate a depletion of protein from their musculature and an associated increase in water content. This gives rise to a much softer and weaker tissue. The myocommata, however, which binds the musculature together becomes much stronger. Collagen content increases during spawning. Probably thickening occurs in this connective tissue. The authors reported that a striking correlation exists between breaking strain of the myocommata and degree of starvation. As the starvation period increased, so did the breaking strain.

During prolonged starvation glucagon remains at higher levels than normal. Aguilar-Parada et al. (1969) indicated from their study that in healthy, young human males there was a modest degree of hyperglucagonemia which could be detected between the hours of 8 a.m. and 9 a.m. during the 4 days of starvation. A rise of 50% was observed. If the amino acid arginine, was given after 3 days of fasting, an exaggerated rise in glucagon was observed. Insulin did not rise in this situation. Unger et al. (1963) showed that dogs, like humans, show a marked increase in glucagon with insulin-induced hypoglycemia. Besides glucagon's glycogenolytic action, there is gluconeogenesis in starvation, which provides a constant flow of glucose to vital-glucose-dependent tissues such as the brain.

During prolonged starvation insulin levels are lower than normal.

Felig et al. (1969) reported that in experimental animals and man there is repletion of liver glycogen as fasting extends beyond 3 days. In Figure 1, a variety of sources are indicated for the glucose molecule that is needed by mammalian organisms. Though adipose tissue constitutes a relatively important source of glucose precursors, the extent of fat breakdown to glucose homeostasis is not extensive.

With regard to the synthesis of glucose from amino acids, with the exception of leucine, all amino acids are potentially glycogenic. The principal amino acid for glucose synthesis in the liver is alanine. It is discharged from cells during fasting, not as a protein breakdown product, but must be synthesized from its immediate precursor, pyruvate (Young and Scrimshaw, 1971).

The requirement of brain tissue for glucose (125-140 g/day in man) is not insulin-dependent and persists even in the presence of increased levels of other body fuels such as ketone bodies and fatty acids (Felig et al., 1969).

Prolonged fasting is characterized by an early period or the first 7 days in which there is rapid gluconeogenesis, and a second or delayed phase occurring after 5 or 6 weeks of starvation. Felig et al. (1969) claim this latter period is characterized by reduced glucose utilization and minimal protein catabolism.

Though corticosteroids are known to activate gluconeogenesis, Sabeh et al. (1969) reported that adrenocortical function of five obese patients during 1 or 2 weeks of starvation was not changed. Prior to the fast,

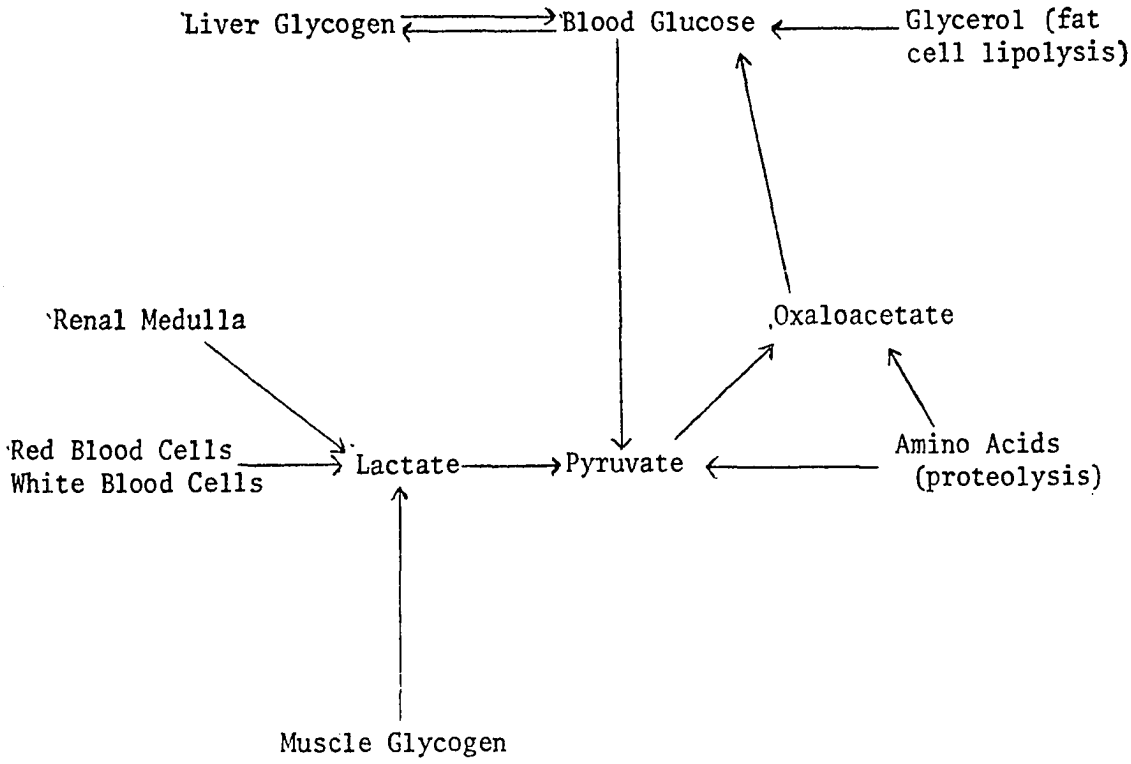

Figure 1. Glucose homeostasis (Felig et al., 1969, p. 294)

cortisol production was 20-39 mg/day. At the end of 7 or 8 days starvation, production was 20-36 mg/day. Glucocorticoid administration during starvation diminishes renal clearance of keto-acids (Sapir et al. 1972). Refeeding only raises blood keto-acid levels.

The cortex of the kidney during prolonged starvation makes a change from the normal, where it served as a minor partner of the liver in the synthesis of glucose, to the main producer (Young and Scrimshaw, 1971; Felig et al., 1969).

Metabolic effects of fasting in man include, also, ketosis, slight metabolic acidosis and accelerated urinary excretion of ammonia. As a consequence to the fall in blood glucose and serum insulin during starvation plasma free fatty acids, blood keto-acids, acetoacetic acid, and its two derivatives, beta-hydroxybutyrate and acetone accumulate. These substances yield energy on oxidation, and the brain will eventually utilize them in place of glucose. Owen et al. (1969) reported that concentrations of beta-hydroxybutyrate and acetoacetate rose slowly in obese humans during the first 3 days of fasting and rose greatly from days 3 to 38. Control values of .07 $\pm$ .01 and .03 $\pm$ .01 millimoles/liter for beta-hydroxybutyrate and acetoacetate, respectively, increased to 5.85 $\pm$ .38 and 1.34 $\pm$ .14 millimoles/liter. Whether ketosis exists in starvation in the dog is argumentative. Crandall et al. (1940) indicated it begins gradually after 2 to 4 days fasting. After 5-11 days, the liver is adding 1.5 to 8.1 millimoles/liter of ketone bodies into the blood. Oxidation of acetone bodies may supply up to 50% of the total caloric requirement of the dog after the fifth day of fasting. It is of special interest that the hepatic energy

output of the liver in the fasting dog (glucose plus ketone bodies) is equivalent to its energy output in the non-fasting state (glucose only).

Lemieux and Plante (1968), however, reported that of six mongrel and four dalmation dogs starved for 12 days, there were none that showed ketone bodies in the plasma or urine. There was no decrease in urinary ammonia excretion. Non-esterified fatty acids rose from an average of 740 to 1000 μeq/liter of blood during fasting and then returned to normal values in the recovery period. This ketone response to fasting differs from that of man.

Canine resistance to development of ketosis during starvation has been previously reported (Morgulis and Edwards, 1924). Possibly the dog is able to reduce its energy requirements during fasting. The marked decrease in urinary nitrogen excretion suggests that metabolic activity is decreased.

Blood sugar and cholesterol levels during complete and partial starvation have been reported as decreased, unchanged, and increased (Keys et al., 1950; Hazelwood and Lorenz, 1959). Morgulis and Edwards (1924) reported unchanged-to-slight decreases in blood glucose levels in fasted dogs. Kornegay et al. (1964) found that in pigs serum cholesterol did not increase immediately upon fasting. However, at 27, 51, 95, and 167 hours, the levels were significantly higher in fasted pigs. Sex was evenly distributed in fasted and nonfasted groups of pigs. Level of cholesterol was not significantly different between groups after 24 and 216 hours refeeding. Keys et al. (1950) concluded that overwhelming evidence indicates that cholesterol concentration in the blood of man decreases during partial starvation and increases during complete starvation.

Reports on the effect of fasting on blood sodium and potassium are conflicting as well (Sunderman, 1947). Kornegay et al. (1964) found serum sodium levels lower in fasted pigs than in controls. Potassium levels were not considerably different in these fasted pigs. Morgulis (1928) has reported no significant change in serum sodium levels in fasted dogs. However, serum potassium levels are reported to be significantly lower in these fasted dogs.

A decrease in thyroid activity has been observed, also, during fasting (Alexander et al., 1964). Uptake and clearance of ^{132}I and serum protein bound iodine were found to be decreased. Fasting, however, has been shown to result in increased secretion of growth hormone by the pituitary (Roth et al., 1963). If refeeding follows a prolonged fast there is an increased capability for protein synthesis and retention of minerals and water (Drenick et al., 1968).

Lipoproteins

There is a great amount of interest at the present time from both a fundamental and medical viewpoint to comprehend more precisely the biosynthesis of lipoproteins. It is the accumulating body of evidence associating hyperlipemia with atherosclerosis in both human and animal that stimulates medical investigators to study the regulatory mechanisms of plasma lipoprotein concentrations.

Physical and chemical properties of lipoproteins

It is now well accepted that the water-insoluble plasma lipids, cholesterol, cholesterol esters, phospholipids, and triglycerides combine

with specific globulins to form lipoproteins. One other plasma lipid, free (non-esterified) fatty acids, is bound to serum albumin. This physiologically active lipid is unstable because of too rapid a turnover and, therefore, does not act as a reliable index for assessing hyperlipidemia. The freely circulating lipoproteins represent the third most abundant group of extracellular entities in the circulation of blood in man and animals. Only albumin and gamma-globulin are present in greater quantities.

Certain functions are attributed to blood lipids. Triglycerides carried by lipoproteins are an available source of fatty acids which can be either oxidized by tissues for energy or can easily be stored in adipose tissue. Phospholipids serve as units of structure in the cell membrane and may promote stability at the water-oil interfaces. Unesterified cholesterol is, also, a cell membrane component and is a precursor of steroid hormones and bile acids as well. The actual role(s) of cholesterol esters is relatively undefined.

A number of plasma lipoproteins, each with characteristic molecular size, density, lipid and protein content, and electrophoretic mobility, have been identified; however, for most purposes, it is adequate to consider only four major somewhat overlapping classes of lipoproteins. All four classes have individual functions and metabolic activities. Type of classification is based upon whether they are fractionated by means of ultracentrifugation or by electrophoresis. In ultracentrifugation, the lipoprotein classes are separated due to differences in their density

(flotation) characteristics which are related to their glyceride concentration. As the relative percentage of triglyceride component increases, the lipoprotein entity demonstrates less of the high density water-soluble characteristics of a protein (average plasma protein moiety, 1.33 g/ml) and assumes more of a water-insoluble, low density property exhibited by fat. Gofman et al. (1949) introduced a nomenclature defining lipoproteins by their flotation characteristics in ultracentrifugal fields. A method involving the addition of concentrated salt solutions to the plasma is followed so as to achieve a desired density of the suspending medium. The solution is then centrifuged at speeds generating more than 100,000 x g. In this manner, the lipoproteins lesser in density than the medium are concentrated at the top of the centrifuge tube and may be selectively removed for analyses. Analytical ultracentrifuges can also be used. They are equipped with an optical system through which concentration gradients created by floating lipoproteins are photographed. Concentrations of density classes are obtained by measuring the areas beneath the migrating peaks seen on the photographs. Flotation rates of lipoproteins of density less than 1.063 g/ml are assigned values expressed in S_f units (Svedberg units of flotation, in 10^{-13} cm/sec/dyne/g).

Table 1 presents the major systems of classification and their relationships. Classification by means of density (d) utilizes the acceptable abbreviations for very low density lipoproteins (VLDL, d = 0.95 to 1.006), low density lipoproteins (LDL, d = 1.006 to 1.063), and high density lipoproteins (HDL, d = 1.063 to 1.21). The VLDL are often combined with the chylomicra which have a density of less than 0.95. Occasionally,

Table 1. Comparison of lipoprotein classification systems[a]

Density		Electrophoretic Behavior		Flotation Characteristics[b]		N-Terminal Amino Acids[c]		Approximate Molecular Weights
Class	Density range (g/ml)	Paper	Starch Block	S_f	$F_{1.21}$	Major	Minor	(in millions)
Chylomicra	<0.95	Origin	α_2,β	>400	——	Ser Thr	Glu Asp	10^3-10^4
VLDL	0.95-1.006	pre-β	α_2	20-400	——	Ser Thr	Glu Asp	5 -100
LDL	1.006-1.063	β	β_1	0-20	——	Glu	Ser Thr	2 -3
HDL	1.063-1.21	α	α_1	——	0-9	Asp		0.25

[a]Schumaker and Adams (1969, p. 116).

[b]S_f designates the negative sedimentation coefficient in Svedbergs in density 1.063 g/ml NaCl solution at 26°C. $F_{1.21}$ is also a negative sedimentation coefficient at density 1.21 g/ml and at 26°C.

[c]Major 30% of detectable end groups and minor 10 to 30% of detectable end groups.

very high density lipoproteins (VHDL, d>1.21) are mentioned in the literature. With a decrease in lipid content and a corresponding increase in protein content, lipoprotein densities increase from 0.95 to 1.21 g/ml. Concomitant with an increase in density are alterations in physiochemical properties, such as decreases in molecular weight in the order of magnitude, 10^{10} to 10^{4}, diminished size of complexes and lowered ultracentifugal flotation rates. Electrophoretic mobility patterns depend upon the supporting medium and have given rise to the terms alpha (corresponding to HDL), beta (corresponding to LDL), pre-beta (corresponding to VLDL), and chylomicra. Though the protein moiety is the predominant factor in determining mobility of lipoproteins, the lipid quantity associated with the protein can alter the migration. Last of all, the major and minor N-terminal amino acids representative of each class are presented. The specific proteins of each class may be referred to as apolipoproteins. Division of proteins into the three classes is made according to their N-terminal amino acids (Schumaker and Adams, 1969).

Structurally, lipoproteins consist of lipid, protein, and some carbohydrate. The latter is less than 5% of the total composition of the lipoprotein entity. The lipid and protein of lipoproteins are loosely associated. Nearly all lipid in the lipoprotein entity can be extracted with appropriate apolar organic solvents. However, there are probable, but small, amounts of lipid that are covalently bound to protein. This bound lipid is most likely intended for structural organization of the lipoprotein.

Two fundamental types of lipoproteins are postulated to occur in the blood: micellar and pseudomolecular lipoproteins (Schumaker and Adams, 1969). The micellar type consists of less than 30% protein and includes the chylomicra, VLDL, and LDL. A hydrophobic core of triglycerides and cholesterol esters surrounded by a hydrophilic covering of protein, phospholipid, and free cholesterol characterizes the micellar type. Representative of HDL and VHDL, the pseudomolecular type contains 30% or more protein. Probably, protein-lipid subunits in some definite quaternary structure comprise the pseudomolecular type. Lipid of this type consists for the most part of phospholipid, lysolecithin in particular. Electron microscopy has shown the quaternary structure to be 2 to 6 subunits.

From a functional viewpoint, circulating lipoproteins have quite an involved biochemistry. They originate from two major sites, the liver and the intestine. In response to fat ingestion, the short-chain fatty acids that are liberated by hydrolysis of triglycerides in the intestine are subsequently absorbed and taken directly to the liver via the portal venous system. Long-chain fatty acids (more than 10 carbons in length), however, are re-esterified into triglycerides in the intestinal mucosal cells. Some specificity of enzymes may discriminate chain length. These re-esterified triglycerides are neatly packaged by proteins, phospholipids (mainly lecithin and sphingomyelin), and small amounts of free and esterified cholesterol. The core, triglycerides and cholesterol esters, is enveloped by these mentioned polar components to form what are known as chylomicra. The adsorbed protein on the chylomicron surface keeps them from sticking to

each other or to the walls of the lacteals, lymphatics, and blood vessels. It is in this form that exogenous fats are transported from the intestine into the lymphatic system and finally into the blood stream by way of the thoracic duct. In the plasma, this fraction of lipoproteins changes in chemical composition and physical character. One such change is an increase in protein content and loss in triglycerides to tissues. Gustafson (1966) has found chylomicra to have a half-life of 17 minutes in man. There is possibly a stepwise reduction in size and increase in density because of the loss in lipids, particularly triglycerides.

Adipose tissue, heart, liver, and other organs are sites to which chylomicra are carried for storage and oxidation. Prior to actual storage, the glyceride moieties are hydrolyzed from their carrier by the action of lipoprotein lipase. Released fatty acids are re-esterified into intracellular esters. If energy levels become low, free fatty acids are released from adipose tissue into the blood. The fatty acids are bound to albumin and are not in the chylomicron form. Such a mobilization of free fatty acids leads to a synthesis of VLDL by the liver. The chylomicra are the largest lipoprotein moiety in the blood and are 0.5 to 1.0 μm in diameter. Electrophoretically, they lack mobility and, therefore, remain at the origin. Also, there is a problem in segregating chylomicra from VLDL. Flotation at d = 1.006 in the ultracentrifugal field, a common characteristic to both fractions, makes it difficult to achieve a good separation. Skipski (1972) claims that isolation of a contamination-free and total chylomicron (S_f>400) lipoprotein fraction requires a more precise special density-gradient procedure.

The VLDL moiety is synthesized in the liver in response to excess dietary carbohydrate or in response to the fatty acids mobilized from adipose tissue. This class of lipoproteins is metabolically involved in the transport of endogenous glycerides. These particles are approximately 320-800 Å in diameter (Searcy and Berquist, 1962). This class, as well as chylomicra, is an important source for energy, by virtue of its triglyceride content.

Low density lipoproteins (LDL) are quantitatively the largest lipoprotein class in man and also the major cholesterol-bearing (both esterified and free) lipoprotein. Metabolically, they may be possibly related to VLDL. The S_f 0-12 lipoproteins, which float between densities 1.019 and 1.063, migrate electrophoretically with beta-globulins. They are spherical, with a diameter of 185 Å. It has been reported by Schumaker and Adams (1969) that hepatectomized and abdominally eviscerated dogs demonstrated that the major site of VLDL and LDL synthesis is the liver. Margolis and Capuzzi (1972) indicated that LDL may appear in the serum solely as an intermediate product from the breakdown of VLDL, and that it is not known whether LDL is secreted from the liver. The liver may only secrete VLDL and HDL under normal dietary conditions.

The protein and phospholipid-rich HDL (alpha-lipoprotein) can be subfractionated into HDL_2 and HDL_3. S_f values are not assigned to HDL, but rather a designated unit for flotation rate, $F_{1.21}$, is given. For HDL_2 and HDL_3, it is $F_{1.21}$ 3.5-9 and $F_{1.21}$ 0-3.5, respectively. In the plasma residue at d>1.21, the infranatant, a phospholipid-protein complex called VHDL, has been identified (Havel et al., 1955). It contains a protein

moiety identical to that of the alpha-lipoprotein, which migrates in electrophoresis with the alpha-globulins. HDL entities are ellipsoidal, 300 by 50 Å. One of the HDL functions is to remove cholesterol from cells. Tangier disease demonstrates an absence of this fraction and an accumulation of cholesterol in the reticulo-endothelial system (Castelli and Moran, 1971).

Human lipoprotein patterns

Composition and distribution of human lipoproteins Great variation in concentrations of lipids and protein in each of the lipoprotein fractions is shown in human plasma. Animals, however, maintain a rather consistent composition (Hillyard et al., 1955). Under normal conditions each class of lipoproteins has a specific pattern for its chemical composition. Lipid content of various classes varies from 98 to 99% in particulate fat, as exists in chylomicra, to 1% or less in albumin-free fatty acid complexes. The size and chemical composition of each of the major human lipoprotein fractions are shown in Table 2. Apart from the lipid component in the lipoprotein entity, there are specific proteins, or apoproteins each which has immunologic differences, i.e., A-reactions and B-reactions. Kuo (1972) reported that the amount of protein in chylomicra varies depending upon the source of chylomicra: chyle or blood plasma. The types of protein in this fraction have been identified by Fredrickson et al. (1967a) to be both A and B apolipoproteins. It was their conclusion that the major portion of the A apolipoprotein was not derived from the serum; it was possibly intestinal derived. Marsh (1969)

Table 2. Average size and composition of major lipoprotein species[a] in human plasma

Lipoproteins	Lipids (% composition)			Proteins (% composition)	Particle diameter (A)
	Cholesterol	Phospholipids	Triglycerides		
High-density (alpha) lipoproteins	20	25	5	50	50X300
Low-density (beta) lipoproteins	50	25	5	20	150X350
Very low-density (prebeta) lipoproteins	13-45	13-25	15-70	4-15	300-500
Chylomicra	3-13	3-13	70-93	1-4	800-5,000

[a]Kuo (1972, p. 9).

indicated that when chylomicra containing labeled A and B protein were injected into dogs, the label persisted long after the chylomicra cleared. HDL proteins showed the label shortly after the injection. The A protein was identified as the HDL apolipoprotein. With regards to the protein moiety, Gustafson (1966) reported that chylomicra metabolize into HDL. An increase in the HDL class during chylomicra clearing in man, also, has been established. Fredrickson et al. (1967a) reported that not only do A and B exist in VLDL, but possibly another may be present, a C apolipoprotein; B is common to LDL.

Human lipoprotein classes are affected both in composition and distribution by sex and age. Genetic determinants for serum lipid, blood pressure, blood sugar, beta-lipoprotein-cholesterol relationship, and enzymes controlling carbohydrate and lipid metabolism play a great role in predisposing a patient to altered lipid patterns. Evidence is clear that some families are predisposed to alterations in lipid metabolism. Patterns are influenced as well by enriched diets and whether the individual lives a sedentary or active lifestyle. Havel et al. (1955) reported that approximately 70% of the total cholesterol in the plasma and 50% of the total plasma phospholipids are in the fraction having a density less than 1.063. Total plasma cholesterol and phospholipid concentration in this fraction was found similar in both young women and men. Men have more lipid, however, in the $d<1.063$ fraction than do women. Women, on the other hand, have more in the $d>1.063$ fraction. Wood et al. (1972) have reported similar findings.

Age also is an influencing factor upon lipoprotein distribution. Barclay (1972) reported that women between 18-35 years of age have more HDL than LDL. In subsequent years, especially at age 45, LDL predominates. Men, however, possess more LDL until age 45, when it peaks and then shows a general decline. The VLDL fraction increases in males during the third and fourth decades of life, peaking in the fifth decade. This fraction correlates well with increases in body weight and triglyceride concentration in the body. Until menopause, females show a lower concentration for VLDL. Searcy and Berquist (1962) indicate that teenagers have lower LDL levels than children less than 10 years old. This reduction in lipoproteins may reflect a surge of endocrine activity, which occurs in the second decade of life.

The likelihood of developing heart disease is greatly increased as cholesterol rises above 220 mg/100 ml blood. In the American male population, average cholesterol in the blood rises to 220 mg/100 ml by age 27 and to 250 mg/100 ml by age 43. One-third of the male population has a blood cholesterol level of 260 mg/100 ml or higher at 40-59 years of age (Consumer Bulletin, 1972). There is a direct relationship between plasma lipid concentration due to dietary fat intake and male sex hormone concentrations (Gass, 1972). The stimulatory effect of dietary fats on the gonads (mediated by the pituitary) increases androgen production which increases the LDL fraction and decreases the HDL fraction (Searcy and Berquist, 1962). Male hypophysectomized animals do not show increases in sex hormone concentrations when fed high dietary fat (Gass, 1972).

Estrogens elevate protein bound iodine (PBI) and androgens depress it. Possibly the effects of sex hormones are mediated through the thyroid gland. Estrogens have been shown to lower both serum total and beta-lipoprotein cholesterol.

Nutritional alterations of composition and distribution of the human lipoproteins When there is no evidence that malnutrition exists, the dietary components which may affect blood lipids and lipoproteins most profoundly are fats and carbohydrates. Barclay (1972) reported the total calories needed to provide energy for adequate nutrition should be derived from a diet that provides 15% from protein, 40% from fat, and 45% from carbohydrate (as little sucrose as possible). These proportions will, of course, vary throughout the world. It is the proportion of calories from fat and carbohydrate, the proportion of saturated to unsaturated fat, type and source of fats, and the manner in which overall energy balance is utilized by the individual that should be carefully observed.

Gas-liquid chromatography has been used to determine the degree of saturation (or unsaturation) in fatty acids, their carbon-chain length, and the branching in the chain. Fatty acids from certain vegetable sources, such as coconut and palm oils, increase serum lipoproteins, whereas those from sunflower seed and corn oils lower them. Animal fats seem to cause an increase in lipoprotein fractions, and those from fish have a hypolipoproteinemic effect (Barclay, 1972).

Lipoproteins of intermediate density are rich in cholesterol and are more responsive to dietary fat. Fats containing large amounts of saturated fatty acids will cause LDL to increase; however, LDL will decrease if fats

containing polyunsaturated fatty acids are substituted isocalorically (Barclay, 1972). Although VLDL are not so sensitive to type of fatty acids, an increase of it and LDL will occur if total fat calories, especially from saturated fats, are increased above 40%. The S_f 0-12 LDL are lower if the fat is unsaturated (Barclay, 1972).

Serum cholesterol has been known for some time to increase in response to saturated dietary fat and to fall when polyunsaturated fat is substituted. Lewis (1971) ascribed a reduction in circulatory cholesterol to the increased catabolism of cholesterol into bile acid and shift to extravascular pools. Possibly, the beta-lipoprotein entity has a decreased capacity for cholesterol. Dietary polyunsaturated fat, also, was indicated to decrease the effect of dietary sucrose on raising plasma triglyceride levels.

Nichols et al. (1957) investigated the effects that diets high in animal fat (42% of total calories), in vegetable fat, or low in fat but high in carbohydrate (73% of total calories) had on serum lipoproteins in five men between 20 and 49 years of age. The LDL fraction was raised only in the men that were fed the diet high in animal fat. Individuals subjected to the low fat, high carbohydrate diet demonstrated an elevation in VLDL. Unfavorable effects upon lipoprotein patterns were attributed to diets high in animal fat, whereas, vegetable oils were considered to have a more favorable influence on lipoprotein composition and distribution.

Elevations in triglyceride concentrations can be readily produced in the normal human by the feeding of diets high in carbohydrate. Alterations of VLDL triglyceride concentration following a carbohydrate diet

(80% carbohydrate and 20% protein) have been reported by Schonfeld (1970) and, also, by Levy et al. (1966). Schonfeld attributes the alterations primarily to overproduction in the liver rather than underutilization by peripheral tissues. There should be no chylomicra with this type of diet. He also found variable total plasma cholesterol but 1.2- to 5.7-fold increases in VLDL cholesterol. Whereas, both authors' investigations showed at least two- to three-fold increases in total plasma triglyceride concentrations, Levy's group found 20-60% decreases in alpha-lipoprotein (HDL) triglycerides. Magnitude and duration of the increase in triglycerides are related to quantity (Lees and Fredrickson, 1965) and type of carbohydrate, age, sex (MacDonald, 1966), presence of obesity, and genetic predisposition. The amount and action of insulin to that specific diet is another factor to consider (Farquhar et al., 1966).

In Great Britain, it has been estimated that of the normal 3000 calories daily intake, 50% is carbohydrate and all but 10% of that is sucrose (Yudkin, 1967). Reaven et al. (1967) gave a formula control diet (15% protein, 42% fat, and 43% carbohydrate), which simulates an average American diet, to 33 individuals for 1 week. Another diet consisting of 15% protein and 85% carbohydrate was administered to individuals for 3 weeks following their equilibration to a control diet. All patients on the special diet demonstrated higher triglycerides than when on the control diet. Fifty percent of these patients had triglyceride concentrations between 300 and 600 mg/100 ml. Two even had values greater than 2400 mg/100 ml. Countries with very high sugar consumption, such as Cuba, Colum-

bia, and Venezuela, however, have a very low incidence of coronary heart disease.

Lees (1965) indicated that reports from at least four different laboratories show that the plasma lipid response to starch diets is much less in triglyceride and cholesterol concentrations than to a sucrose diet. In his own investigation, six females and one male, 19-22 years of age, were maintained on a normal diet for 1 week and then subjected to a diet of 10% protein and 90% cooked starch (wheat or rice) for 4-14 days. A period of 3-7 days of normal diet was allowed before a second diet consisting of 90% sucrose was administered to the subjects for another 4-14 days. Plasma triglycerides and cholesterol for the subjects after starch feeding showed average increases of 109 mg/100 ml and 120 mg/100 ml, respectively. Average increases for the same lipids after high-sugar feeding were 88 mg/100 ml and 119 mg/100 ml, respectively. Lipid concentrations in these patients on the two diets were higher than those of the control patients. Similar responses in lipid concentrations were shown from both diets.

Anderson (1967) has suggested that fructose or sucrose may be more potent than other carbohydrates in raising triglycerides in males and postmenopausal women. Whereas, men on high carbohydrate diets show highly significant increases in triglycerides, young women demonstrate far more resistance to such diets. Nestel et al. (1970) have detected no differentiation between fructose and glucose on the basis of labeled palmitate incorporation into serum triglycerides. Although the hyperglyceridemic

response to sucrose has been attributed to the fructose moiety, there have been reports of stimulation of triglyceride turnover with diets in which glucose was the only sugar.

Pleshkov (1964) reported that the easily soluble sugar is more rapidly digested and absorbed than other types of carbohydrates, thus, raising the blood sugar level and increasing insulin production. Hodges et al. (1967) have demonstrated that carbohydrates can influence fat metabolism. Starch, in contrast to sucrose, increases fecal excretion of bile acids and lowers plasma cholesterol.

High carbohydrate diets not only regulate endogenous hepatic triglyceride formation but stimulate the production of the LDL protein moiety (Eaton and Kipnis, 1969). Fasting was related to a pronounced decrease in both triglycerides and the low density lipoprotein-protein moiety.

Human cardiovascular disease has been suggested to be more closely associated with the dietary carbohydrates than with fats. Keys et al. (1950) have reported that starvation-refeeding accelerates the onset of this disease in man. Smith et al. (1964) found the same to exist with swine which were fed diets of 70% glucose.

Prolonged nutritional deprivation results in mobilization of unesterified fatty acids (UFA) and increases in beta-lipoproteins. After 72 hours of starvation, serum UFA may triple in concentration, whereas, triglyceride and cholesterol concentrations remain relatively constant. Havel (1957) reported that UFA may account for the increased fatty acid transport during fasting and during the chylomicron removal from plasma. Levels of

VLDL increased between 24 to 72 hours of fasting so as to transport the released free fatty acids or triglycerides. After 72 hours, VLDL had not increased. Searcy and Berquist (1962) reported that plasma LDL rises appreciably after several days of fasting. The HDL fraction is not significantly altered. The LDL fraction reverts to pre-fasting levels within 24 hours after resumption of a normal diet. Plasma UFA levels promptly dropped with carbohydrate ingestion. Rubin and Aladjem (1954) have reported the same results on lipoproteins in man fasted for 4 or 5 days. Also, if sucrose ingestion followed the fast, no significant change occurred in serum lipoprotein patterns in 3 hours.

Genetic and disease alterations of human lipoproteins Fredrickson et al. (1967b, c, d, e) have suggested a classification by which serum lipoproteins can be categorized on the basis of phenotypes that were devised for genetically determined abnormalities. With a certain amount of reservation, other lipoprotein patterns that are abnormal due to acquired and nonfamilial disorders of lipid metabolism can be classified on this system. Two main classes of phenotypes exist: hyperlipoproteinemia and hypolipoproteinemia.

Hyperlipoproteinemias may be either primary or secondary. A primary hyperlipoproteinemia is inherited as a simple recessive and is rare. The secondary hyperlipoproteinemia is due to disease. As a class, the hyperlipoproteinemias are divided into five types. Type I is characterized by the development of marked hyperlipemia, hypertriglyceridemia, even on a normal diet. A low level of plasma post-heparin lipolytic activity (PHLA)

indicates the enzyme, lipoprotein lipase, may be absent or defective (Levy and Fredrickson), 1968). An exogenous hyperlipemia is one in which the chylomicra remain elevated 14-16 hours after the last meal. This disorder is generally symptomatic before 10 years of age, and a low fat diet is desired for therapy. Type II (hyper-beta-lipoproteinemia) is familial and perhaps the most common of all types. Biochemically, it is characterized by an increase in the plasma beta or LDL fraction. The condition is characterized by high cholesterol levels and atherosclerotic development in childhood. In type III, or broad beta disease, the LDL lipoproteins are abnormally laden with triglyceride and can be found in the VLDL (pre-beta) fraction. The VLDL demonstrate abnormal beta migrating particles in electrophoresis. Sensitivity to high fat or high carbohydrate diets and premature vascular disease are associated with this hyperlipoproteinemia. Increased concentrations of VLDL and triglycerides and normal to elevated concentrations of cholesterol exist in type IV hyperlipoproteinemia. High intakes of dietary fat seem not to increase plasma glycerides. Also, coronary artery heart disease proneness in middle life and defective glucose metabolism are demonstrated in this type (Kuo, 1972). Type V is represented by increased levels of chylomicra and VLDL (Fredrickson et al., 1967e). It is an endogenous hyperlipemia and quite intolerant to normal amounts of dietary fat. Triglyceride levels are increased by high carbohydrate diets (Levy and Fredrickson, 1968).

The hypolipoproteinemias include three rare hereditary diseases. The chemical composition of affected lipoproteins have been thoroughly

investigated. Tangier disease, also called familial hypo-alpha-lipoproteinemia, is characterized by the complete absence of HDL. Also absent is the VLDL fraction. The LDL (beta-lipoproteins) in this disease accumulates a large amount of triglyceride and hence enriches the total serum to concentrations of 600 mg/100 ml. Cholesterol and phospholipids are subnormal (Barclay, 1972). A second hypolipoproteinemia, abeta-lipoproteinemia, is represented by the complete absence of LDL and inability to synthesize VLDL (Fredrickson et al., 1967b). The plasma cholesterol levels are low because the LDL carries most of the cholesterol in the human. Hypo-beta-lipoproteinemia is characterized by low levels of plasma LDL, cholesterol, phospholipids, glycerides, and essential fatty acids. Fat absorption and chylomicron formation are generally normal. The VLDL are, also, usually synthesized (Fredrickson et al., 1967b).

Relationship of human lipoprotein alterations and etiology of heart and blood vessel diseases An elevation in plasma lipids, high blood pressure, heavy cigarette smoking, obesity, and physical inactivity have been associated as risk factors for heart disease. A report from the Inter-Society Commission for Heart Disease Resources (1970) states heart disease to be the number one cause of deaths (54% of all deaths) in the United States. Coronary artery heart disease (CAHD) shares the largest proportion of these deaths and possesses an incidence rate of three per 1000 persons. Many studies that have been done to ascertain these risk factors indicate cholesterol concentration as the single most important factor in development of CAHD (Kannel et al., 1964). Kannel et al. (1971)

further concluded from the Framingham Study, a 14-year study of 2282 men and 2845 women, that increased risk of CAHD was evident as the total serum cholesterol became equal to or greater than 280 mg/100 ml. Normal serum cholesterol values range from 150 to 280 mg/100 ml. Since CAHD has been observed more often in young men than in young women, an association of serum lipoprotein levels and hormones seems apparent (Barclay, 1972). Women over 45 years of age do not demonstrate any correlation between their serum cholesterol and incidence of CAHD (Kannel et al., 1971). The triglyceride concentration of VLDL is suggested by these authors to be possibly a better indicator of risk of CAHD in older women than is serum cholesterol concentration. Elevation of triglycerides imposes an increased risk to CAHD. Often an elevation of this lipid is associated with elevation of plasma cholesterol. A serum triglyceride value of 158 mg/100 ml and cholesterol value of 260 mg/100 ml have been suggested by many as the dividing line to separate normal from cardiac-prone individuals. Wright (1971) has reported that many investigators feel that a much better correlation exists between elevated triglyceride levels and CAHD than between cholesterol levels of less than 260 mg/100 ml and this disease. Hypertension was also an increased risk factor associated with individuals with CAHD.

Little et al. (1967) have shown that coronary patients appear to react differently to hyperlipidemic foods. The S_f 20-400 fraction (VLDL) showed no important correlation with carbohydrate and sucrose diets. Dietary fat correlated positively with serum cholesterol, phospholipids, and S_f 0-20 lipoproteins (LDL). The serum lipid levels of coronary-prone

men apparently are more influenced by dietary lipid levels than by carbohydrate concentrations.

Kalugina (1963) reported on a study demonstrating lower HDL and higher LDL in patients 15 to 37 years of age. The increase in LDL was attributed to hypertensive disease. In patients in which hypertension was in combination with atherosclerosis the increase in LDL was more consistent. The more advanced the hypertension, the greater elevations there were in LDL. Dietary therapy reduced LDL fraction but not the blood pressure.

Just as people with the same plasma triglyceride or cholesterol concentrations may have different lipoprotein patterns, so will they react differently to dietary therapy. Diets low in cholesterol and polyunsaturated fats may help reduce coronary attack rate in middle-aged man. Plasma triglycerides, as well, may be modified by dietary intervention. Physical activity, too, will reduce hypertriglyceridemia.

Reduction of serum cholesterol by dextro-thyroxine in man has been reported (Engelberg, 1962). Forty-three normal and 14 CAHD subjects were given the drug in doses from 2 to 16 mg/day for 2 years. During the course of the experiment 8 of the 14 CAHD subjects quit treatment because of increased angina. Forty-three controls and 6 remaining CAHD patients showed average decreases in serum cholesterol from 322 mg/100 ml to 243 mg/100 ml, a decline of 25%. The LDL fraction showed a 24% decline in S_f 0-12. An additional group of four hypothyroid patients with an initial average cholesterol value of 310 mg/100 ml showed a decrease in cholesterol to 240 mg/100 ml after given adequate thyroid therapy (3 g of thyroid).

When 8 mg of sodium dextro-thyroxine was administered in addition to the already given thyroid therapy, a further decrease to 180 mg/100 ml was demonstrated. Thus, the predominately cholesterol-bearing lipoprotein class, S_f 0-12, was affected by this drug therapy.

Lipoprotein can penetrate arterial endothelium through small vesicles. A rather constant amount of whole plasma may be entering the intima, transporting a variable amount of lipoprotein, dependent on its cholesterol content. Increased concentrations of lipoprotein have been found in vessels from hypertensive individuals; these vessels, also, have increased intimal thickening. Lipids can penetrate the endothelial barrier to come in contact with the internal elastic lamina (Smith and Slater, 1972). The movement of the LDL fraction into the human arterial wall has been evidenced by histochemical, immunoelectrophoretic, and immunochemical techniques (Scott and Hurley, 1970).

Ross and Glomset (1973) performed in vitro experiments to describe more effectively the growth promoting substances which affect growing arterial smooth muscle cells. Thoracic aorta smooth muscle cells from macaques (M. nemestrina) were grown in a Dulbecco-Vogt modification of Eagle's medium containing the species' own complete serum. Stationary growth or attainment of a maximum population was observed to occur with cells growing in 5% serum after 10-14 days of logarithmic growth. Elastic fibers, proteins, and collagen were produced during the logarithmic period of growth. Proteins were found to be the growth promoting factors. Of all the lipoprotein fractions used, LDL gave better growth promoting effects. With respect to arterial disease, LDL is able to penetrate the

intima where it causes cell proliferation or interferes with metabolism of cells. It may associate with extracellular glycosaminoglycans or with elastin and interfere with matrix turnover. Lipoproteins, too, could become entrapped, denature in time, and yield insoluble deposits. Hopefully, presentation of such correlation and other revealing information will bring a better comprehension of the way heart and blood vessels are affected by faulty lipid metabolism.

Dog lipoprotein patterns

Composition and distribution of dog lipoproteins Dog lipoprotein patterns differ from the human's in some respects. The lipid and protein composition of human and dog plasma lipoproteins is presented in Table 3. Usually, the chylomicron fraction exists in such low concentrations that it is not separated as such but is included with the VLDL fraction. The VLDL fraction is not quantitatively a large fraction in the dog. The LDL fraction comprises the majority of triglycerides in dog's plasma. Dog lipoproteins of $d<1.063$ g/ml (VLDL and LDL) contain approximately 15% of the cholesterol, 8% of the phospholipid, and more than 60% of the triglyceride found in the serum (Solyom et al., 1971). The protein content of the serum VLDL is significantly higher in dogs than has been observed in man. The existence of a unique apolipoprotein, the X-component, in the dog's VLDL fraction has been reported by this same author. This component is thought to correspond to the apolipoprotein C which exists in human serum VLDL and chyle VLDL. Lipoproteins of $d>1.063$ g/ml contain approximately 85% of the serum cholesterol and 90% of the serum phospholipid. The

Table 3. Lipid composition of human and dog plasma

Protein and lipids	Human[a]					Dog[b]			
	Chylo-micra	VLDL	LDL[c]	HDL	VHDL	VLDL[d]	LDL	HDL	VHDL[e]
	(% of total)								
Apolipoprotein	2	9	21	33	57	13	31	44	—
Phospholipid	7	18	22	29	21	18	31	35	—
Cholesterol	8	22	46	30	17	9	22	16	—
Triglyceride	83	51	11	8	5	60	16	5	—

[a]White et al. (1964, p. 435).

[b]Solyom et al. (1971, p. 476).

[c]The LDL fraction comprises 50-60% of the total lipoproteins. Chylomicra usually are negligible. VLDL and HDL constitute 0-20% and 10-40% of the total, respectively.

[d]Dog VLDL is equivalent here to VLDL plus chylomicra.

[e]Insufficient amount of sample for all analyses.

authors not only found that HDL (d>1.063<1.21 g/ml) carried 79% of the total cholesterol, but that it was the principal lipoprotein in dog plasma. The percentage distribution of dog serum lipids is shown in Table 4. Cholesterol/phospholipid ratios in the HDL fraction of dogs have been reported to be similar to man's (Havel et al., 1955).

On the basis of other species, including the human, age, sex, and breed would be expected to affect the lipoprotein pattern in dogs. No effects of age and breed on dog lipoprotein patterns have been reported.

Table 4. Distribution of serum lipids among dog lipoprotein fractions[a] values, expressed as percentage of serum concentrations, are presented as the mean ± standard error. Figures in parentheses are number of samples analyzed

Serum lipid	Distribution of dog serum lipids among						
	VLDL[b]	LDL		HDL			VHDL
	d<1.006	d>1.006 <1.019	d>1.019 <1.063	d>1.063 <1.110	d>1.110 <1.160	d>1.160 <1.210	d>1.210
Cholesterol	1.7±0.9(3)	0.7±0.5(6)	12.7±2.7(6)	22.9±5.8(8)	37.0±9.8(7)	19.3±3.8(6)	5.8±1.6(7)
Phospholipid	1.1±0.5(4)	0.4±0.1(6)	6.2±3.3(6)	22.4±5.7(8)	33.8±7.1(5)	22.0±6.0(8)	13.8±3.0(8)
Triglycerides	20.5±7.2(5)	6.8±6.4(6)	35.6±6.0(6)	6.1±4.3(8)	17.4±8.8(6)	9.3±2.8(8)	4.0±2.4(8)

[a]Solyom et al. (1971, p. 474).

[b]Dog VLDL is equivalent here to VLDL, plus chylomicra.

Nutritional alterations of composition and distribution of dog lipoproteins Types and levels of nutrition have been found to affect the composition and distribution of total blood lipids and lipoprotein fractions in the dog. Grande and Schultz (1966) reported that normal dogs given 40% of their caloric intake in coconut oil demonstrated large increases in total cholesterol, phospholipids, and triglycerides. A broader variety of dietary oils was used by Lindall et al. (1971) to assess the effects of different dietary oils on serum lipoproteins of the dog. After a low fat control maintenance diet was given for 2 weeks, 16 male dogs were given four special diets each of which consisted of 84 parts of low fat diet and 16 parts of one of the oils: menhaden; coconut; safflower; and olive. Each experimental diet derived 40% of the calories from its particular fat (oil). Four groups of four dogs each were rotated in such a way that during any 2-week period all four diets were being fed. Each diet was fed for 2 weeks and for a terminal control period. No changes in relative concentrations of protein, cholesterol, or phospholipid in HDL (the major lipoprotein of the dog) were observed from the experimental diets with the exception of menhaden oil's reduction of phospholipids. LDL cholesterol levels increased more than three-fold with the coconut oil diet. This diet caused two-fold increases in LDL protein and phospholipid. Olive and safflower oils caused small increases in LDL cholesterol and phospholipid. Menhaden oil had no significant effect in reducing or increasing protein or cholesterol in low density lipoproteins. VLDL showed very little change due to the different diets. Though the LDL

fraction of dog and man respond similarly to these diets, HDL, the major fraction in the dog, is irresponsive.

The effects of nutritional deprivation and diets high in carbohydrate on dog lipoproteins have not been investigated.

Dog lipoproteins in relation to thyroidectomy, hypertension, and the arterial intima Thyroidectomy in the dog causes elevation of serum lipids. When thyroidectomized male dogs were fed a low fat diet, serum triglycerides increased (Grande and Schultz, 1968). Grande and Schultz (1966) showed that significant increases in serum cholesterol are possible when thyroidectomized dogs are fed a diet consisting of 80% low fat and 20% coconut oil. These dogs have slightly higher plasma phospholipid concentrations than do control dogs fed a low fat diet. The triglyceride levels in the treatment animals were not significantly different from the controls. The synthesis of phospholipids is stimulated by thyroid hormone. These authors postulated that the lack of increase in serum phospholipids of thyroidectomized dogs fed coconut oil over those thyroidectomized dogs fed a low fat diet may have been due to a deficiency in phospholipid synthesis. If these dogs were later treated with thyroxine, they showed significant decreases in serum cholesterol.

There has been some supportive evidence that administering thiouracil to cholesterol-fed dogs will induce atherosclerosis that is similar to that observed in man (Milch et al., 1958). Milch and his colleagues fed dogs of both sexes thiouracil for 14-15 months. Though cholesterol concentrations of 394 mg/100 ml resulted, no lesions were observed. Other dogs which were fed only cholesterol for a period of 16 months had plaques and

cholesterol concentrations of 429 mg/100 ml. Females showed significantly higher concentrations of the S_f 0-12 fraction in thiouracil- and cholesterol-fed dogs. Lewis et al. (1952) found in normal dogs made hypothyroid by giving a 10 mCi dose of ^{131}I an increased concentration of VLDL and LDL. When diets high in cholesterol were administered to these dogs, the LDL fraction further increased. The abnormal lipoprotein pattern was corrected by feeding dessicated thyroid.

Malmros and Sternby (1968) have assessed the relative resistance of dogs to dietary increases of cholesterol to be due to newly absorbed dietary cholesterol rapidly suppressing the intrinsic high rate of cholesterol synthesis in the liver. Strong hypercholesterolemia can be produced if the diet, apart from cholesterol, contains saturated fat, e.g., hydrogenated coconut oil. No marked increases in triglycerides resulted from this diet of coconut oil.

Schultz and Grande (1968) conducted an experiment to study the effects of starch versus sucrose diets on dogs before and after thyroidectomy. The diets consisted of 55 parts of a low fat diet (1% fat) and 45 parts of either cornstarch or sucrose. Nine dogs were assigned to each of the two diets for a period of 2 weeks. At the end of the time period, those dogs receiving a starch diet were changed to sucrose and those receiving a sucrose diet were changed to a starch diet. This new dietary scheme lasted for 2 weeks. All 18 dogs were then thyroidectomized and allowed 2 months for recovery before the same dietary scheme was again presented to them. Lipids values are shown in Table 5. Lipid levels with respect to diet before thyroidectomy did not show any significant changes. This may be

Table 5. Serum lipid values of dogs fed sucrose and starch diets before and after thyroidectomy[a]

	Diets		Difference	
	Starch	Sucrose	Sucrose minus Starch	
	(mg/100 ml)		(mg/100 ml)	P value[b]
Before thyroidectomy				
Total cholesterol	137.0± 7.5[c]	137.0± 8.0	0.0± 3.7	
Phospholipids	310.0±15.7	314.0±12.2	4.0±13.7	0.80
Triglycerides	47.0± 2.3	50.0± 2.0	2.0± 1.9	0.30
After thyroidectomy				
Total cholesterol	304.0±21.4	292.0±20.9	-12.0±20.8	1.55
Phospholipids	468.0±17.1	442.0±11.4	-26.0±17.6	0.15
Triglycerides	100.0± 6.0	113.0± 5.9	13.0± 4.3	0.006*

[a]Schultz and Grande (1968, p. 72).

[b]Significant difference between effects of diets before and after thyroidectomy.

[c]Means ± SE for 18 dogs.

*Difference is statistically significant at $P<0.05$.

due to the low cholesterol content in the diets. The sucrose diet caused significantly higher levels of triglycerides than starch after thyroidectomy. In the absence of the thyroid gland, sucrose does not raise individual lipid levels, with the exception of triglycerides, any more than does starch.

The effects of arterial pressure on lipoprotein patterns have been demonstrated in experimentally neurogenic and renal hypertensive dogs (Lewis et al., 1952). Dogs with renal hypertension showed a high concentration of VLDL. The results from this experiment showed that the increase in this fraction did not precede the development of the hypertension. The

LDL concentration of normal and renal hypertensive dogs was nearly the same. Experimental neurogenic dogs showed relatively small changes in lipoproteins. Of seven neurogenic hypertensive dogs, which showed after 10 months blood pressures from 200 to 260 mm Hg, only one had an increase in lipoprotein concentration (LDL). Following buffer nerve section in dogs with arterial hypertension, there was little or no change of the lipoprotein pattern. Since there were no significant changes observed in lipoprotein patterns of dogs with neurogenic hypertension or in dogs with buffer nerve section, an elevated blood pressure may not be responsible for the abnormal lipoprotein pattern in the renal hypertensive dogs.

Prolonged mental and emotional stresses often accompany the hypertensive patient. The adrenal medulla in the stressed dog will secrete epinephrine which will induce hyperlipidemia and hypercholesterolemia. Experimentally elevated cholesterol can be maintained by exogenous epinephrine. The overall percentage increase of serum lipid in response to epinephrine occurs greatest in the cholesterol and phospholipid-rich fraction of $d>1.019<1.063$ g/ml and to a lesser extent in the cholesterol and phospholipid-rich fraction of $d>1.063<1.21$ g/ml (Shafrir et al., 1959).

An elevation of blood pressure can cause a marked increase of entry rate of lipoproteins through the dog aortic wall. In areas where circumferential tension of the arterial wall is high, the endothelial cells may be stretched. This may permit an inflow of lipoproteins either by way of the intercellular junctions or by the vesicles in the endothelium. Labeled LDL enters the inner layer of the aortic wall at a faster rate in the ascending thoracic aorta than in the descending thoracic and slower

yet in the abdominal aorta. This gradient is similar to that shown in the inner layer of the aorta during the first month of developing atherosclerosis in dogs fed thiouracil and cholesterol (Duncan et al., 1963).

MATERIALS AND METHODS

Experimental Design

Animals

Nineteen Specific Pathogen Free (SPF) beagles,[1] consisting of 10 females and 9 males, were acquired at 8 months of age.

Each animal was vaccinated[2] for distemper, hepatitis, and leptospirosis. Individual stainless steel cages and indoor housing were provided for them. The animals were randomized for sex and cage position in the room. All dogs were maintained on a balanced diet[3] for the first 6 months for diet stabilization prior to the implementation of the experiment. The control animals were maintained on this diet through all treatment regimens.

Treatment preparation and regimens

Subsequent to 2 months of adjustment to their surroundings and handling, each dog had an exteriorized carotid artery loop surgically prepared (O'Brien et al., 1971) to provide a convenient and direct method to measure blood pressure by needle puncture. After 3 months of healing of the preparation, there were 15 animals with excellent loops ready for the direct needle puncture technique. For a period of 2 months approximately

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[1]Obtained from the beagle colony at Veterinary Medical Research Institute, Iowa State University, Ames, Iowa.

[2]Fromm Laboratories, Inc., Grafton, Wisconsin.

[3]Friskies Dry Dog Food, Carnation Co., Los Angeles, California. Composition includes 23.0% crude protein, 7.0% crude fat, 5.0% crude fiber, 12.0% moisture and 10.0% ash. Iodized salt (0.94%) and vitamins are included.

a dozen blood pressures were obtained from each dog. An average of these pressures was used as a stable representative pressure for that dog. The average blood pressures for all dogs were allocated into two groups, with nearly equal sex representation in each group, such that both groups would possess a similar average blood pressure. The control group consisted of five females and five males. The treatment group consisted of five females and four males.

Both groups of animals were placed on the experimental regimen on May 18, 1972. The control group was allowed the regular maintenance diet and water ad libitum. This diet was removed, however, from the treatment animals for a period of 30 days. Water was allowed ad libitum to this group through all treatment regimens. On June 17, 1972, the treatment group was given a specially formulated, semi-moist high carbohydrate diet.[1] Realimentation continued for this group of dogs for 20 days. Seventy percent of the energy in this formula diet was derived from carbohydrate. Sucrose represented 27.0% and rice and other starches represented the remaining 43.0% (dry matter basis). The caloric content of the special diet was 679 Cal/lb and the diet's digestibility was 85.0%. Table 6 summarizes the composition of this diet.

The quantity of food fed to each treatment animal was partially determined by the individual animal's body surface area. Surface area was calculated from a nomogram which correlated body weight and length with surface. Body weights were acquired weekly throughout the entire experi-

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[1]Theracon, Inc., Topeka, Kansas.

Table 6. Chemical composition of the high carbohydrate diet

Nutrients[a]	Wet weight basis %
Moisture	60.75
Dry Matter	39.25
Protein[b]	8.64
Fat[c]	1.15
Ash	1.53
Calcium	0.41
Phosphorus	0.29
Sodium[d]	0.14
Potassium	0.06
Nitrogen Free Extract	27.93

[a]The vitamins and minerals in this diet meet the requirements of the Committee on Animal Nutrition (1962, pp. 2-3).

[b]Four parts horsemeat and one part casein.

[c]Corn oil.

[d]Sodium content of the high carbohydrate diet and commercial diet (Friskies) was 350 mg/100 g dry weight and 359 mg/100 g dry weight, respectively.

mental regimens (Appendix Tables B-1 and B-2). A daily luxus consumption energy level of 120 Cal/m^2/hour was utilized along with surface area to help determine the quantity of food to be fed to each treatment animal.

The treatment group was placed on a second starvation regimen on July 7, 1972, and continued through July 23, 1972 (17 days). A second refeeding with the high carbohydrate diet was then resumed on July 24, 1972, and continued until the dogs were sacrificed on August 23, 1972 (31 days). The treatment regimens are illustrated in Table 7.

Table 7. Treatment regimens

Group	Time Continuum (in days)	Diet
Control	1-98	Maintenance diet (Friskies)
Treatment		
Starvation I	1-30	None
Refeeding I	31-50	70.0% carbohydrate
Starvation II	51-67	None
Refeeding II	68-98	70.0% carbohydrate

Experimental Investigations

Fine structure

Tissues were collected for electron microscopic studies from both groups of animals. A 1 cm segment of left or right femoral artery, depending on the random selection for that dog, was surgically removed 40 days before the first treatment regimen of the experiment. A similar segment from the contralateral artery was surgically removed at the termination of the experiment.

Although one of the main emphases of this experiment was the fine structure of the femoral artery, appropriate light microscopy was deemed necessary to determine any areas of pathological significance due to the treatment regimen. One-half of each 1 cm vessel segment was fixed in 10% phosphate-buffered formalin solution, dehydrated in a graded ethyl alcohol series, cleared in xylene, and embedded in paraffin. Sections were cut at 8 μm in thickness and stained with Gomori's aldehyde fuchsin (McManus and

Mowray, 1965). Additional sections were taken from paraffin blocks of selected animals and were stained with Harris' hematoxylin and counter-stained with eosin (Luna, 1968).

The remaining 0.5 cm of each animal's vessel segment was cut into 1 mm long circumferential rings that, in turn, were cut diameter-wise into 4 arcs. These tissues were cut in dishes containing 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (Sabatini et al., 1963) or 0.1 M Millonig's phosphate buffer and later placed in vials of the fixative and buffer combinations (pH 7.3) at 4 C. All tissues were divided equally between the combinations to allow for further investigations, if desired, of influences that buffers and dehydration agents have on tissues. After fixation for 2 hours, the tissues were rinsed briefly in cold 0.1 M cacodylate and 0.23 M sucrose buffer or in the 0.1 M Millonig's phosphate buffer. For better membrane stability and clarity, all tissues were postfixed for 1 hour at 4 C in 1% osmium tetroxide with either of the buffers. One-half the material, representing both buffer-fixative combinations, was dehydrated in the following grades of alcohol for 10 minutes each: 50, 70, 85, 95%, and twice in absolute ethyl alcohol. The other one-half of material was dehydrated using the following grades of acetone: 30, 60, and 90% at 5 minutes each and three changes of 100% at 10 minutes each. The alcohol-dehydrated, pre-treatment tissues were then processed through propylene oxide and stepped ratios of resin and propylene oxide until the tissues were finally embedded in pure Epon 812. The arcs of femoral vessel were orientated at the tip of the embedding molds so that a cross-section of the

vessel could be made. The acetone-dehydrated tissues were processed utilizing stepped ratios of resin and acetone until the final embedding. The post-treatment tissues were similarly processed, with the exception that an Epon-Araldite resin was used for infiltration and embedding.

As a matter of routine, plastic sections of approximately 1 μm in thickness were cut with a glass knife on an LKB 4801A ultratome.[1] When stained with toluidine blue,[2] the information from the sections was used to determine if it was worthwhile to do thin sectioning. These thick sections were taken from representative blocks of pre-treatment tissue of each dog. Due to time requirements, only eight post-treatment dogs were randomly selected to have representative blocks of tissues used for electron microscopy. There were two females and two males in each of the control and treatment groups. Consequently, 1 μm sections on post-treatment tissues were made only on these selected animals. The condition (softness) of the Epon prevented the cutting of thin sections on the pre-treatment samples.

Sections 500 to 700 Å in thickness were cut with a glass knife on the ultratome and then picked up on uncoated 300 mesh copper grids. Two percent uranyl acetate[3] (Stempak and Ward, 1964) and lead citrate[4] (Reynold, 1963) were used as stains, so as to enhance contrast to the sectioned

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[1] L.K.B. Produkter A.B., Stockholm, Sweden.

[2] Polysciences, Rydal, Pennsylvania.

[3] Vaughn, Inc., Memphis, Tennessee.

[4] Fisher Scientific Co., Fairlawn, New Jersey.

material. The sections were examined on a RCA-EMU4[1] electron microscope.[2]

Other supporting histology

A right or left adrenal gland was randomly taken from each sacrificed animal. These tissues were processed and sectioned similarly to the femoral arteries for light microscopy observation. Sections were stained with hematoxylin and counterstained with eosin. Additional sections were stained with oil red O[3] (Luna, 1968).

Blood chemistry

Eighteen-hour preprandial blood samples were drawn from the internal jugular vein of control and treatment animals at the beginning of the experiment and at the end of each treatment regimen. A disodium ethylenediamine tetra-acetic acid (EDTA) solution (120 mg/100 ml) was used to prevent clotting. Additional blood samples for the fractionation of lipoproteins were collected from six randomly selected animals that would undergo the treatment regimen. Three of each sex were represented. Sampling was done prior to the experiment, at the end of the first starvation regimen, 3 days after the first refeeding regimen, and at the conclusion of the last treatment (second refeeding regimen). Whole (total) blood samples were centrifuged as soon after collection as possible in a Sorvall

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[1]Radio Corporation of America, Scientific Instruments, Camden, New Jersey.

[2]Available through the courtesy of Dr. Marvin Stromer, Food Technology, Iowa State University, Ames, Iowa.

[3]Allied Chemical, Morristown, New Jersey.

Superspeed Model RC2-B automatic refrigerated centrifuge[1] for 30 minutes (1478 x g, 1 C). The plasma samples were immediately withdrawn by Pasteur pipettes and placed in plastic tubes. All but the plasma samples for lipoprotein determination were stored in a freezer (-20 C) until the completion of the experiment, at which time total plasma sodium, potassium, glucose, urea nitrogen, cholesterol, and triglycerides were analyzed.

Plasma electrolytes Sodium and potassium were simultaneously analyzed on the Technicon AutoAnalyzer.[2] Lithium was used as an internal standard. The technique is described in the Technicon AutoAnalyzer Methodology-Method File N-20b I/II.

Blood plasma glucose and urea nitrogen Blood glucose and urea nitrogen were run simultaneously on the Technicon AutoAnalyzer. The description of this method can be found in the Technicon AutoAnalyzer Methodology-Method File N-16.

Plasma cholesterol Whole plasma cholesterol determinations were carried out on a Technicon AutoAnalyzer, as described in the Technicon AutoAnalyzer Methodology-Method File N-24a. The description of the quantitative procedure for the determination of cholesterol (Block et al., 1965) is based on the reaction of a concentrated sulfuric acid and ferrous chloride in acetic acid with the steroids having a 5-ene, 3 beta-ol group. A colored reaction product is developed and read at 550 nm in a tubular flowcell.

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[1] Ivan Sorvall, Inc., Norwalk, Connecticut.

[2] Technicon Instruments Corporation, Tarrytown, New York.

Plasma triglycerides Whole plasma triglyceride determinations were carried out on the Technicon AutoAnalyzer simultaneously with cholesterol. A description of this method is described by Technicon AutoAnalyzer Methodology-Method File N-70. This semi-automated procedure, which is based on the work of Kessler and Lederer (1965), demonstrates that a plasma sample is first extracted with isopropanol in the presence of a slurry containing zeolite, copper lime, and Lloyd reagent. The lipid extract is then sampled into an air-segmented alcoholic potassium hydroxide solution. Saponification of triglycerides to glycerol occurs on-stream in the 50 C heating bath. After saponification, periodate reagent is added to the reaction mixture to oxidize the glycerol to formaldehyde. The formaldehyde is then condensed with diacetylacetone and ammonia to form a fluorescent condensation product (3, 5-diacetyl-1, 4-dihydrolutidine). After heating, the reaction mixture enters the fluorometer where air is removed, the product is activated by the light coming through the 400 nm interference filter, and the fluorescent product is measured at 485 nm.

Fractionation and lipid determinations of lipoproteins

Isolation and purification of lipoproteins Sequential preparative ultracentrifugation was used to separate dog plasma lipoproteins into the following four density ranges: $d<1.006$ g/ml, very low density (VLDL); $d>1.006<1.063$ g/ml, low density (LDL); $d>1.063<1.21$ g/ml, high density (HDL); and $d>1.21$ g/ml, very high density (VHDL). Such separations were made according to the method of Havel et al. (1955). Five ml of plasma from each of the selected six animals were placed in polycarbonate ultracentrifuge tubes and were layered over with 1.5 ml of 0.15 M NaCl solution

(d=1.006 g/ml). Solutions and the general procedures for density adjustment are seen in Appendix Table A-1 and Figure 2, respectively. All solutions that were used contained EDTA at a concentration of 10 mg/100 ml.

The separation of VLDL and LDL required that ultracentrifugation be carried out for 24 hours at 104,500 x g at 4 C. A Beckman Model L-2[1] preparative ultracentrifuge[2] and a Spinco 40 rotor[1] were utilized for this procedure. Calculations for centrifugal force are in Appendix A. Longer periods of time such as these were necessitated to separate LDL and HDL in the dog. After the rotor had stopped, great care was exercised to avoid any abrupt movement of the rotor or of each individual tube during manipulation prior to pipetting. After centrifugation, lipoproteins of less than solvent density were concentrated in a layer at the top of the tube. Beneath this was a clear, colorless zone occupying one-quarter of the length of the tube. Consequently, the S_f 20-400 VLDL fraction was quantitatively removed in the top 1.0 ml and the lipoprotein-free salt background reference was in the 1.5 ml below. The removal was done immediately as certain fractions, upon standing, will buoy up or settle out with time.

Although the use of a Spinco tube cutter[1] has some advantages in special applications and was used to some extent in this work, it was not found effective and is not recommended for lipoprotein work (Lindgren et al., 1972). Pipetting provides the best quantitative removal of lipoprotein fractions, with visual evaluation of completeness and with minimal

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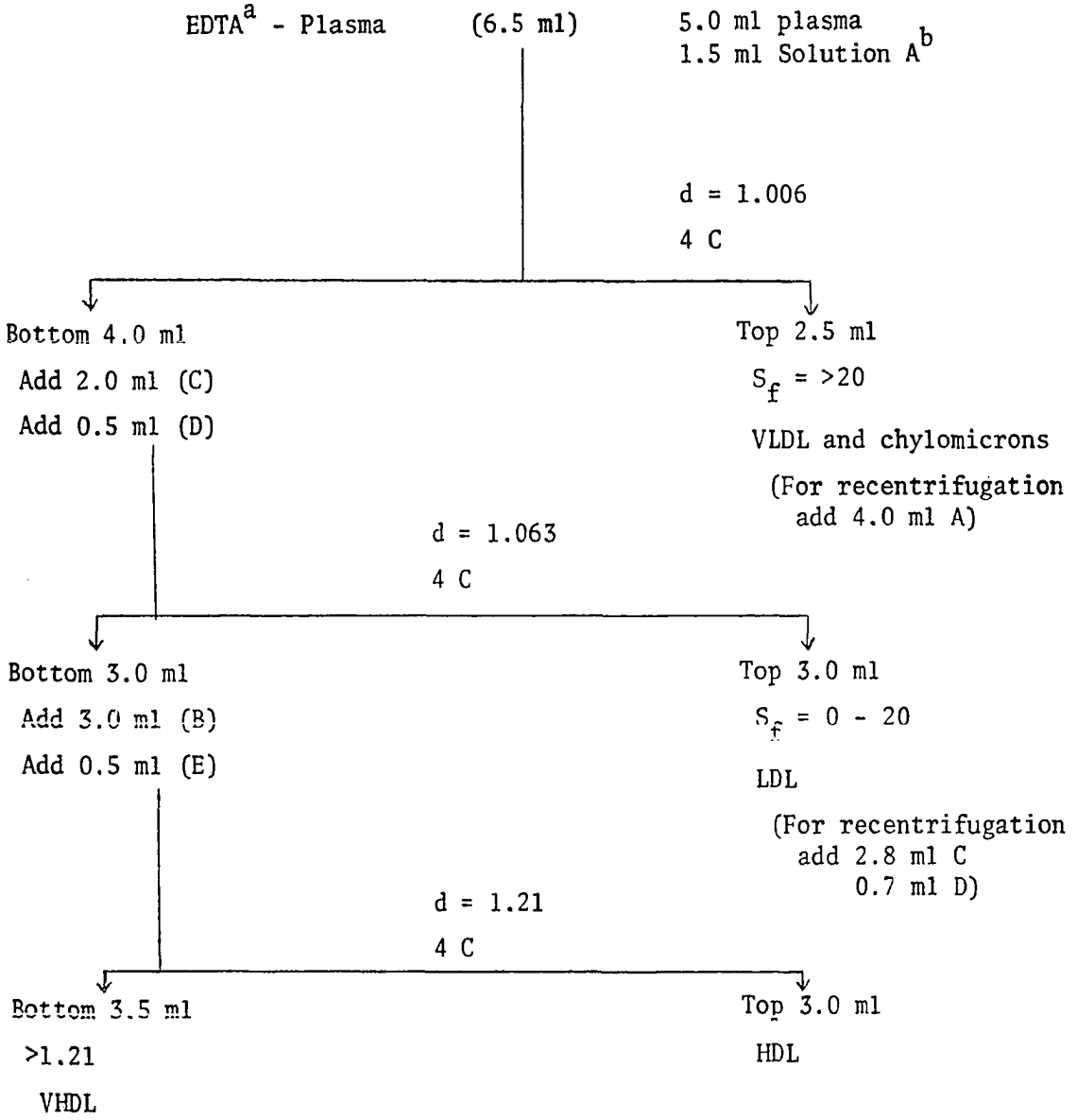
[1]Spinco Division, Beckman Instruments, Inc., Palo Alto, California.

[2]Available through the courtesy of Dr. Donald Beitz, Nutritional Physiology, Animal Science, Iowa State University, Ames, Iowa.

Each ultracentrifugation was carried out at 104,500 x g (39,800 rpm) for 24 hours at 4 C. The scheme for fractionation (Havel et al., 1955) is presented below.



[a]EDTA concentration = 120 mg/100 ml plasma.

[b]Solutions A, B, C, D, and E shown in Appendix Table A-1.

Figure 2. Lipoprotein fractionation with density adjustments

disturbance to the remainder of the preparative tube. A special thin-walled Pasteur pipette with an inside bore of 0.4 to 0.6 mm was used. Pipetting was done in the darkened cold room on a fixture equipped with a focused light beam allowing visualization of the lipoproteins by Tyndall scattering. Fractions were placed in calibrated 0.5, 1.0, 2.0, and 2.5 ml vials. The 2.5 ml of VLDL fraction and reference zone were washed by resuspending them with 0.15 M NaCl solution to a volume of 6.5 ml in an ultracentrifuge tube and recentrifuging them to obtain homogeneity and purity of the fraction. The fraction was then stored in a calibrated vial. The infranatant from the first ultracentrifugation was adjusted to d>1.006 d=1.063 by the addition of an appropriate NaCl and KBr solution and then centrifuged for 24 hours at the same speed. Subsequent to this ultracentrifugation, the top 3.0 ml were removed, adjusted again to 6.5 ml with the appropriate solution of density 1.063 g/ml, and recentrifuged. The infranatant of the first ultracentrifugation at density 1.063 g/ml was adjusted to d>1.063 d=1.21 with the proper NaCl and KBr solutions and then ultracentrifuged. After this run, the 3.0 ml supernatant was again increased in volume with a salt solution to density of 1.21 g/ml and recentrifuged. The infranatant of the former centrifugation at density 1.21 g/ml was classified as d>1.21 g/ml.

Cholesterol determination of lipoprotein fractions Amounts of cholesterol in whole plasma and lipoprotein fractions were determined in triplicate. Consequently, three values were obtained on each sample of plasma and each lipoprotein fraction of the six animals. The method used for this determination was developed by Mann (1961). Reagents and procedures are described in Appendix A. Alcoholic alkaline digestion of the

plasma and lipoprotein samples was followed by an extraction of the mixture with redistilled petroleum ether and water. Subsequent to the evaporation of an aliquot of the petroleum ether, a solution of $FeCl_3 \cdot 6H_2O$ in H_2SO_4 (FeSac) and acetic acid was mixed with the residue-containing cholesterol. After completion of the reaction, the optical densities of the samples were determined with a Gilford recording spectrophotometer, utilizing a wavelength of 560 nm. The optical densities of the samples were compared with those of the solutions containing known amounts of cholesterol. The color complex was stable for 15 to 60 minutes following the addition of the FeSac reagent. The method was very reproducible.

Triglyceride determination of lipoprotein fractions The concentration of triglycerides in the whole plasma and each of the lipoprotein fractions obtained from ultracentrifugation was determined in triplicate. Thus, three values were obtained on each sample of plasma and each lipoprotein fraction of the six animals used in this part of the experiment. A colorimetric method (Fletcher, 1968) for the estimation of serum triglycerides was used. The reagents and detailed procedures are included in Appendix A. The glycerides in the plasma and lipoprotein fractions were isolated from the phospholipids by mixing them with a slurry of silicic acid mixture (100 g silicic acid, 5 g $CuSO_4$, and 10 g $Ca[OH]_2$) in redistilled isopropanol. Redistillation was necessary for all triglyceride work, as some fluorescing contaminant gave an unstable base line on the semi-automated technique (Technicon AutoAnalyzer) and wide variation in optical density readings. Whereas, the phospholipids remained adhered to

the silicic acid mixture, the assay for glycerides was made from the phospholipid-free extract containing isopropanol. The glycerides in the extract were then saponified to free glycerol by using 1.1 N KOH. The glycerol was oxidized by sodium metaperiodate to form formaldehyde which, in turn, was condensed with acetylacetone (2, 4-pentanedione and isopropanol in ammonium acetate) to form a yellow, fluorescent product (3, 5 diacetyl-1, 4-dihydrolutidine) absorbing at 405 nm. The optical density of each sample was compared to solutions containing a known concentration of triolein. A Gilford recording spectrophotometer[1] was used in obtaining optical density readings on the samples. Such qualities as excellent recovery, high reproducibility, and good correlation with other reliable automated and manual methods make this technique especially valuable for the determination of triglycerides in lipoprotein fractions.

Electrophoresis of whole plasma and lipoprotein fractions

Electrophoresis was done on all lipoprotein fractions to demonstrate the extent of purity in each fraction separated by the ultracentrifugation procedure and, also, to visualize the mobility patterns of dog lipoproteins. Plasma samples were electrophoresed immediately to benefit most from the charged groups in the proteins. Neither plasma nor lipoprotein fractions should be frozen as freezing denatures the lipoproteins and prevents the charged groups from exhibiting good migration. Since LDL, HDL, and VHDL possessed greater densities of salt solution than normal, these samples were dialyzed to remove the salts. Protein fractions, dissolved in

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[1]Gilford Instrument Laboratories, Inc., Oberlin, Ohio

concentrated salt solutions, have been shown to exhibit electrophoretic mobilities lower than those obtained in whole serum (Sakagami and Zilversmit, 1961). Samples were placed in dialysis tubing and then placed for 12 hours in 4.0 liters of 0.15 M NaCl solution which was under constant agitation.

Cellulose acetate[1] strips were coded for information and marked 1 inch from the end, rinsed briefly with distilled water, and dipped into 0.05 M Electra HR Tris-barbital-sodium-barbital buffer[2] (1 package dissolved in 750 ml distilled water and adjusted to pH 8.8) for 10 minutes. Each of the two electrophoresis chambers[2] was filled with 50.0 ml of the buffer, and the wicks were soaked. Strips were individually removed, blotted, and given a sample application of plasma or fraction. The application was made at the 1 inch mark and was 2 and 4 µl, respectively, for plasma and fraction. The strips were placed application side down with application sites at the cathodal end of chamber. The chamber was covered and the samples were electrophoresed for 15-18 minutes at 205-210 volts with a special power supply.[3] The amperage was maintained below 7 mA by using ice in the outer two chambers.

Strips were then removed and placed in a slide dish of oil red O (0.5 g/liter methanol) in a 35 C oven for 0-5 hours. Two baths of 50% ethanol were used for destaining. The strips were stored in either a slide dish of 15% glycerol or in glycerinated bags.

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[1]Millipore Corporation, Bedford, Massachusetts.

[2]Helena Laboratories, Beaumont, Texas. No information on exact amount in package was available.

[3]Buchler Instruments, Fort Lee, New Jersey.

RESULTS AND DISCUSSION

Arterial Fine Structure

Appropriate light microscopy was deemed necessary prior to the fine structure study to determine if there were any areas of pathological significance due to time and treatment. A histopathological examination of paraffin sections (8 μm) of femoral artery stained with either Gomori's aldehyde fuchsin or hematoxylin and eosin revealed no remarkable changes in the arterial wall structure of control dogs or dogs periodically starved and refed a high carbohydrate diet. The Gomori's aldehyde fuchsin stains elastin specifically. Amount and arrangement of elastin in the femoral arteries of all dogs at the beginning of the experiment showed no apparent differences with those femoral arteries of either control or treatment dogs at the end of the experiment. Similarly, 8 μm sections of adrenal glands stained with either hematoxylin and eosin or oil red O exhibited no histological differences between control and treatment dogs at the end of the experiment.

Plastic thick sections (1 μm) of dog femoral arteries revealed upon staining with toluidine blue no differences in arterial structure between all dogs at the beginning of the experiment and control dogs at the end of the experiment. Dogs periodically starved and refed the high carbohydrate diet showed no differences in arterial structure when compared to those dogs of the control group. The Epon-embedded pre-treatment tissues possessed softness of the plastic, thus, preventing the thin sectioning of these samples. Since no apparent differences in structure existed at 1 μm

thickness in control dogs for pre- and post-treatment arterial samples, it was assumed the time duration (98 days) of the experiment contributed no aging effect on the femoral arteries of the dogs. Consequently, only post-treatment tissues were used for electron microscopy study. Two female and two male dogs of both control and treatment groups were randonly selected to provide arterial tissues at the end of the experiment. Five or six tissue samples representative of different locations along the vessel segment were sectioned and then observed at the fine structure level.

Electron micrographs of control and treatment dogs' femoral arteries are shown in Figures 3-9.

Figures 3 and 4 represent transverse-sectional views of normal fine structure of the femoral artery in the four control dogs (nos. 4, 10, 23, and 28). No observable differences in structure were shown to exist between female and male control dogs. The intima of the dog's femoral artery is especially thin and contains little more than a single layer of endothelial cells. Each endothelial cell contains a nucleus which bulges from the inner surface of the vessel. Vesicles are shown lining the plasma membrane of the endothelial cell. They are not as prominent a feature in these cells as in smooth muscle cells. Mitochondria, tonofilaments, vacuoles, and endoplasmic reticulum are shown as well.

A basement membrane is shown lying immediately below the endothelial layer. Whereas, it is usual for the endothelium of a smaller muscular artery or arteriole to lie directly on the internal elastic lamina (Ham, 1969), the dog's muscular artery contains a small amount of fibrous connective tissue between the endothelium and internal elastic lamina (Waters,

Figure 3. Fine structure of a normal femoral artery from a control dog

An endothelial cell (EC), nucleus (N), smooth muscle cell (S), elastic fibers (E), collagen (C), internal elastic lamina (I), elastic lamina (EL), spicules (SP), lumen (L), and media (M) are identified. Transverse section. 1 μm is equal to 2.85 cm (line)


I
L
EC
E
SP
EL
M
C

Figure 4. Characteristic fine structure of the intimal layer in a control dog's femoral artery

An endothelial cell (EC), nucleus (N), vesicles (V), vacuoles (U), mitochondria (MN), endoplasmic reticulum (ER), tonofilaments (T), basement membrane (B), fringed buried pockets (F), lumen (L), internal elastic lamina (I), elastic fibers (E), dense bodies (DB), proelastin fibrils (P), and reticulum (R) are shown. Transverse section. 0.5 μm is equal to 2.92 cm (line)


L
V
B
R
E
P
F
I

1965). A subendothelial space exists in the arterial intima shown in both figures. Such spaces contain reticulum which is thought to be partly mucopolysaccharide. A few smooth muscle cells are common to this space, but are normally aligned parallel with the internal elastic lamina. The lack of this orientation in Figure 3 is due to contraction following the excision of the arterial segment. Elastin and collagen fibrils are shown as well in this space. The layer beneath the subendothelial space is the internal elastic lamina.

The internal elastic lamina shown in Figure 3 appears folded or wrinkled (black areas in the lamina). Such distortion is a common difficulty experienced by many investigators working with arterial tissues (Keech, 1960; Parker, 1958). This washboard appearance is partly due to the different cutting properties contributed by the tenacious and homogeneous matrix of elastin, smooth muscle cells, and the endothelium.

Certain small diverticula or buried pockets with dark fringes (Figure 4) show a patchwork appearance alongside the internal elastic lamina (Fyfe et al., 1968). These pockets may widen as fibrils are added to their sides and blend in with neighboring ones to thicken the internal elastic lamina. They indicated that the pockets persist in the adult animal and have been observed to create and abolish fenestrae of the lamina. Proelastin fibrils emanate about their edges. There are fewer of these dark-fringed pockets produced on the endothelial side of the internal elastic lamina than in the laminae throughout the media. They are known as spicules in the media, or attachment sites of smooth muscle cells to the laminae.

The media is shown in Figure 3 to be adjacent to the internal elastic lamina. Fine fibrillar material, as well as amorphous material, is shown between the basement membranes of the smooth muscle cells and the neighboring internal elastic lamina. The elastin components here in this area are circular profiles with a thin rim of densely staining material (Pease and Molinari, 1960). These authors reported that elastin appears only as islands in the remainder of the medial layer. The smooth muscle cells are attached at multiple points to these islands by way of connections through the basement membranes. Collagen, though separated from the basement membrane-elastin complex, is a part of the extracellular matrix.

There are mitochondria concentrated at the ends of the elongate nucleus of the smooth muscle cell (Figure 5). Golgi complexes, endoplasmic reticulum, and absence of myofilaments exist in the areas of the mitochondrion. Pinocytotic vesicles are found along the smooth muscle cell surface wherever dense bodies are absent. Both longitudinal and cross-sections of myofilaments are shown. Longitudinally orientated myofilaments are seen entering and possibly terminating in these dense bodies. Medial smooth muscle cells and extracellular matrix did not appear to be different between femoral arteries of control and treatment dogs.

Figure 6 is a transverse-sectional view of the endothelium and subendothelial space of a male dog (no. 25) periodically starved and refed a high carbohydrate diet. The usual organelles are present in the intima. Some rather unique characteristics are shown, however. A lipid droplet is shown in the intercellular spaces of the endothelium. A vacuolated mitochondria is indicated. An electron-dense filamentous or flocculent-

Figure 5. Electron micrograph of a normal smooth muscle cell in the femoral artery of a treatment dog

A nucleus (N), Golgi apparatus (G), mitochondria (MN), endoplasmic reticulum and associated ribosomes (ER), myofilaments (K), and dense bodies (DB) are identified. Transverse section. 0.5 μm is equal to 2.92 cm (line)

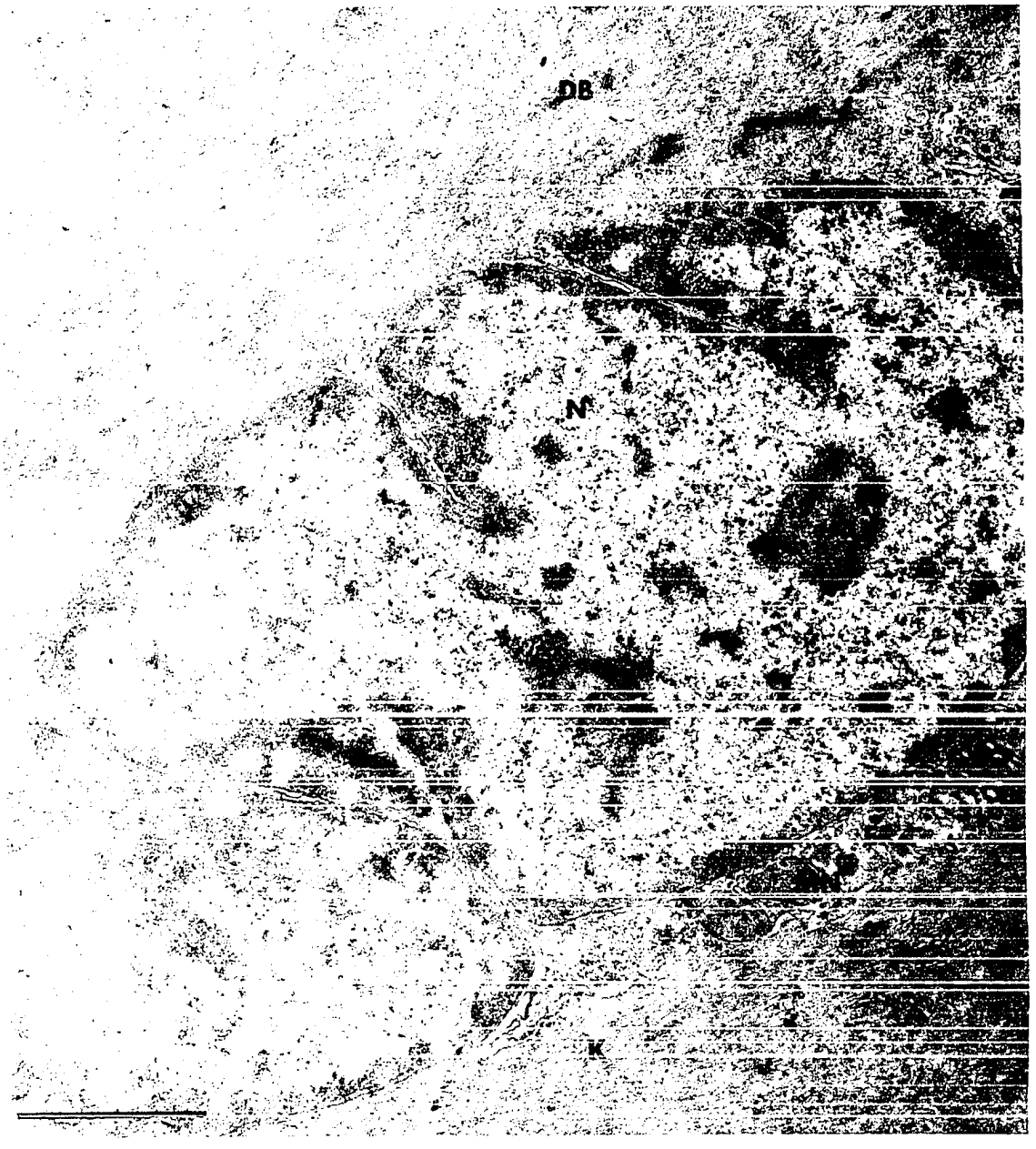
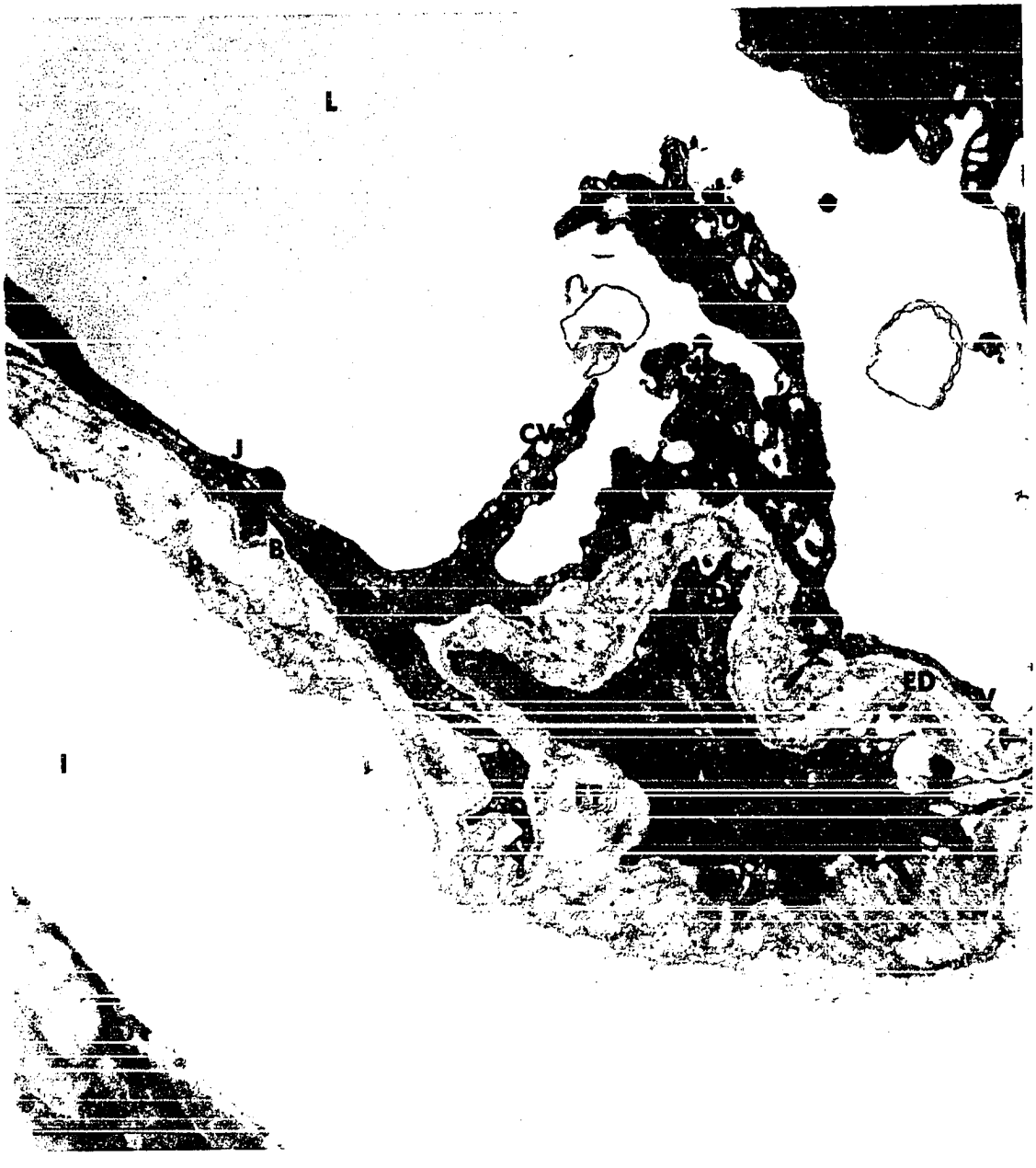

DB
N
K

Figure 6. Fine structure of the intima in the femoral artery of a male dog periodically starved and refed a high carbohydrate diet

An intercellular junction (J), lumen (L), nucleus (N), internal elastic lamina (I), vesicles (V), lipid droplet (LD), basement membrane (B), reticulum (R), fine filaments of connective tissue (FT), caveolae (CV), vacuoles (U), smooth muscle cell (S), dense fibrillar material (DF), Golgi vacuoles (GV), vacuolated mitochondria (W), flocculent or filamentous-appearing material in circular profiles of endoplasmic reticulum (O), and extracellular lipid (ED) are shown. Transverse section. 0.5 μm is equal to 2.13 cm (line)


L
J
B
I
ED

appearing material within the profiles of granular endoplasmic reticulum of both smooth muscle cells and the endothelium indicates an increased cellular activity. None of these cellular activities were observed in arterial tissues from control animals.

Figure 7 shows a longitudinal view of the arterial intima in a female dog (no. 5) that was periodically starved and refed a high carbohydrate diet. This is a representative picture of the arterial intima (in longitudinal-section) of the other female (no. 7) and two male treatment dogs (nos. 25 and 29). Endothelial cells contain lipid-filled vesicles and vacuoles filled with dense fibrillar material. Filamentous or flocculent-containing profiles of granular endoplasmic reticulum are shown. Electron-dense mitochondria indicate increased lipid accumulation and metabolism. Possible lysosomes exist in the endothelial cells. Some of the smooth muscle cells of the subendothelial space contain cytoplasmic lipid inclusions. These inclusions have a relatively pale center and a narrow osmophilic rim. The subendothelial space contains lipid in the extracellular matrix as well. Reticulum, elastic fibers, and collagen fibrils are shown in this space.

Figure 8 represents a transverse-sectional view of a female treatment dog's arterial media (no. 5), adjacent internal elastic lamina, and subendothelial space. A degenerating smooth muscle cell exists in the subendothelial space. Some lipid-filled vesicles are shown in the space. Otherwise, the alternating areas of pinocytotic vesicles and dense bodies of the smooth muscle cells in the media, as well as the medial extracellular matrix, appear much like the normal dog's tunica media.

Figure 7. Longitudinal view of intimal fine structure in the femoral artery of a dog periodically starved and refed a high carbohydrate diet

An endothelial cell (EC), lipid-filled vesicles (LV), large cytoplasmic vacuolations (CL), vacuolated mitochondria (W), flocculent-appearing material in endoplasmic reticulum profiles (O), mitochondria (MN), primary lysosomes (LY), lumen (L), basement membrane (B), internal elastic lamina (I), subendothelial space (SE), collagen fibrils (C), extracellular lipid (ED), cytoplasmic lipid inclusions (CI) in a smooth muscle cell, elastic fibers (E), and reticulum (R) are shown. Longitudinal-section. 1 μm is equal to 2.85 cm (line)


C
L
GL
E
E
ED
I

Figure 8. Characteristic fine structure of the medial layer in the femoral artery of a dog periodically starved and refed a high carbohydrate diet

A nucleus (N), pinocytotic vesicles (PV), dense bodies (DB), degenerating smooth muscle cell (DS), internal elastic lamina (I), extracellular lipid (ED), and subendothelial space (SE) are indicated. Transverse-section. 1 μm is equal to 2.85 cm (line)

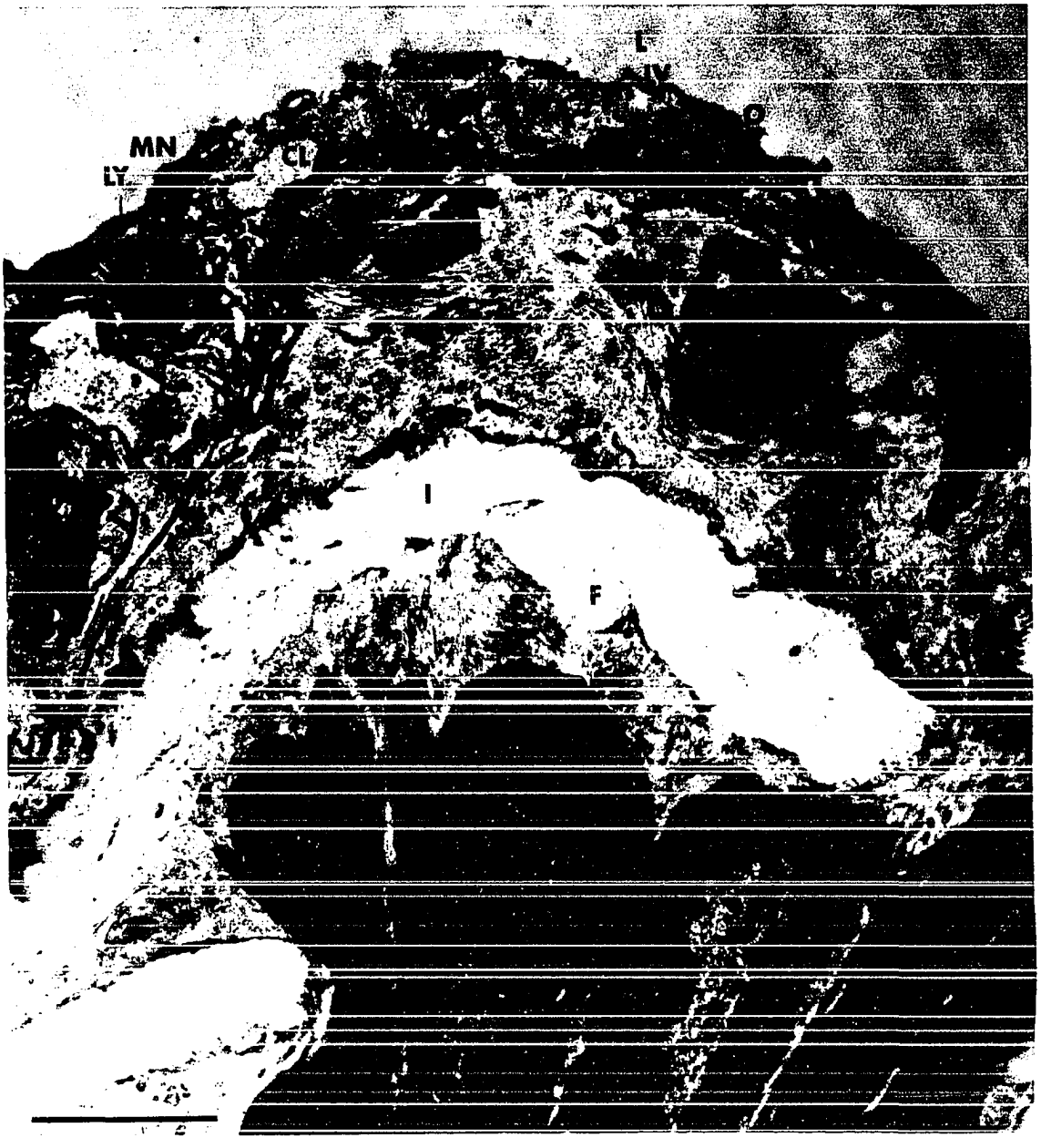

ED
I
SE

Figure 9 is an electron micrograph of a male treatment dog's femoral artery (no. 29) which responded to periodic starvation and refeeding a high carbohydrate diet with greater changes in fine structure. The endothelial layer contains many lipid-filled vesicles. Large vacuolated areas, also, appear in the cytoplasm. The cisternae of the agranular endoplasmic reticulum are laden with lipid, as are the mitochondria of both endothelial and smooth muscle cells. Granular endoplasmic reticulum is quite apparent in the endothelium. Some dense fibrillar material appears in the subendothelial space. Increased connective tissue, principally collagen fibrils, is present in all areas including a penetration of the endothelium. In the intima, there are places along the internal elastic lamina which are lined with amorphous electron-dense material resembling extracellular lipid. Some cytoplasmic lipid inclusions are found in what looks like a smooth muscle cell. Few of the cytoplasmic organelles common to smooth muscle cells are shown in these ovoid, or modified smooth muscle cells of the subendothelial space. A duplicated internal elastic lamina and fenestra are shown. A radial reorientation of smooth muscle cells appears inside the fenestra of the internal elastic lamina. This is interpreted by Buck (1961) to be a cell in the act of migration.

The fine structural features of the control dogs' femoral arteries are similar to those found in the pial vessels of the cat and monkey (Pease and Molinari, 1960), femoral artery of the mouse (Rhodin, 1962), iliac artery of the dog (Suzuki et al., 1964), and aorta of the dog (Geer, 1965). The femoral arteries of dogs periodically starved and refed a high

Figure 9. Fine structure of the femoral artery from a male dog periodically starved and refed a high carbohydrate diet

Lumen (L), duplicated internal elastic lamina (I), lipid fenestra (A), cytoplasmic vacuolations (CL), lipid-filled vesicles (LV) and vacuoles (LU), agranular endoplasmic reticulum (arrow), lipid-laden mitochondria (MN), primary lysosome (LY-arrow), extracellular lipid (ED), cytoplasmic lipid inclusions (CI), flocculent-appearing material in endoplasmic reticulum profiles (O), granular endoplasmic reticulum (ER), dense fibrillar material (DF), collagen fibrils (C), fringed buried pockets (F), radially-oriented smooth muscle cells (S), dense bodies (DB), and modified smooth muscle cell (X) are shown. Transverse-section. 1 μm is equal to 2.85 cm (line)


MN
LY
CL
I
F

carbohydrate diet, however, provided some observable changes in structure of the endothelium and subendothelial space. The membrane-bounded vesicles and vacuoles in the endothelium that are shown filled with electron-dense material (Figures 7 and 9) are thought to contain lipid. Lipid inclusions have not been found in normal vascular tissue in dogs (Geer, 1965). The bulk of lipid that accumulates in these vesicles and vacuoles is derived from the blood and enters the vessel wall through the endothelium. Transport of the blood lipids as lipoproteins from the bloodstream into the arterial wall requires passage across the endothelium (Duncan et al., 1963). The lipoprotein passes either through the cytoplasm or at an endothelial junction (Figure 6).

Small vesicular structures of 300 to 500 Å diameter are shown associated with luminal, outer, and intercellular surfaces of the 50 to 100 Å thick endothelial cell plasma membrane. There were many of these vesicles filled with lipid material (Figures 7 and 9). However, Buck (1958) demonstrated that an intravenous injection of colloidal thorium dioxide into rats, rabbits, cats, ferrets, and puppies produced within 3 hours an incorporation of colloidal particles in only the vacuoles of endothelial cells in large muscular arteries, such as the femoral. No vesicular structures in the endothelium of these thorium dioxide-treated animals contained the particles. The vacuoles are limited by a single membrane and are about the size of a mitochondria. They were termed by Buck to be inclusions and concerned with phagocytosis. Though Buck reported no findings of thorium dioxide colloidal particles in the intercellular spaces, Figure 6 shows a

lipid droplet between two endothelial cells. Figures 7-9 exhibit dense deposits throughout the intima. Perhaps, the lipids in the form of lipoproteins enter the endothelial cells through the small vesicles. The lipid-containing cisternae of the agranular endoplasmic reticulum (Figure 9) and the electron-dense mitochondria (Figures 7 and 9) indicate that the endothelium is the seat of increased metabolic activity. The homogeneous cytoplasmic lipid inclusions (Figures 7 and 9) of the smooth muscle or modified-like smooth muscle cells appeared to be the result of active lipid synthesis in the cells' agranular endoplasmic reticulum. These inclusions may have been formed by the coalescence of smaller cisternae of lipid-containing endoplasmic reticulum (Geer, 1965). In addition to the inclusions shown in the modified-like smooth muscle cell in Figure 9, electron-dense material is seen surrounding the pinocytotic vesicles associated with the plasma membranes of smooth muscle cells in the subendothelial space and fenestra of the internal elastic lamina. Phagocytosis of this dense material (extracellular lipid) by smooth muscle cells might occur. Other extracellular lipid appears in the intimal space and near the internal elastic lamina.

Though proof that the electron-dense extracellular material contains lipid is incomplete, this substance correlates well with that stainable lipid observed in sections at the light microscopy level (Geer, 1965).

Counts of elastic fibers in the subendothelial spaces were made from the micrographs of many arterial samples. No differences in fiber number and size could be shown between normal arteries and those subjected to

repeated episodes of starvation and refeeding. There is a flocculent or filamentous-appearing material in profiles of granular endoplasmic reticulum in all treatment dogs (Figures 6, 7, and 9). The filamentous material resembles the abundant fibrillar material associated with the basement membranes and reticulum. The filamentous material is, also, shown bordering the internal elastic lamina, especially near the fenestra. The very small dots along the internal elastic lamina of treatment male dog no. 29 (Figure 9) meet the diameter specifications for proelastin fibrils (100-200 Å). A tremendous quantity of these fibrils exists in comparison to all other control and treatment arteries observed. They emanate about and from the buried pockets to give dark-fringed appearances. Possibly, remodeling of the internal elastic lamina is occurring as a result of the starvation and refeeding regimens. In treatment male dog no. 29 there is a tremendous quantity of collagen fibrils in the endothelial cells, subendothelial space, and in the area of the fenestra of the internal elastic lamina. The collagen fibrils appear in the form of whorls.

These fine structural changes in the quantity of extracellular material in the arterial intima of treatment male dog no. 29 may stem from starvation, high carbohydrate feeding, or the combination of both dietary stresses. The increase in the number of collagen fibrils may not have resulted from an absolute increase in collagen synthesis but rather from a reduced production of mucopolysaccharides in starvation which would then precipitate collagen or its precursors from a relatively supersaturated solution. An increase in collagen fibrillarity has been shown in the cartilaginous matrix of female mice fasted for 2-3 days (Silberberg et al.,

1967). Collagen content has been shown to increase in the cod during spawning (actual starvation period). Lavéty and Love (1972) claim this increased collagen content to account for an increased breaking strain in the connective tissue (*myocommata*) of the cod.

It may be that disintegrating cells or cell parts resulting from starvation provided the necessary building stones for collagen synthesis. Thickened fibrils have been shown in the bays at the cell periphery of chondrocytes (Silberberg et al., 1967). This evidence seems to support the latter method of fibrillogenesis, wherein building stones were made available by disintegrating cells for collagen synthesis.

Starvation may have caused some fraying of arterial elastic fibers, and refeeding may have induced a reparative process involving a prolific synthesis of collagen. Fuller et al. (1972) reported a similar sequence of injury and reparation in senescence. Frayed elastic fibers were not shown, however, in any of the micrographs obtained in this experiment. Had arterial tissue samples been removed at starvation, one would be able to qualify any injurious effects starvation had on elastic fibers. Marked elaboration of function in the Golgi apparatus and in both agranular and granular endoplasmic reticulum are definitely shown in treatment animals, especially in treatment male dog no. 29 (Figure 9).

The arterial fine structure in treatment dogs was definitely altered, though in a varying extent to each animal. The effects which diet and dietary-influenced lipoprotein contributed to such alterations in fine structure are discussed at the end of the lipoprotein discussion.

Blood Chemistry

Statistical analyses of blood chemistry data

Data were statistically analyzed with a computer.[1] Means for each group within sex and each group (consisting of both sexes) at each regimen were computed for blood constituents. Appendix Tables B-3 through B-35 contain individual dog data, the computed means, and their statistical analysis. A standard error was calculated for each mean. An analysis of variance program was used to evaluate the data obtained from analyses of plasma sodium, potassium, glucose, urea nitrogen, lipids, and lipoprotein concentrations. F-tests at a maximal level of 1% were conducted on the results. All variables were subjected to the Student t-test (1% level) to examine the effects of treatment and sex. Correlation coefficients were computed to help explain association among variables.

Plasma sodium analysis

Analysis of the data in Appendix Table B-3 gives Control regimen mean plasma values for sodium concentration of 164.0 ± 2.8 and 164.4 ± 2.8 meq/liter in the female control and treatment dogs, respectively. Control regimen mean plasma values for sodium concentration in the male control and treatment dogs were 166.0 ± 2.8 and 167.8 ± 3.1 meq/liter, respectively. No significant difference in sodium concentration was found between females and males in the Control regimen. Control regimen mean concentrations of sodium in overall control and treatment groups were 165.0 ± 2.0 and

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[1]Computer Center, Iowa State University, Ames, Iowa.

165.9 ± 2.1 meq/liter, respectively; these means accounted for both sexes. Some established normal serum values for sodium and other blood constituents are listed in Table 8.

Table 8. Blood serum values in the beagle dog[a]

Constituent	U. of Calif., Davis[b]	U. of Rochester[b]
Sodium (meq/liter)	182.0±14.0(69)	147.3±3.1
Potassium (meq/liter)	4.7± 0.5(65)	4.7±0.3
Glucose (mg/100 ml)	99.0±22.0(931)	
Urea nitrogen (mg/100 ml)	15.6± 7.1(937)	12.2±4.0
Cholesterol (mg/100 ml)	218.0±65.0(936)	

[a]McKelvie (1970, p. 282).

[b]Values are expressed as the mean ± SD, number of samples in parentheses. Age ranges: U. of Calif. at Davis study -- 120 to 720 days (293 dogs); U of Rochester study -- 1/2 to 7 years (63 dogs). Recorded measurements include dogs of both sexes.

Significant differences existed among means of the various treatment regimens. The probability for a larger F value is 0.005 (Appendix Table B-5). The overall treatment group exhibited a mean plasma sodium concentration at Starvation I to be significantly higher ($P<0.01$) than the mean sodium value at Control regimen. Refeeding I, however, showed for all treatment dogs a decrease ($P<0.01$) in sodium values from the Control regimen. Significant increases characterized both female and male treatment groups at Starvation I with respect to Control regimen values. Starvation II sodium values showed no significant differences with those of the Control regimen. Morgulis (1928) reported no change in plasma

sodium concentration when dogs were subjected to lengthy periods of starvation. Crouch (1968) found no significant effect of repeated starvation and refeeding on plasma sodium concentrations.

Increased sodium concentration of the plasma in starvation may be due to greatly increased sodium reabsorption by the kidney. An increased plasma volume associated with a decreased blood volume exists, also, in starvation. The human kidney is active in gluconeogenesis during fasting. Unlike the human, the dog shows no ketosis during starvation. This is due to the dog's high degree of efficiency in utilizing ketone bodies. Ketone bodies are thought to stimulate renal glucose production in the dog during starvation. Lemieux and Plante (1968) reported that dogs which fasted for 12 days showed a renal conservation of sodium accompanying gluconeogenesis. It appears to be that the dog conserves sodium far more efficiently than does man. Possibly, the more lengthy time of Starvation I over II caused a need for conservation of sodium, thus, providing a significant increase in plasma sodium.

Katz et al. (1968) reported that when dietary carbohydrate was fed to men who were fasting, sodium excretion was promptly diminished. Non-carbohydrate meals, however, did not reduce excretion of sodium. The significant decrease ($P<0.01$) of mean sodium concentration in the overall treatment group during Refeeding I with respect to the mean sodium concentration of the overall treatment group during the Control regimen may have been due to a compensatory mechanism of returning electrolyte concentrations to normal. Normal plasma sodium concentrations are expected when dogs are refed a maintenance diet after fasting (Lemieux and Plante, 1968).

Plasma potassium analysis

Analysis of the data in Appendix Table B-4 provides Control regimen mean plasma values for potassium concentration of 4.5 ± 0.1 and 4.4 ± 0.1 meq/liter in female control and treatment dogs, respectively. Control regimen mean plasma potassium concentrations in the male control and treatment dogs were 4.7 ± 0.1 and 4.6 ± 0.2 meq/liter, respectively. There was no significant difference in potassium concentration with respect to sex in the Control regimen. Control regimen mean concentrations of plasma potassium in overall control and treatment groups were 4.6 ± 0.1 and 4.5 ± 0.1 meq/liter, respectively; these means accounted for both sexes. Normal serum values for potassium are shown in Table 8.

Though variation in potassium concentrations existed for the control group of dogs during treatment regimens, the values definitely fell within the normal range reported for beagle dogs. Stewart and Longwell (1969) found a normal range in beagles of 4.2 to 5.5 meq/liter. The variation in potassium concentrations may have been due in part to environmental factors such as temperature and humidity.

The experiment has shown evidence of real differences among means of treatment regimens. The probability of a larger F value is 0.005 (Appendix Table B-6). Significant differences existed among group means. The probability of a larger F value in this case is 0.05. A group and treatment regimen interaction existed with highly significant differences among its means. Such a probability for a larger F value here is 0.01.

The treatment group, consisting of both sexes, showed mean plasma potassium concentrations to be significantly decreased ($P<0.01$) at

Refeedings I and II from the overall treatment group mean at the Control regimen. The overall treatment group exhibited significantly less ($P<0.01$) plasma potassium concentration at Refeeding II with respect to the overall control group potassium concentration at same regimen. Potassium concentrations in treatment dogs were not any lower than those in control dogs in Starvations I and II. Morgulis (1928) reported lower serum potassium concentrations for fasted dogs. Lemieux and Plante (1968) found that within 48 hours after the onset of starvation in the dog potassium excretion fell rapidly and continued to fall throughout the remainder of the 12 days of starvation. As was indicated for sodium, renal conservation of potassium appeared to be more efficient in the dog than in man. This conservation mechanism possibly is an explanation for near normal values in the treatment dogs. It was, also, shown by Lemieux and Plante that potassium excretion was greatly increased during refeeding of a control (maintenance) diet. Therefore, lower plasma potassium concentrations resulted.

This increased excretion of potassium during refeeding could explain the significantly lower potassium values shown in treatment dogs during refeeding regimens in this experiment.

Plasma glucose analysis

Appendix Table B-7 gives the plasma glucose data for control and treatment dogs. Control regimen mean plasma values for glucose were 112.0 ± 3.1 and 109.4 ± 3.1 mg/100 ml in female control and treatment dogs,

respectively. Control regimen mean plasma values for glucose concentration in male control and treatment dogs were 111.8 ± 3.1 and 110.2 ± 3.5 mg/100 ml, respectively. There was no significant difference in glucose concentration with respect to sex in the Control regimen. A Control regimen mean concentration of plasma glucose for all control dogs was 111.9 ± 2.2 mg/100 ml. All treatment dogs showed at this same regimen a mean plasma glucose concentration of 109.8 ± 2.3 mg/100 ml. Normal serum glucose values are shown in Table 8.

Significant differences existed among means of the various treatment regimens. The probability for a larger F value is 0.005 (Appendix Table B-9). Mean plasma glucose concentrations of the overall control group at Refeeding I and Starvation II were significantly lower ($P<0.01$) than the mean plasma glucose concentration of the Control regimen for all control dogs. The mean plasma glucose concentration of the overall treatment group at Starvation II showed a significant decrease ($P<0.01$) from that of the Control regimen mean plasma glucose concentration. Mean plasma glucose concentration in the female control group showed a significant decrease ($P<0.01$) in Refeeding I and Starvation II. Such variation in plasma glucose concentrations which existed in the control dogs may be attributed to environmental influences. Nonetheless, values were in the normal range.

Both female and male treatment groups showed at Starvation II significant decreases ($P<0.01$) with respect to their Control regimens.

Lemieux and Plante (1968) reported that blood glucose showed no sig-

nificant changes in dogs starved for 12 days and then refed a maintenance diet. Control, fasting, and refeeding blood glucose concentrations were 80, 86, and 87 mg/100 ml, respectively. Unchanged-to-slight decreases in blood glucose levels in the dog were reported by Morgulis and Edwards (1924). The lack of significant decreases reported in most studies is possibly due to short periods of starvation (3-12 days) rather than longer ones such as exist in this experiment (17-30 days).

Crandall et al. (1940) reported that as early as 3 days fasting, there was a 50% reduction in the amount of glucose liberated from the liver. After 5 to 11 days fasting, the liver was adding 1.5 to 8.1 mg of ketone bodies to each 100 ml of blood. Oral administration of glucose to these dogs promptly reduced ketogenesis in the liver.

Blood plasma urea nitrogen analysis

Analysis of the data in Appendix Table B-8 gives blood plasma urea nitrogen concentrations for control and treatment dogs. The mean urea nitrogen values in the Control regimen were 12.8 $\pm$ 1.9 and 13.4 $\pm$ 1.9 mg/100 ml in female control and treatment dogs, respectively. Control regimen mean blood plasma urea nitrogen concentrations in male control and treatment dogs were 12.3 $\pm$ 1.9 and 13.2 $\pm$ 2.1 mg/100 ml, respectively. There was no significant difference in urea nitrogen concentration with respect to sex in the Control regimen. A Control regimen mean concentration of blood plasma urea nitrogen was 12.6 $\pm$ 1.3 and 13.3 $\pm$ 1.4 mg/100 ml for overall control and overall treatment dogs, respectively; both sexes are accounted for in these means. Normal serum urea nitrogen values are shown in Table 8.

The experiment gave evidence of real differences among means of groups, treatment regimens, and groups-treatment regimen interaction. The probability of a larger F value here is 0.005 (Appendix Table B-10). The overall control group blood plasma urea nitrogen concentrations varied little throughout the experiment and were within normal physiological limits. Female and male treatment dogs both showed highly significant increases ($P<0.01$) in urea nitrogen concentration at Starvations I and II with respect to the mean values of their respective control groups and Control regimens. Composite means representative of both sexes are shown for each group and treatment regimen in Figure 10.

The changes in blood plasma urea nitrogen concentration in Starvations I and II indicated a change in living tissue. With an absence of dietary carbohydrate, glucose must be produced. Synthesis of glucose is generally at the expense of proteins, thus, producing increased levels of nitrogen in the blood. The magnitude and direction of the change in urea nitrogen concentration during partial and complete fasting have been reported to be variable. Sunderman (1947) reported increased blood urea nitrogen in man fasted for 45 days.

It would be expected during starvation that a change of concentration in one plasma constituent would be associated with a change in other constituents. Excretion of ketoacids and nitrogen in the starving subject is associated with decreased plasma glucose and insulin. Such excretion products accumulate in the blood and tend to produce metabolic acidosis. Increased potassium concentration is evident in acidosis. The dog, however,

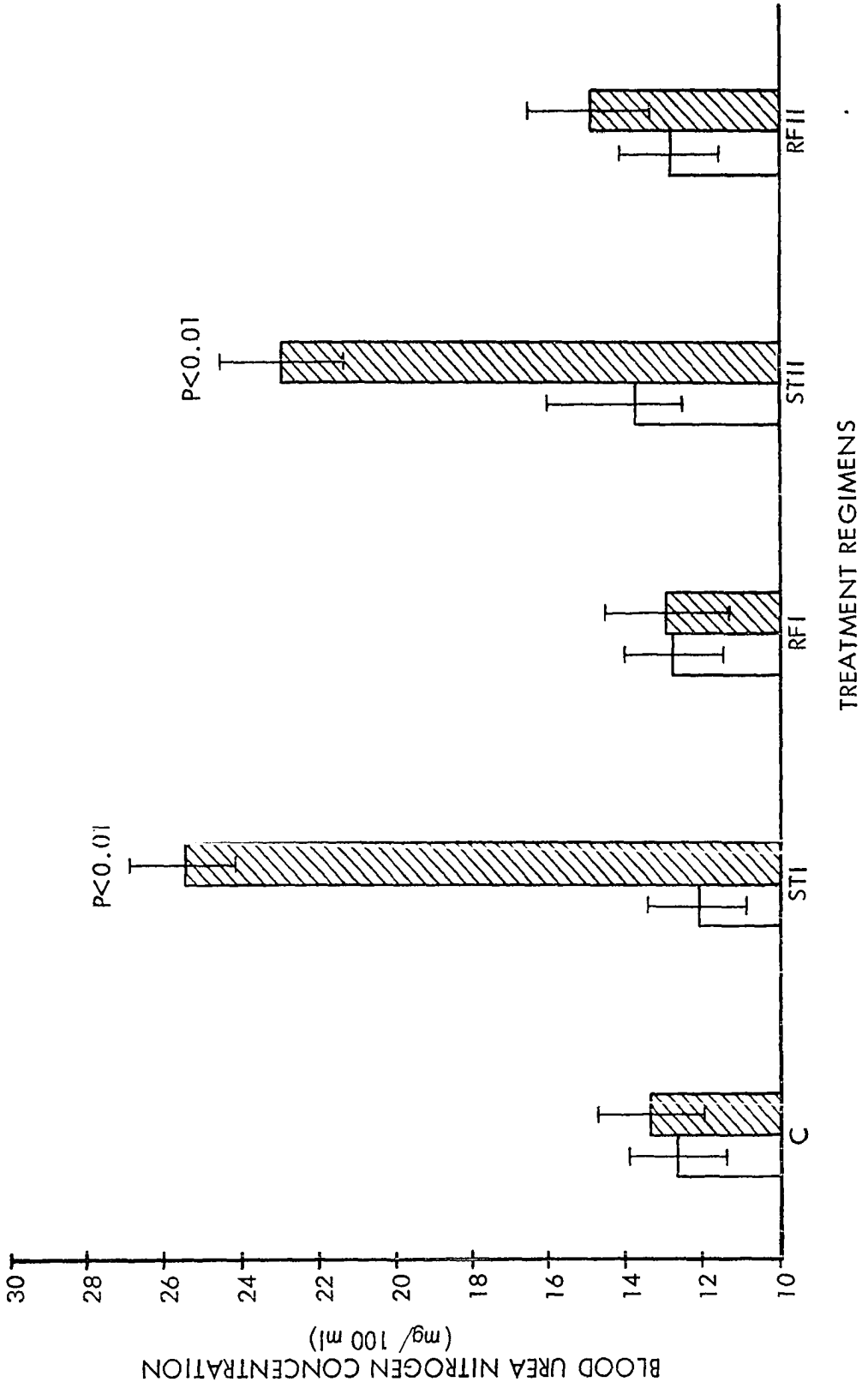
Figure 10. Mean blood urea nitrogen concentrations in control dogs maintained on a commercial diet and in treatment dogs periodically starved and refed a high carbohydrate diet

Values represent mean plasma concentrations at the end of each regimen. SEM are indicated.

Control Regimen	- C	Day 0
Starvation I	- STI	Days 1-30
Refeeding I	- RFI	Days 31-50
Starvation II	- STII	Days 51-67
Refeeding II	- RFII	Days 68-98

Control group 

Treatment group 


BLOOD UREA NITROGEN CONCENTRATION
(mg/ 100 ml)
30
28
26
24
22
20
18
16
14
12
10
C
STI
RFI
STII
RFII
P<0.01
P<0.01
TREATMENT REGIMENS

is thought to have resistance to the development of ketosis in starvation (Morgulis and Edwards, 1924). Sodium is conserved by the kidney during starvation. Some correlation coefficients for these constituents are shown in Appendix Table B-11.

Whole plasma cholesterol analysis

Appendix Table B-12 gives the data for whole (total) plasma concentrations of cholesterol. Mean whole plasma cholesterol concentrations in the Control regimen were 164.8 ± 8.5 and 203.5 ± 9.5 mg/100 ml in female control and treatment dogs, respectively. Mean whole plasma cholesterol concentrations for male control and treatment dogs in the Control regimen were 162.9 ± 9.5 and 133.9 ± 10.6 mg/100 ml, respectively. A significant difference in plasma cholesterol concentration was found between females and males in the Control regimen. The probability of a larger F value is 0.005 (Appendix Table B-14). However, mean values for plasma cholesterol in the control groups did not show significant sex differences at any treatment regimen. The control groups exhibited in the Control regimen mean cholesterol concentrations of 164.8 ± 9.5 mg/100 ml for females, 162.9 ± 9.5 mg/100 ml for males, and 163.8 ± 6.7 mg/100 ml for a composite of both sexes. There was no significant difference between overall control and overall treatment groups in Control regimen. It is evident that the treatment group contributed the significant difference between sexes at Control regimen. These cholesterol concentrations were well below the value listed in Table 8 and values reported by Stewart and Longwell (1969).

Significant differences existed among means of the various treatment regimens. The probability for a larger F value here, however, is only 0.05. The mean effects from interaction of sex, group, and treatment regimen were shown to be significantly different. The probability for a larger F value is 0.005. Although starvation was the only treatment that showed apparent differences in mean cholesterol concentration between the overall treatment group and overall control group, the differences were not significant. Sex differences were shown in treatment dogs by an inverse relationship of mean whole plasma cholesterol concentrations. Female treatment dogs showed a significant decrease ($P<0.01$) in plasma cholesterol concentration from the Control regimen to Starvation I. A near Control regimen value was evidenced at Refeeding I and significant decreases ($P<0.01$) occurred at Starvation II and Refeeding II. Although not significant, differences in mean plasma cholesterol concentration were shown to exist between female control and treatment groups at Refeedings I and II and Starvation II. Male dogs showed a highly significant increase ($P<0.01$) in mean whole plasma cholesterol concentration at Starvation I. Differences in cholesterol concentration due to sex seemed to average out and give mean values for the overall treatment group that were not different from one another, except for the value in Refeeding II. Means for whole (total) plasma cholesterol concentrations of both sexes and groups are shown for each treatment regimen in Figure 11.

The behavior of cholesterol concentration during complete and partial starvation has been reported in the literature to be decreased, unchanged,

Figure 11. Mean total plasma cholesterol concentrations in control dogs maintained on a commercial diet and in treatment dogs periodically starved and refed a high carbohydrate diet

Values are representative of mean plasma concentrations at the end of each regimen. SEM are indicated.

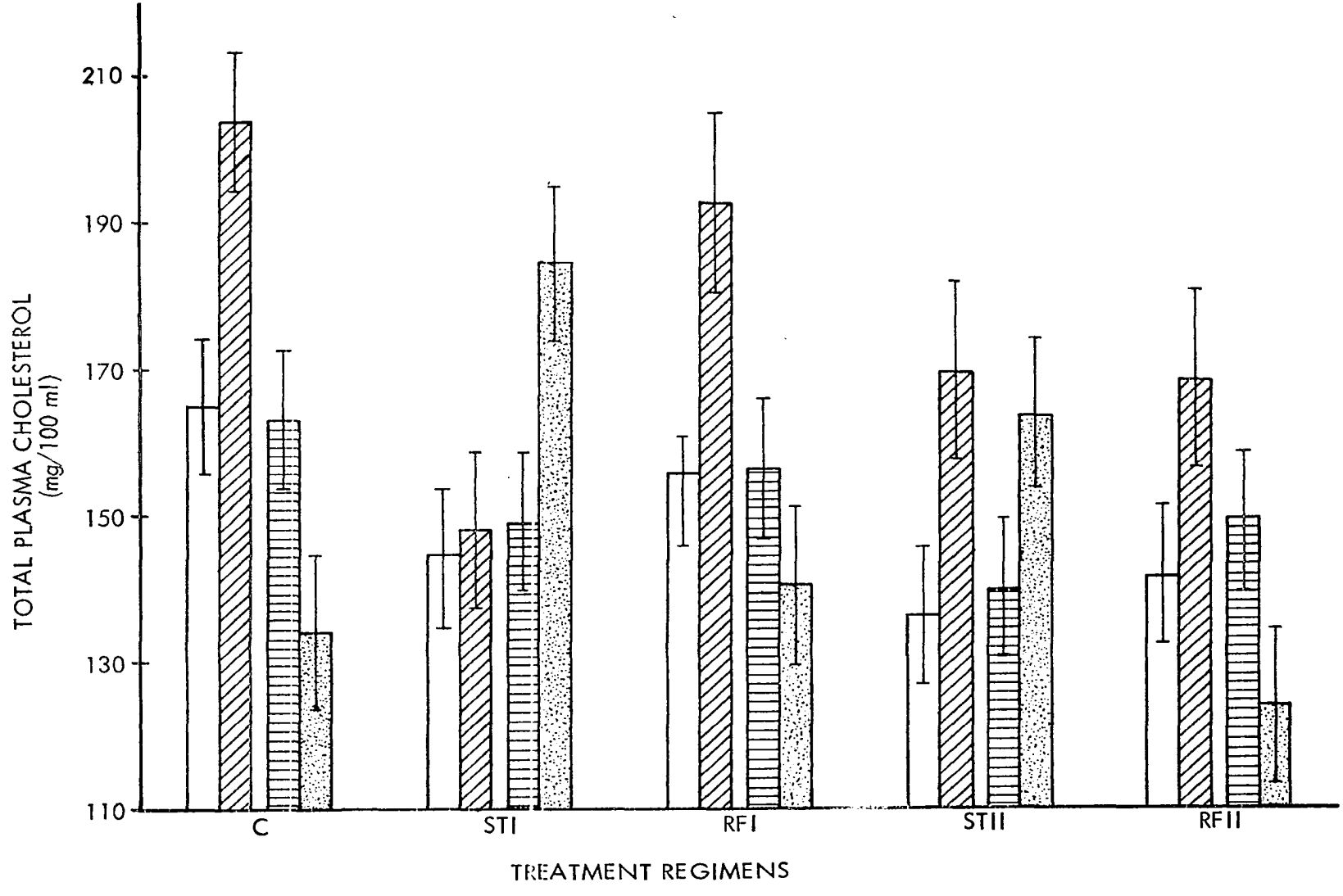
Control Regimen	- C	Day 0
Starvation I	- STI	Days 1-30
Refeeding I	- RFI	Days 31-50
Starvation II	- STII	Days 51-67
Refeeding II	- RFII	Days 68-98

Control females 

Treatment females 

Control males 

Treatment males 


TOTAL PLASMA CHOLESTEROL
(mg/100 ml)
210
190
170
150
130
110
C
STI
RFI
STII
RFII
TREATMENT REGIMENS

or increased (Keys et al., 1950). Keys concluded that the preponderance of evidence indicates that the concentration of cholesterol in the blood decreases during partial starvation and increases during complete starvation.

Cholesterol concentrations of the whole (total) plasma and LDL fraction can be raised by mental stress (Searcy and Berquist, 1962). Stress causes a stimulation of the sympathetic nervous system which results in an increased synthesis and release of epinephrine from the medulla of the adrenal gland. Epinephrine causes the release and increase of unesterified fatty acids. Epinephrine can maintain elevated cholesterol concentrations for some time (Shafrir et al., 1959). Under stress conditions adrenocorticotropic hormone (ACTH) is secreted from the pituitary to act upon the adrenal gland where adrenocorticosteroids are released. The secretory capacity of the rat's adrenal cortex and its response to stress are greater in the female than in the male (D'Angelo, 1968; Baker, 1952). Estradiol has been shown to enhance pituitary ACTH secretion and steroid clearance, whereas, testosterone depresses both (D'Angelo, 1968). Since the effects of starvation and adrenal hypercorticism on various tissues and organs have been shown to be similar in many ways (Baker, 1952), starvation may act as a stress agent and cause some of these phenomena to occur. Starvation may act upon the body to cause the release of ACTH which in turn acts upon the adrenal gland where an elaboration of cholesterol into androgens and estrogens takes place. Thus, after the processes of elaboration, the circulating cholesterol concentration is lowered

(Searcy and Berquist, 1962). The newly synthesized female estrogens (estradiol) could have a feedback effect upon the pituitary to further reduce the cholesterol concentration. Testosterone, however, would allow cholesterol concentrations to rise.

The refeeding of a high carbohydrate diet rich in sucrose brought plasma cholesterol concentrations in male treatment dogs to the near Control regimen value. The supply of free fatty acids from adipose tissue is low in starvation (Searcy and Berquist, 1962). However, concomitant to refeeding, hepatic lipogenesis attempts to adequately maintain levels of esterified fatty acids. Plasma cholesterol concentrations returned to normal for females as well. Possibly, with females, the ingestion of such a diet high in sucrose depressed the normally rapid conversion of cholesterol to bile acids (Searcy and Berquist, 1962). Inhibition of fatty acid mobilization may have been a part of the female response to refeeding.

Whole plasma triglyceride analysis

Analysis of the data in Appendix Table B-13 gives mean whole (total) plasma triglyceride concentrations for the Control regimen of 59.5 $\pm$ 3.2 and 55.7 $\pm$ 3.2 mg/100 ml for female control and treatment dogs, respectively. Mean whole plasma triglyceride concentrations for male control and treatment dogs at Control regimen were 66.8 $\pm$ 3.2 and 60.6 $\pm$ 3.6 mg/100 ml, respectively. No significant differences existed for triglyceride concentrations because of sex. Significant differences existed among means of control and treatment groups. Significant differ-

ences existed among means of treatment regimens and, also, among mean effects from the interaction of groups and treatment regimens. For these differences, the probability for a larger F value is 0.005 (Appendix Table B-15). The overall control group (consisting of both sexes) showed at Starvations I and II and Refeeding II significant decreases ($P<0.01$) in mean triglyceride concentration from the Control regimen. Such variation among mean values in control dogs may have been due to environmental factors. The overall treatment group showed at Refeedings I and II mean whole plasma triglyceride concentrations to be increased significantly ($P<0.01$) over the Control regimen value. Mean whole plasma triglyceride concentrations of the overall treatment group were significantly higher ($P<0.01$) at Starvation I and Refeedings I and II than in respective regimens of the overall control group. Means for whole (total) plasma triglyceride concentrations of both sexes and groups are shown for each of the treatment regimens in Figure 12.

Lemieux and Plante (1968) reported that in dogs fasted for 12 days there were no significant changes in whole plasma triglyceride concentrations when compared to triglyceride values obtained from previous and subsequent periods of feeding on a maintenance diet. Seventy-two hours or more of fasting in the human caused serum unesterified fatty acids (UFA) to triple in concentration, whereas, triglyceride concentrations remained relatively constant (Searcy and Berquist, 1962). The mean whole plasma triglyceride concentrations in treatment dogs of this experiment showed no significant changes at starvation regimens with respect to their Control regimen value.

Figure 12. Mean total plasma triglyceride concentrations in control dogs maintained on a commercial diet and in dogs periodically starved and refed a high carbohydrate diet

Values are representative of mean plasma concentrations at the end of each regimen. SEM are indicated.

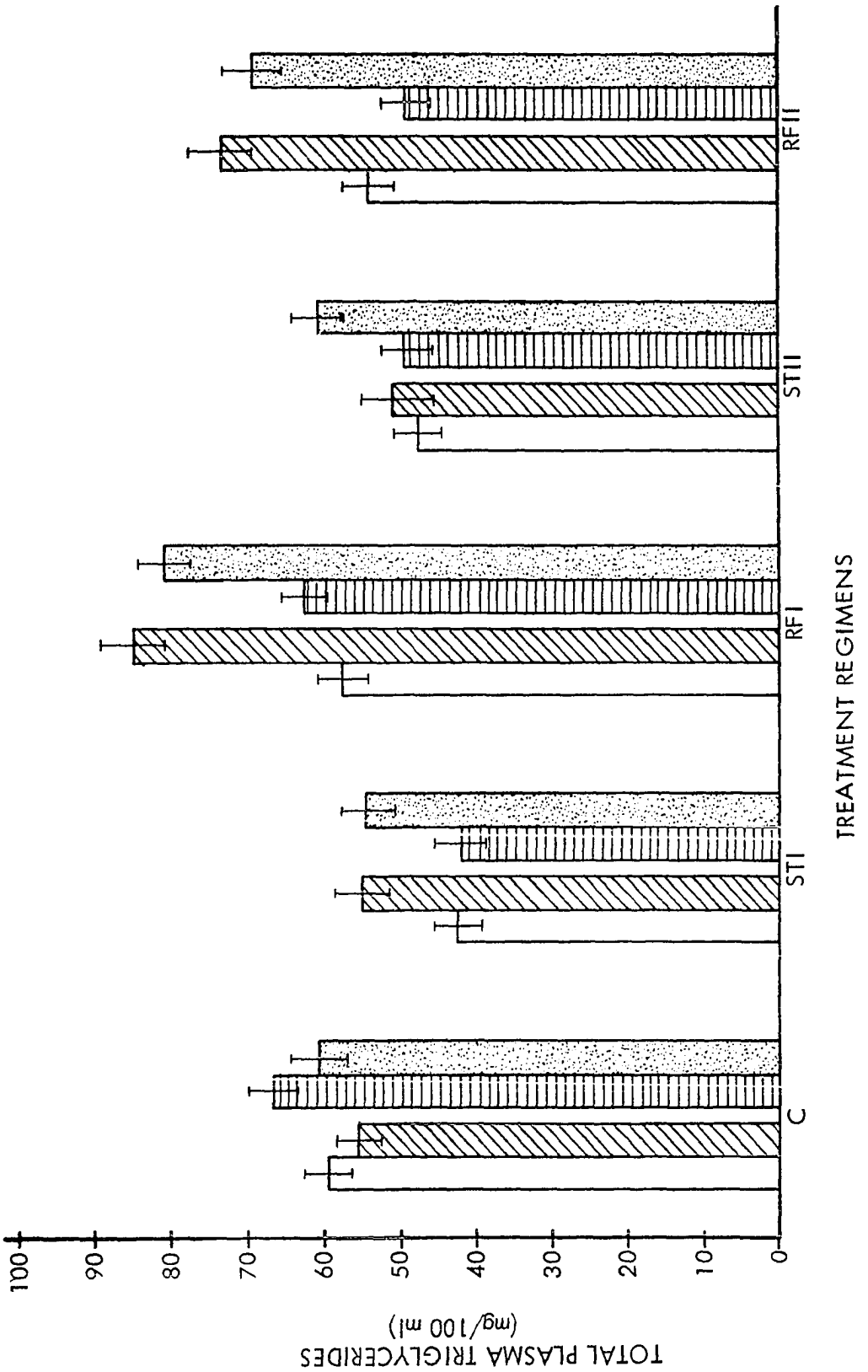
Control Regimen	- C	Day 0
Starvation I	- STI	Days 1-30
Refeeding I	- RFI	Days 31-50
Starvation II	- STII	Days 51-67
Refeeding II	- RFII	Days 68-98

Control females

Treatment females

Control males

Treatment males


TOTAL PLASMA TRIGLYCERIDES
(mg/100 ml)
0
10
20
30
40
50
60
70
80
90
100
C
STI
RFI
STII
RFII
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Plasma triglycerides are elevated when a high carbohydrate diet is fed to man (Levy et al., 1966; Schonfeld, 1970). Sucrose or fructose may be more potent than other carbohydrates in raising triglyceride concentrations in the plasma (Anderson, 1967). This was the response of the treatment dogs in this experiment when they were refed a high carbohydrate diet (high sucrose content) after prolonged starvation. Reaven et al. (1967) claimed the degree to which a high carbohydrate diet stimulates hepatic triglyceride secretion is directly related to the insulin response produced by that diet. Reaven and his associates hypothesized that insulin secretion could be stimulated to maintain cellular glucose uptake. The resulting hyperinsulinemia and hyperlipoproteinemia (hypertriglyceridemia) are the price to be paid to maintain a normal transcellular transport and utilization of glucose.

Determination of cholesterol and triglyceride concentrations in plasma lipoprotein fractions of the dog

The mean concentrations of plasma cholesterol and triglycerides for all dogs have been aforementioned. These values were obtained using the Technicon AutoAnalyzer. However, micromethods (Mann, 1961; Fletcher, 1968) were more suitable for lipid determinations of lipoprotein fractions because of the small number of samples and rapidity with which they should be analyzed. It was necessary to obtain values for whole (total) plasma cholesterol and triglycerides so that percentage recovery and composition of fractions could be evaluated correctly. Therefore, a brief presentation of the whole plasma cholesterol and triglyceride data obtained by

micromethods is necessary. The mean concentrations of cholesterol and triglycerides of three selected female and three selected male dogs are shown for each of the treatment regimens in Table 9 and Figures 13 and 14. These means resulted from the analysis of the data in Appendix Table B-16. The differences in concentration that existed among values between the two methods may be due to the different sensitivity of the two methods to lipid concentrations and effect of dietary changes on lipid concentrations. Reproducibility in analyzed plasma samples for each method was good.

The Control regimen mean plasma cholesterol concentrations were 188.5 $\pm$ 15.5 mg/100 ml for female dogs and 131.7 $\pm$ 15.5 mg/100 ml for male dogs. Each dog served as its own control. No significant difference in cholesterol values was shown for the sexes. Individual cholesterol values in females were similar in many instances for the micromethod and Technicon AutoAnalyzer (automated) method. The value at Refeeding I was different from the automated value because of the earlier sampling of plasma for the micromethod. Three days into Refeeding I were estimated to demonstrate the peak of stress in refeeding. For males, only Control and Starvation I regimens exhibited similarity in values between the two methods. Significant differences existed among mean effects of the sex and treatment regimen interaction. The probability of a larger F value is 0.005 (Appendix Table B-17). Plasma mean cholesterol concentrations for females were significantly lower ($P<0.01$) at Starvation I and Refeeding I than at the Control regimen. Males showed a significantly greater ($P<0.01$) cholesterol concentration at Starvation I than did females.

Table 9. Cholesterol and triglyceride concentrations of plasma and plasma lipoproteins[a] in female and male dogs

Treatment Regimen	Plasma		Lipoprotein Fractions							
			VLDL[b]		LDL		HDL		VHDL	
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
					Cholesterol (mg/100 ml)					
Control	188.5	131.7	0.0	1.6	22.1	29.3	162.2	91.8	0.0	0.7
Starvation I	106.2	188.6	1.6	2.2	10.6	48.0	85.4	133.6	1.0	1.3
Refeeding I	107.6	161.1	1.9	2.0	19.0	41.4	80.0	107.6	3.3	0.0
Refeeding II	152.0	100.7	0.6	0.4	29.2	15.6	99.4	79.7	1.5	0.6
					Triglycerides (mg/100 ml)					
Control	36.0	29.6	6.7	7.4	14.3	9.8	8.1	4.4	3.7	4.1
Starvation I	55.3	72.0	6.1	6.8	8.2	18.9	21.5	27.0	17.2	16.3
Refeeding I	86.2	94.3	19.4	10.6	25.4	36.8	19.6	20.8	18.3	18.4
Refeeding II	79.5	72.3	10.0	11.8	25.9	25.1	18.4	16.7	15.4	15.1

[a]Each value is an average of those cholesterol and triglyceride concentrations from three dogs of each sex, except in females at Refeeding II (two dogs). SEM are shown in Appendix Tables B-16, B-19, B-22, B-25, and B-28. The value for each fraction represents the concentration of cholesterol and triglycerides contributed to the total plasma cholesterol and triglyceride concentrations.

[b]Includes both chylomicra and VLDL fraction.

Figure 13. Micromethod determined total plasma cholesterol concentrations in dogs periodically starved and refed a high carbohydrate diet

Each dog served as its own control. Values are representative of mean plasma concentrations at the end of each regimen, except Refeeding I. Values for Refeeding I represent mean total cholesterol concentrations at 3 days refeeding. SEM are indicated.

Control Regimen	- C	Day 0
Starvation I	- STI	Days 1-30
Refeeding I	- RFI	Days 31-50
Refeeding II	- RFII	Days 68-90

Females 

Males 

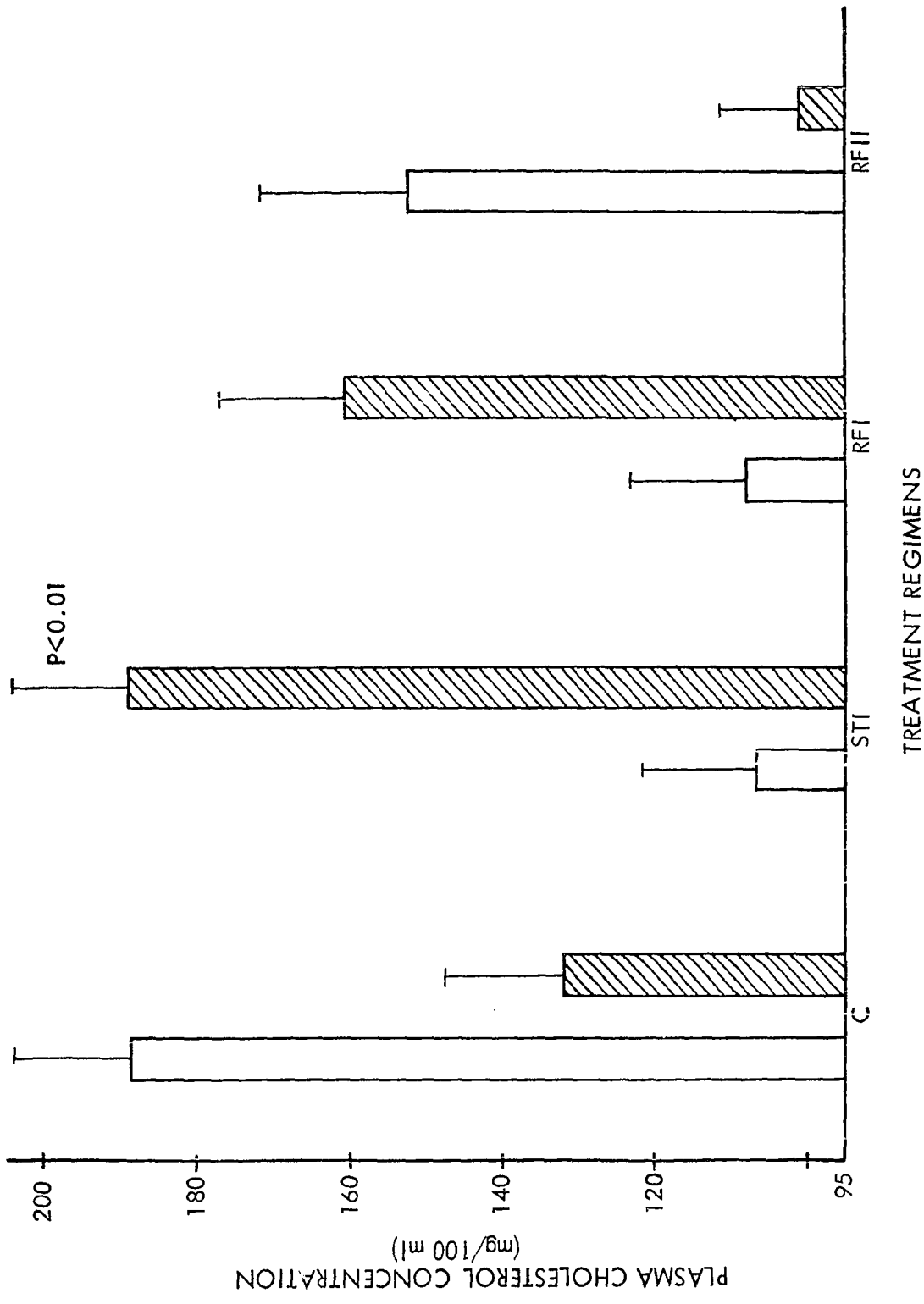

PLASMA CHOLESTEROL CONCENTRATION
(mg/100 ml)
200
180
160
140
120
95
P<0.01
C
STI
RFI
RFII
TREATMENT REGIMENS

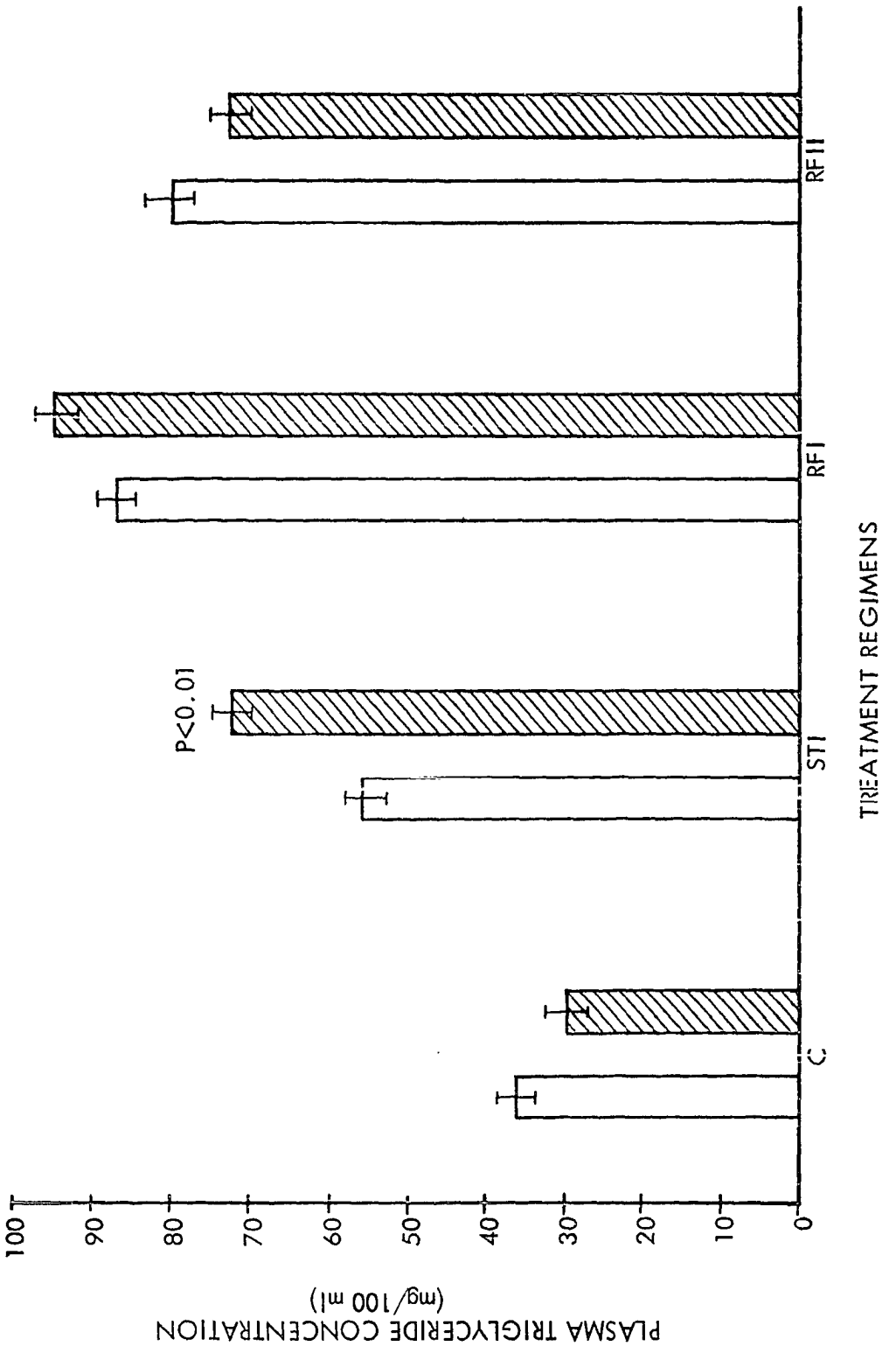
Figure 14. Micromethod determined total plasma triglyceride concentrations in dogs periodically starved and refed a high carbohydrate diet

Each dog served as its own control. Values are representative of mean plasma concentrations at the end of each regimen, except Refeeding I. Values for Refeeding I represent mean total triglyceride concentrations at 3 days refeeding. SEM are indicated.

Control Regimen	- C	Day 0
Starvation I	- STI	Days 1-30
Refeeding I	- RFI	Days 31-50
Refeeding II	- RFII	Days 68-98

Females ☐

Males ▨


PLASMA TRIGLYCERIDE CONCENTRATION
(mg/100 ml)
100
90
80
70
60
50
40
30
20
10
0
C
STI
RFI
RFII
P<0.01
TREATMENT REGIMENS

The females did not show any restoration of cholesterol concentrations at 3 days into refeeding. Males responded with a decrease in plasma cholesterol concentration, just as was demonstrated by automated data of all treatment males.

The Control regimen mean plasma triglyceride concentrations were 36.0 ± 2.6 mg/100 ml for female dogs and 29.6 ± 2.6 mg/100 ml for male dogs. No significant difference in triglyceride values was shown between sexes. Mean plasma triglyceride concentrations in females at Starvation I and both refeedings were similar when measured by either the micromethod or automated method. Males only showed similarity of triglyceride values at Refeeding II. Significant differences existed among means of treatment regimens, and the sex and treatment interaction. The probability for a larger F value is 0.005 (Appendix Table B-18). Plasma mean triglyceride concentrations for both female and male dogs were significantly greater ($P<0.01$) at Starvation I and both refeedings than at their respective Control regimens. Male dogs, also, showed a significantly greater ($P<0.01$) plasma triglyceride concentration at Starvation I than did female dogs. The reasons for such overall triglyceride responses in these six dogs to the different treatment regimens are the same as those mentioned for all treatment dogs in the previous section on whole plasma triglyceride analysis.

Cholesterol concentration of the lipoprotein fractions The concentrations of cholesterol in the individual lipoprotein fractions were determined to reveal the contribution that individual fractions were making to the total plasma cholesterol in dogs periodically starved and refed a high

carbohydrate diet. Plasma lipoprotein cholesterol concentrations for each fraction are shown in Appendix Tables B-19, B-22, B-25, and B-28. Mean fraction cholesterol concentrations for each sex at each treatment regimen are shown in Table 9. A graphic illustration of the data in this table is shown in Figure 15. When the sum of cholesterols from all fractions was compared to the total plasma cholesterol concentration, the percentage recoveries (±SEM) for the fractionation method for the different treatment regimens were 95.7 ± 3.5 for Control, 94.9 ± 5.1 for Starvation I, 95.4 ± 4.0 for Refeeding I, and 91.6 ± 6.4 for Refeeding II.

Starvation and the refeeding of a high carbohydrate diet produced significant effects upon the cholesterol concentrations of different plasma lipoprotein fractions. The effects produced by these dietary stresses were modified by sex. The Student t-test was performed only whenever significance ($P<0.01$) was shown by the F-test to exist among mean effects of treatment regimen, sex, and any interaction of these two (Appendix Tables B-20, B-23, B-26, and B-29).

The concentration of the chylomicra and VLDL fraction cholesterol was minute when compared to other plasma lipoprotein fractions in the dog. Though meager in amount, cholesterol concentrations of this fraction in all dogs were significantly increased ($P<0.01$) by Starvation I and Refeeding I. A Control regimen mean cholesterol for all dogs was 0.8 ± 0.2 mg/100 ml (Appendix Table B-19). This was increased over two-fold (1.9 ± 0.2 mg/100 ml) in both Starvation I and Refeeding I. The mean chylomicra and VLDL cholesterol concentrations for both sexes at each treatment regimen are shown in Table 9 and Figure 15-A.

Figure 15. Mean cholesterol concentrations of the plasma lipoprotein fractions in dogs periodically starved and refed a high carbohydrate diet

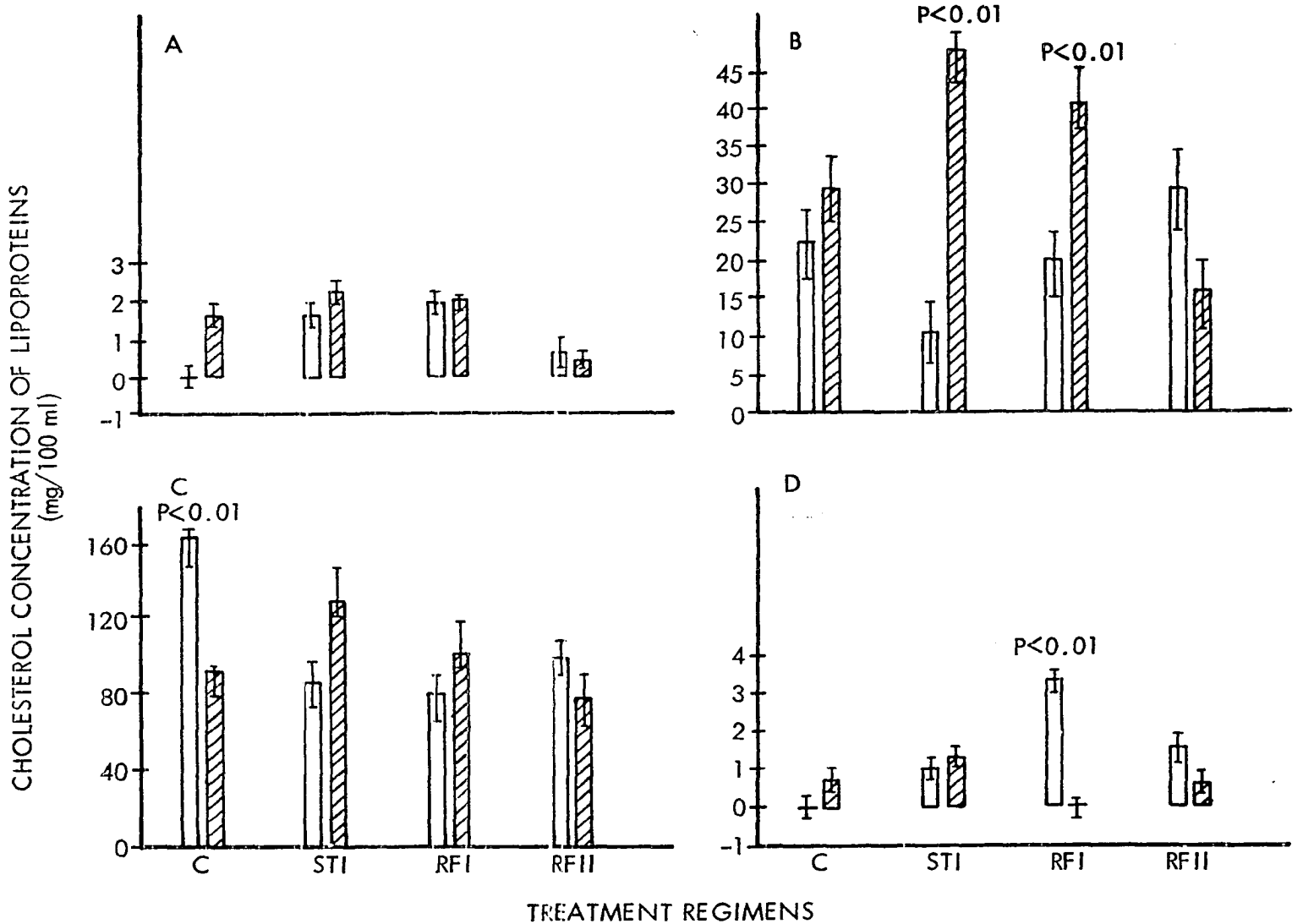
Values are representative of mean concentrations at the end of each regimen, except in Refeeding I. Values for Refeeding I represent mean cholesterol concentrations at 3 days refeeding. SEM are indicated.

A) Mean cholesterol concentration in the chylomicra and VLDL fraction.
B) Mean cholesterol concentration in the LDL fraction.
C) Mean cholesterol concentration in the HDL fraction.
D) Mean cholesterol concentration in the VHDL fraction.

Control Regimen	- C	Day 0
Starvation I	- STI	Days 1-30
Refeeding I	- RFI	Days 31-50
Refeeding II	- RFII	Days 68-98

Females 

Males 


A
B
C
D
P<0.01
P<0.01
P<0.01
P<0.01
CHOLESTEROL CONCENTRATION OF LIPOPROTEINS
(mg/100 ml)
TREATMENT REGIMENS
C
STI
RFI
RFII

Morris et al. (1972) reported that an increase in VLDL cholesterol was produced in ponies fasted for 8 days. Feeding an 80% carbohydrate and 20% protein diet to 4 women and 13 men resulted in 1.2- to 5.7-fold increase of VLDL cholesterol (Schonfeld, 1970). The women showed a lesser increase in VLDL cholesterol than did the men. No chylomicra were present in the plasma of these individuals.

Though agreement exists between the results of these authors and this experiment's significant increases in VLDL cholesterol concentration during Starvation I and Refeeding I, it would be difficult to conclude much on the basis of the extremely small amounts of VLDL cholesterol existing in the dogs' plasma.

The mean cholesterol concentration in the LDL fraction during the treatment regimens is shown for both sexes in Table 9 and Figure 15-B. During Starvation I, mean cholesterol concentration in LDL of male dogs responded differently from female dogs. Females showed a 50% decrease in cholesterol concentration at Starvation I (10.6 mg/100 ml) from the Control regimen value (22.1 mg/100 ml). Males, however, exhibited a 65% increase of LDL cholesterol concentration over the Control regimen, 29.3 to 48.0 mg/100 ml. Upon refeeding, the mean cholesterol concentration of females returned nearly to a Control regimen value, whereas, the males continued to show a higher than Control regimen value (41.4 mg/100 ml). Refeeding II values were 29.2 mg/100 ml for females and 15.6 mg/100 ml for males. None of these increases or decreases were significant. Male mean plasma LDL cholesterol concentrations were significantly greater ($P<0.01$)

than those of females at Starvation I and Refeeding I.

Concentrations of S_f 0-12 (LDL), as well as S_f 12-400 (VLDL) lipoproteins have been shown to increase significantly in humans fasted for 96 hours (Havel, 1957; Rubin and Aladjem, 1954). Mental stress results in plasma LDL increases in the human (Searcy and Berquist, 1962). This fraction is the major cholesterol-bearing lipoprotein of the human. If ACTH or corticosteroids are administered to humans, there are prompt reductions in serum total and LDL cholesterol. HDL cholesterol was found to increase, however (Searcy and Berquist, 1962). Since the analogous cholesterol-bearing lipoprotein in dog plasma is the HDL fraction, it would be reasonable to produce a similar reaction but in reversed order, i.e., ACTH or corticosteroids reduce HDL cholesterol but increase LDL cholesterol. Such a change in the HDL fraction cholesterol concentration to LDL cholesterol concentration did not occur with the dogs utilized in this starvation and refeeding experiment.

The major LDL fraction (S_f 0-10 lipoproteins) shows a slight fall in the mean value of cholesterol in man after ingestion of a carbohydrate diet (Havel, 1957). Decreases in LDL cholesterol concentration were shown in this experiment in only male dogs after Refeeding I. The manner in which sex hormones contributed to the significant differences shown between cholesterol concentrations of males and females during Starvation I and Refeeding I is alluded to more fully under the discussion of whole plasma cholesterol analysis. The cholesterol concentrations of the LDL fraction in male and female treatment dogs responded similarly as they

each did in the whole plasma.

The cholesterol concentration of the HDL fraction was shown in the females to be significantly lower ($P<0.01$) in Starvation I (85.4 $\pm$ 12.7 mg/100 ml) and Refeeding I (80.0 $\pm$ 12.7 mg/100 ml) than in the Control regimen (162.2 $\pm$ 12.7 mg/100 ml); this is approximately a 50% decrease. Refeeding II for females showed slightly higher cholesterol concentrations than observed in the previous two treatment regimens. Mean cholesterol concentration of plasma in females was significantly greater ($P<0.01$) at the Control regimen (162.2 $\pm$ 12.7 mg/100 ml) than the male mean cholesterol concentration at the same regimen (91.8 $\pm$ 12.7 mg/100 ml). Cholesterol concentrations for the HDL fraction are shown in Table 9 and Figure 15-C.

Rubin and Aladjem (1954) reported that the concentration of high density serum lipoproteins in six humans was not significantly altered during a period of 4 to 5 days fasting. This was basically true of the male dogs of this experiment. Though the males did increase their HDL cholesterol during starvation, it was nonsignificant. Male and female dogs reacted to starvation stress differently. Males exhibited an increase in HDL cholesterol, whereas, the females showed a significant decrease ($P<0.01$). Since the major cholesterol-bearing fraction in the dog is HDL, the explanation for such different responses in cholesterol concentration of both sexes probably is similar to that discussion given in the section on whole plasma cholesterol analysis.

Refeeding of a high carbohydrate diet did not significantly affect the HDL cholesterol content in plasma of male dogs. Barclay (1972) claimed the same response in normal human subjects fed a similar diet high in

carbohydrate. The significant decrease ($P<0.01$) shown in female mean cholesterol concentration after starvation was not altered much from refeedings.

As shown in Figure 15-D, the cholesterol concentration within the VHDL fraction of male dogs was not affected by either starvation or refeeding. Female dogs, also, did not exhibit any significant differences in VHDL cholesterol concentration due to starvation. However, the mean Control regimen cholesterol concentration of VHDL in female dogs (0.0 ± 0.3 mg/100 ml) was significantly less ($P<0.01$) than the values at both refeedings. Female mean cholesterol concentration at Refeeding I was significantly greater than the male mean cholesterol concentration at that same regimen. Such minute amounts of cholesterol in the VHDL fraction and any associated changes due to treatment are not held important, as VHDL is considered a residual cholesterol-containing protein (Lindall et al., 1972).

The relationship of each fraction's mean cholesterol concentration to the total plasma cholesterol or to that of mean cholesterol concentrations of other fractions is shown in Table 10. Individual dog values are in Appendix Tables B-31 to B-34. The effect of starvation and refeeding showed a lowering effect in mean plasma cholesterol concentration (Table 9). In females, there was a 10.3% increase of mean LDL cholesterol from Control regimen to Refeeding II, and a 11.9% decrease in HDL cholesterol for the same treatment regimens. Males, however, showed a 7.5% decrease in mean LDL cholesterol concentration and 8.4% increase in HDL cholesterol concentration from Control regimen to Refeeding II. Though a small increase, the male dogs showed a similar response as the one proposed for

Table 10. Percentage of total cholesterol or total triglycerides in each lipoprotein fraction[a]

Fraction	Chylomicra and VLDL		LDL		HDL		VHDL	
Treatment Regimen	Female	Male	Female	Male	Female	Male	Female	Male
			Cholesterol (% of total)					
Control	0.0	1.3	12.0	23.7	88.0	74.4	0.0	0.6
Starvation I	1.6	1.2	10.8	25.9	86.6	72.2	1.0	0.7
Refeeding I	1.8	1.3	18.2	27.4	76.8	71.3	3.2	0.0
Refeeding II	0.5	0.4	22.3	16.2	76.1	82.8	1.1	0.6
			Triglycerides (% of total)					
Control	20.4	28.8	43.6	38.1	24.7	17.1	11.3	16.0
Starvation I	11.5	9.8	15.5	27.4	40.6	39.2	32.4	23.6
Refeeding I	23.5	12.3	30.7	42.5	23.7	24.0	22.1	21.2
Refeeding II	14.3	17.2	37.2	36.5	26.4	24.3	22.1	22.0

[a]The percentage was determined by the summation of the particular lipid in all four fractions and calculating the amount of the lipid each fraction contributed to the total of that particular lipid. Each value listed is a mean of three animals in each sex class.

the effect of ACTH on the dog's cholesterol concentrations in these two fractions. The chylomicra and VLDL fraction and VHDL fraction cholesterols, which contribute a minute percentage of the total plasma cholesterol in the dog, did not exhibit much change.

The HDL and LDL cholesterols were positively correlated with whole plasma cholesterol. The correlation coefficients were 0.9449 (significant at the 0.01% level) and 0.7342 (significant at the 0.02% level), respectively (Appendix Table B-35). Such a high correlation coefficient for HDL cholesterol was consistent with the fact that HDL is the major cholesterol-bearing fraction.

Triglyceride concentration of the lipoprotein fractions The mean percentage recoveries (±SEM) of triglycerides were 89.0 ± 5.2 for the Control regimen, 95.9 ± 3.2 for Starvation I, 93.9 ± 3.0 for Refeeding I, and 92.1 ± 4.7 for Refeeding II. The percentage recoveries mentioned here and those for cholesterol content of lipoprotein fractions were excellent when compared to reports in the literature of 80% or better (Solyom et al., 1971). These authors as well as others allude to difficulties in the clean separation of LDL from HDL in dog serum. This then presents some quantitation problems of lipids. Sakagami and Zilversmit (1961) reported that smaller plasma samples (5 ml) and longer periods of centrifugation of time (24 hours) give a satisfactory separation of dog plasma lipoproteins. Such criteria were used in this experiment. Mean plasma lipoprotein fraction triglyceride concentrations are shown in Table 9. These data are graphically illustrated in Figure 16.

Starvation and refeeding of a high carbohydrate diet produced significant changes upon triglyceride concentrations of different plasma fractions. The plasma lipoprotein triglyceride concentrations for each fraction are shown in Appendix Tables B-19, B-22, B-25, and B-28. The Student t-test was performed only whenever significance ($P<0.01$) was shown by the F-test to exist among mean effects of treatment regimen, sex, and any interaction of these two (Appendix Tables B-21, B-24, B-27, and B-30).

The mean triglyceride concentration of the chylomicra and VLDL fraction of all dogs was significantly changed only during the first refeeding; it was an increase from 7 mg/100 ml in the Control regimen to 15 mg/100 ml in Refeeding I. The absence of an elevation in triglyceride concentration

Figure 16. Mean triglyceride concentrations of the plasma lipoprotein fractions in dogs periodically starved and refed a high carbohydrate diet

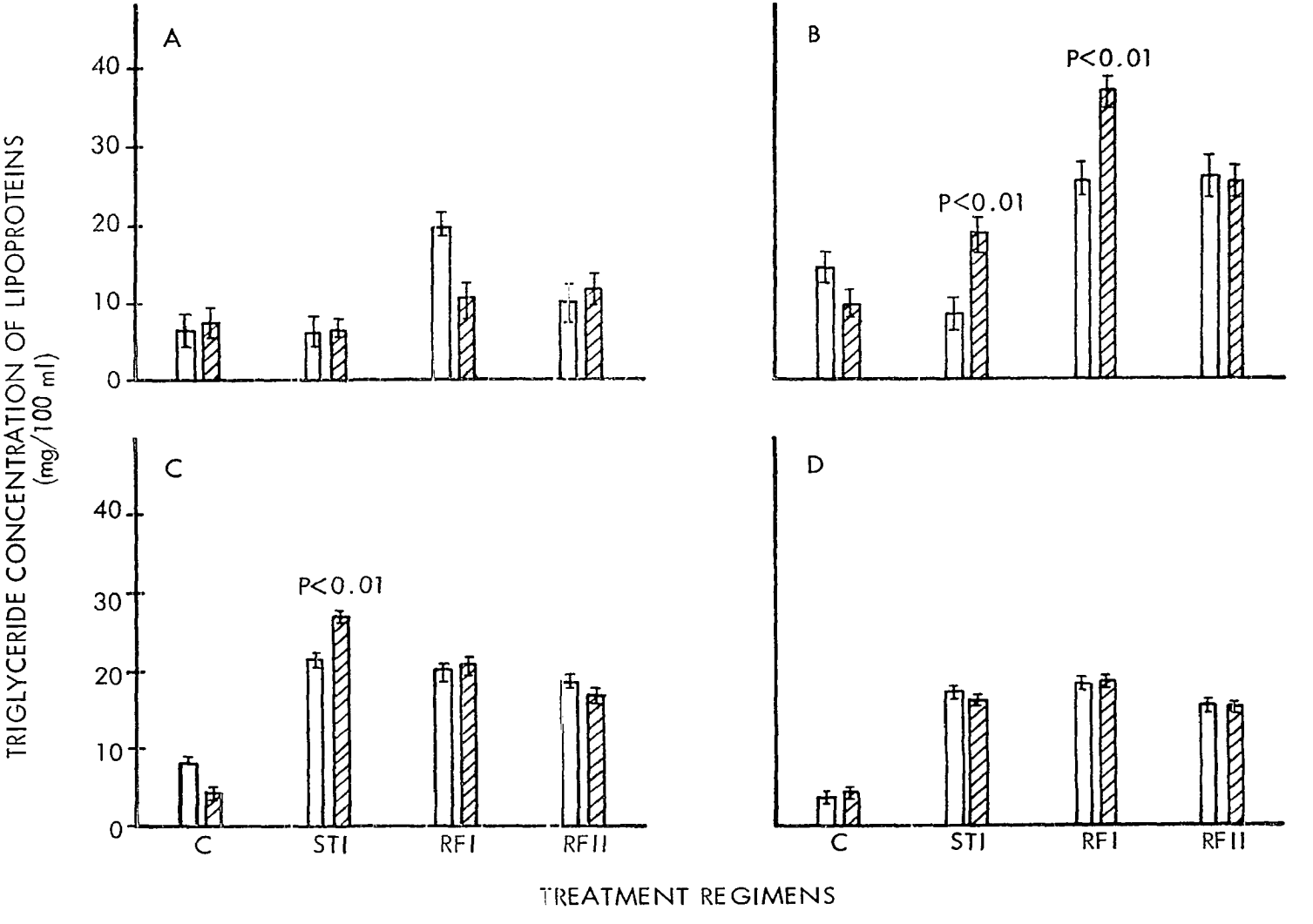
Values are representative of mean concentrations at the end of each regimen, except in Refeeding I. Values for Refeeding I represent mean triglyceride concentrations at 3 days refeeding. SEM are indicated.

A) Mean triglyceride concentration in the chylomicra and VLDL fraction.
B) Mean triglyceride concentration in the LDL fraction.
C) Mean triglyceride concentration in the HDL fraction.
D) Mean triglyceride concentration in the VHDL fraction.

Control Regimen	- C	Day 0
Starvation I	- STI	Days 1-30
Refeeding I	- RFI	Days 31-50
Refeeding II	- RFII	Days 68-98

Females 

Males 


A
B
C
D
P<0.01
P<0.01
P<0.01
40
30
20
10
0
TRIGLYCERIDE CONCENTRATION OF LIPOPROTEINS
(mg/100 ml)
C
STI
RFI
RFII
TREATMENT REGIMENS

at Starvation I is consistent with the concepts presented by Searcy and Berquist (1962). They reported that after 72 hours of fasting, serum UFA tripled while triglyceride concentrations remained relatively constant. In human starvation, levels of VLDL increase in order to transport the triglycerides just removed from adipose tissue (Barclay, 1972). The dog apparently maintains a relatively constant triglyceride level in VLDL because of rapid turnover time of triglycerides in this fraction (Bates, 1967). The rise in triglyceride concentration due to a high carbohydrate diet at Refeeding I is in agreement with the work of Lewis (1971). The triglyceride data for this fraction are shown in Table 9 and Figure 16-A.

The mean triglyceride concentration of the LDL fraction during the treatment regimens is shown for both sexes in Table 9 and Figure 16-B. During Starvation I, mean triglyceride concentration in LDL of female dogs responded differently from male dogs. Females showed a 57% decrease in LDL triglyceride concentration at Starvation I (8.2 mg/100 ml) from the Control regimen value (14.3 mg/100 ml). Males, however, showed almost a two-fold increase over the Control regimen, 9.8 to 18.9 mg/100 ml. This increase in mean LDL triglyceride concentration for the males was significant at $P<0.01$ (Appendix Table B-22). At Refeedings I and II the mean LDL triglyceride concentrations for both females and males were significantly higher ($P<0.01$) than their respective values at the Control regimen. Male mean LDL triglyceride concentrations at Starvation I and Refeeding I were significantly higher ($P<0.01$) than those of females.

Margolis and Capuzzi (1972) reported that in man the level of LDL may

be decreased by estrogens and increased by androgens. The stress from starvation may cause an increased amount of these hormones, which in turn cause these indicated effects. Because the low density fraction in the dog carries a good quantity of triglycerides, the decrease in triglyceride concentration with females and increase with males may be explained by this hormonal effect.

The significant increases in mean triglyceride concentrations at both refeedings are similar to those found in experiments with rats fed a diet high in carbohydrates which not only produced a marked increase in hepatic triglyceride synthesis, but also produced a comparable stimulation of LDL-protein synthesis (Eaton and Kipnis, 1969).

The HDL triglyceride concentration in the plasma of females was significantly increased ($P<0.01$) in Starvation I and both refeedings (Appendix Table B-25). Two- to 2.6-fold increases over Control regimen values were produced in the plasma HDL triglycerides of females at starvation and refeeding regimens. In males at Starvation I there was a six-fold increase in mean HDL triglyceride concentration. Female and male mean Control regimen HDL triglyceride concentrations were 8.1 and 4.4 mg/100 ml, respectively (Table 9). The male mean triglyceride concentration at Starvation I was significantly greater ($P<0.01$) than the female mean triglyceride value at the same regimen. Graphic illustration of these data is shown in Figure 16-C.

An explanation for such increases in dog plasma HDL triglcyeride concentrations due to starvation may be related to the manner in which HDL

cholesterol was changed at starvation. With an increase in HDL cholesterol concentration in male dogs, an associated increase in HDL triglyceride would be required.

Barclay (1972) reported that individuals fed high carbohydrate diets showed elevations of VLDL and reductions in HDL. This was found, also, to be evident in this experiment during the refeeding regimens.

The mean VHDL triglyceride concentration for all dogs (3.9 $\pm$ 0.4 mg/100 ml) was significantly less ($P<0.01$) than mean VHDL triglyceride concentrations at Starvation I and both refeedings (Appendix Table B-28). As shown in Table 9 and Figure 16-D, a 4.3-fold increase in VHDL triglyceride concentration occurred from Control regimen to Starvation I. An incremental increase and decrease of VHDL triglyceride concentration occurred, respectively, with Refeeding I and Refeeding II.

These results may be similarly explained by that discussed for HDL triglyceride responses, since much dog lipoprotein work now includes the VHDL fraction with the HDL fraction (Lindall et al., 1971).

The relationship of each fraction's mean triglyceride concentration to the total plasma triglycerides or to that of mean triglyceride concentrations of other fractions is shown in Table 10. Individual dog values are listed in Appendix Tables B-31 through B-34. The effect of starvation and refeeding showed a two-fold or better increase in whole plasma triglyceride concentration (Table 9). All fractions exhibited changes in percentage composition of triglycerides. The greatest alterations were at Starvation I with decreases of VLDL and LDL triglyceride composition and

increases in the high density fractions. Female dogs tended to return to Control regimen VLDL and LDL triglyceride compositions at Refeeding I. At Refeeding II, LDL and HDL triglycerides in both females and males showed somewhat similar percentage compositions to their Control regimen values. Percentage composition of VLDL triglycerides at the end of the experiment showed a decrease from that percentage composition at the beginning of the experiment. Percentage composition of VHDL showed an increase from the beginning to the end of the experiment. The VLDL, LDL, HDL, and VHDL triglyceride concentrations were all positively correlated with whole plasma triglycerides. The correlation coefficients were 0.5421, 0.8290, 0.7410, and 0.8788, respectively. All were significant at 0.02% or less (Appendix Table B-35). The higher correlation coefficients are consistent with the fact that the majority of plasma triglycerides in the dog are in the LDL and high density fractions. VLDL triglycerides have too rapid a turnover rate to allow a large triglyceride concentration to exist in this fraction.

Triglyceride-to-cholesterol ratios were calculated to better demonstrate the relationship between these two lipids. The values are shown in Table 11 and Figure 17. Inverse relationships of the ratio exist between males and females. The ratio of triglyceride to cholesterol exhibited increases from Control regimen to Starvation I in whole plasma and all fractions with exception to chylomicra-VLDL and VHDL for female dogs. This would indicate an overall increase in triglycerides. During both refeedings there was a substantial increase of the triglyceride-to-cholesterol ratio

Table 11. Triglyceride-to-cholesterol ratio[a]

Treatment Regimen	Plasma		Lipoprotein Fraction							
			Chylomicra and VLDL		LDL		HDL		VHDL	
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
Control	0.2	0.2	∞	4.6	0.6	0.3	0.0	0.0	∞	5.8
Starvation I	0.5	0.4	3.8	3.1	0.8	0.4	0.2	0.2	17.2	12.5
Refeeding I	0.8	0.6	10.2	5.3	1.3	0.9	0.2	0.2	5.5	∞
Refeeding II	0.5	0.7	16.7	29.5	0.9	1.6	0.2	0.2	10.3	25.2

[a]Calculated by dividing the mean triglyceride concentration in the plasma or fraction by the plasma or fraction's corresponding cholesterol concentration.

in the chylomicra and VLDL fraction. From Starvation I to Refeeding II, male dogs showed a range in ratios from 3.1 to 29.5. No definite change took place in the triglyceride-to-cholesterol ratio in the HDL fraction during both refeedings. The LDL fraction showed ratios for males to have generally increased from 0.3 at Control regimen, to 0.4 at Starvation I, and finally to 0.9 and 1.6 for refeedings. The females exhibited a similar overall increase, except for a decrease at Refeeding II. Ratios such as these can be helpful in determining the cholesterol-bearing capacity of a lipoprotein. Increases in plasma cholesterol necessitate increases in plasma triglyceride concentration. Increases of plasma cholesterol, therefore, might be inhibited if plasma triglyceride concentrations were not adequate.

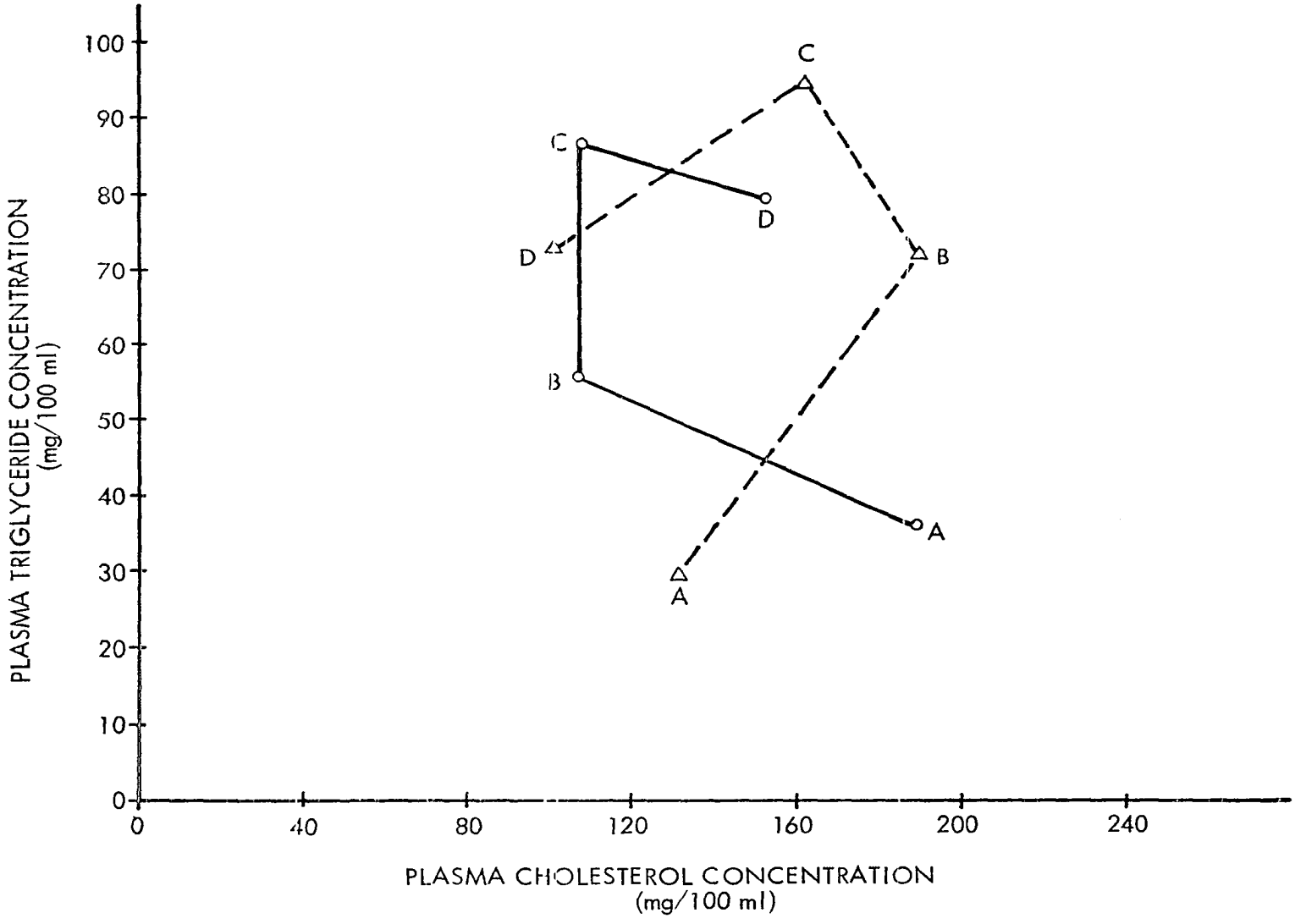
Figure 17. Mean total plasma cholesterol concentration as a function of mean total plasma triglycerides in dogs periodically starved and refed a high carbohydrate diet

Values at each point represent mean concentrations at the end of each regimen, except in Refeeding I. Values for Refeeding I represent mean concentrations at 3 days refeeding.

Control Regimen	- A	Day 0
Starvation I	- B	Days 1-30
Refeeding I	- C	Days 31-50
Refeeding II	- D	Days 68-98

Females o——·——o

Males △— — —△


PLASMA TRIGLYCERIDE CONCENTRATION
(mg/100 ml)
100
90
80
70
60
50
40
30
20
10
0
0
40
80
120
160
200
240
PLASMA CHOLESTEROL CONCENTRATION
(mg/100 ml)
C
D
B
A
C
D
B
A

Electrophoretic demonstration of lipoprotein fractions The use of electrophoresis in this experiment was intended for demonstrating the purity in lipoprotein fractions obtained from ultracentrifugation. Duplicate samples from whole plasma and each fraction were electrophoresed as soon as possible after sampling at Control, Starvation I, and Refeedings I and II regimens. Satisfactory lipoprotein patterns were observed for control samples of both whole plasma and lipoprotein fractions. The stained patterns were, however, so faint that photographs were impossible. Patterns for control dog plasma and individual lipoprotein fractions were like those normal dog lipoprotein patterns shown on polyacrylamide gel by Lindall et al., (1971), with one exception. An interchange of pre-beta and beta band positions is necessary for patterns on polyacrylamide gel to look similar to those on the cellulose acetate used in this experiment. The alpha band was either absent or faint in many plasma and lipoprotein fractions samples taken at Starvation I. A physical change involving a net reduction in the negative charges on or in the lipoprotein probably occurred, resulting in a reduction in electrophoretic mobility. At Refeeding I the alpha band was restored.

Experimentally Altered Lipoproteins in Relation to Changes in Arterial Fine Structure

Plasma lipoproteins have long been implicated to participate in atherogenesis. Ross and Glomset (1973) reported that differentiation of elastic fibers and collagen in growing arterial smooth muscle cells in vitro depends upon proteins as growth promoting substances. It would be expected that

during starvation the liver would not synthesize sufficient protein for lipoprotein formation. During starvation protein synthesis is inhibited or retarded. Electrophoresis of treatment dogs' whole plasma and HDL fractions showed either an absence of the alpha band or a faint band in starvation. Both starvation regimens showed significantly increased ($P<0.01$) blood urea nitrogen concentrations and normal blood glucose concentrations. This indicated that protein catabolism and gluconeogenesis, respectively, were involved in starvation. The constituency of the connective tissue matrix in the femoral artery would be expected to be affected by such a dietary stress and inadequacy of protein.

Vesiculated endoplasmic reticulum, large vacuolated areas in the cytoplasm, some vacuolated mitochondria, and primary lysosomes are shown in the intima of the treatment dogs that were starved and refed a high carbohydrate diet. Lysosomes may be involved in intracellular digestion of degenerating tissues. It is these cellular components that suggest some injury to the intimal layer of the artery during treatment. If vessel segments had been removed at starvation, the direct effects of starvation could be better evaluated. Though all the selected treatment dogs for fine structure studies exhibited many of these changes in cellular components and conditions in the endothelium and/or the subendothelial space, male treatment dog (no. 29) showed the most changes in arterial structure of the intima. Both female treatment dogs (nos. 5 and 7) showed similar but less extensive changes in the intima. Male treatment dog (no. 26) had comparatively little change in fine structure. Peculiar to the significant

decrease ($P<0.01$) in mean cholesterol concentrations in females at Starvations I and II, male treatment dog (no. 29) responded similarly in direction, but not magnitude. The cholesterol concentration of the HDL fraction in this male dog showed a response that was similar to the females. The triglyceride concentrations of the whole plasma in male dog no. 29 were like the males of his treatment group at Starvation I. They were higher than those of treatment female dogs. At Starvation I, there were significant increases ($P<0.01$) in plasma triglyceride concentrations in the HDL fraction for both sexes. A nonsignificant decrease in plasma LDL triglyceride concentration at Starvation I was shown for female treatment dogs, whereas, a significant increase ($P<0.01$) in plasma LDL triglyceride concentrations occurred for male treatment dogs. Blood lipids were not altered enough during starvation to speculate on the role of lipoproteins in these arterial changes.

A proliferative response in collagen synthesis, as is shown in male treatment dog no. 29 (Figure 9), may stem from a diet high in carbohydrate. Evidence in the intima indicates cellular and extracellular reactions to the diet. Lipid droplets, vacuoles, and inclusions were evident in treatment dogs that were starved and refed a high carbohydrate diet. Searcy and Berquist (1962) reported that ingestion of simple sugars, such as glucose or sucrose (the main constituent of this refeeding diet), depresses the conversion of cholesterol to bile acids, thus, contributing to hypercholesterolemia. Plasma and lipoprotein fraction cholesterol concentrations for females and males at the end of the experiment (Refeeding II) were not significantly different from those of the Control regimen, with two exceptions. The mean plasma cholesterol and HDL cholesterol concentrations for

females were significantly decreased ($P<0.01$). It would, therefore, not be reasonable to classify the lipid droplets, inclusions, and vacuoles as cholesterol on the basis of the plasma concentrations. The lipid inclusions were membrane bound, which excludes cholesterol. However, arterial lipids may have arisen in situ by a de novo synthesis, or by elaboration of lipids obtained from the plasma.

The ingestion of a high carbohydrate diet, such as the one used in this experimental refeeding (70%), increases the triglyceride pool in the body of man. This increased availability of triglycerides is largely due to an increased rate of triglyceride synthesis. Triglyceride concentrations in the VLDL fraction of carbohydrate-fed dogs subsequent to starvation were significantly increased ($P<0.01$) for both female and male treatment dogs during Refeedings I and II when compared to the triglyceride values during the Control regimen. The significantly higher ($P<0.01$) triglyceride concentrations for whole plasma in treatment female and male dogs during both refeedings were reflective of highly significant increases ($P<0.01$) in LDL, HDL, and VHDL triglyceride concentrations. Though the triglyceride concentrations were increased, hypertriglyceridemia was not induced by such a diet. Hypertriglyceridemia does occur in man if given diets high in carbohydrate. However, the dog has been shown to possess a very rapid turnover time for plasma VLDL triglycerides (Bates, 1967). The LDL fraction shows comparatively a lesser turnover time, and HDL is very slow. The dog apparently possesses the unique ability to clear the plasma of triglyceride fatty acids by VLDL transport. Such a rapid turnover reduces the possibility for cholesterol accumulation. These aspects

were substantiated by the VLDL concentrations of triglycerides in this experiment.

The increased amounts of collagen fibrils and filamentous-appearing material in profiles of endoplasmic reticulum are associated with an increased amount of ribosomes and lipid in the endothelial cells. The significantly increased concentrations of triglycerides ($P<0.01$) in both females and males at refeedings were evidenced in the LDL fraction. Scott and Hurley (1970) reported the peptide component of this lipoprotein enters an exchangeable pool in the human arterial wall by crossing the intima. It is not known whether the lipid and peptide components of the LDL fraction dissociate at the endothelial surface, or if the cholesterol in the lipoprotein vehicle is most important.

The chemical alterations that occurred during the starvation regimens caused damage to the intimal cells. In turn, these injured cells released substances which induced reparation, i.e., collagen synthesis. Such disturbances in protein metabolism during starvation might have caused a structural defect in the arterial walls of treatment dogs (Figures 6, 7, and 9), weakened the walls, and produced an increased distensibility. Increased collagen synthesis was shown only in male treatment dog no. 29 (Figure 9). Whorls of collagen are most impressive of repair processes occurring in this dog. If little repair occurred in respect to what was needed, normal hemodynamic stresses which are submitted to the wall, will in due time cause further injury to the wall. It is expected that increased amounts of collagen synthesized during such reparative processes should decrease the

distensibility of the arterial wall and, thus, increase blood pressure. A few more episodes of starvation and refeeding might have induced in the other dogs an increased collagen synthesis and more extensive intimal responses as are shown in male treatment dog no. 29.

CONCLUSIONS

1. The fine structural responses of the intima in femoral arteries of dogs subjected to long term starvation were cytoplasmic vacuolations and lysosomes which were indicative of malnutrition and intracellular digestion of damaged tissues.

2. Intimal lipid deposits resulted from the high carbohydrate diet refed after starvation. Filamentous or flocculent-containing profiles of endoplasmic reticulum and collagen whorls indicated protein synthesis during or subsequent to the severe dietary stresses. Such activity suggests an extensive repair process to the arterial wall.

3. The greatly increased plasma cholesterol concentrations for whole plasma and the HDL fraction in males at Starvation I and the inverse response for females suggest a sex hormonal effect upon cholesterol concentration during such a stress.

4. The responsiveness of lipoproteins to the high carbohydrate diet was shown to be greatest in the LDL fraction, the major lipoprotein carrier of triglycerides in the dog. The significant increases in LDL triglyceride concentrations could account for the increase in intimal lipid deposits. Endothelial injury due to starvation possibly facilitated penetration of triglycerides through the endothelial barrier.

5. The triglyceride response to the high carbohydrate diet in this experiment agrees with the concept that triglyceride concentration controls cholesterol-bearing capacity of each lipoprotein fraction.

SUMMARY

The present investigation was undertaken to determine the effects of long term starvation and the refeeding of a high carbohydrate diet on the fine structure of the femoral artery and blood chemistry in dogs of both sexes. An identification of the cells participating in the formation of alterations at the fine structural level was necessary for the purpose of providing indications of pathological change. The working hypothesis was that during starvation some rather debilitating effects occur in elastic tissues of the intima and internal elastic membrane. It was, also, conjectured that subsequent refeeding would provide to the damaged cells and matrix a prolific synthesis of collagen, which would, after repeated episodes of starvation-refeeding, produce decreased arterial distensibility, increased blood pressure, and eventual hypertension.

Ten female and 9 male Specific Pathogen Free beagle dogs were assigned on a nearly equal basis to either a control or treatment group. Each of the four sex-group combinations had a similar mean blood pressure. The control and treatment groups were given a commercial maintenance diet until the dogs became acclimated to their surroundings. Whereas, the control group was maintained on this same diet throughout the remainder of the experiment, the treatment group was subjected to episodes of starvation and refeeding of a diet high in carbohydrate. Vessel segments of the femoral artery in each dog were removed before the experiment began and segments from the contralateral femoral artery were removed at the end of the experiment. Both light and electron microscopy were conducted on

arterial tissue samples. Light microscopy was, also, done on the adrenal gland. Blood samples were collected at the end of each treatment regimen so that chemical analyses could be made for the selected blood constituents.

Femoral arteries of control dogs and treatment dogs subjected to periodic starvation and refeeding a high carbohydrate diet showed no structural differences at the light microscopy level between vessel segments collected at the beginning and end of the experiment. Similar findings were shown for adrenocortical tissues.

Fine structure studies of femoral arteries subjected to periodic starvation and refeeding a high carbohydrate diet revealed vesiculated endoplasmic reticulum, large vacuolated areas in the endothelium, and some primary lysosomes. Cytoplasmic vacuolation resulted from malnutrition, and the lysosomal bodies are indicative of intracellular digestion of damaged tissues. It is these alterations that indicate the changes to be associated with starvation.

Refeeding a high carbohydrate diet after Starvations I and II revealed not only these changes associated with starvation but, also, showed vesicles, inclusions, and vacuoles of lipid in endothelial and smooth muscle cells. All treatment dogs showed filamentous or flocculent-appearing material in circular profiles of granular endoplasmic reticulum. One dog in particular, male treatment no. 29, had endothelial cells with abundant ribosomes, lipid-containing cisternae of the agranular endoplasmic reticulum, and infiltrating collagen fibrils. The subendothelial space of this dog's artery had increased quantities of collagen in whorl patterns.

Abundant filamentous material associated with the basement membranes and internal elastic lamina indicates a pronounced response to stress. Radial orientation of smooth muscle cells, their penetration of the duplicated and fenestrated internal elastic lamina, and intense dense bodies in the smooth muscle cells indicate migration of medial cells and possible synthesis of myofibrillar proteins. The alterations in structure of these intimal constituents may indicate the need for the prolific synthesis of collagen in response to the debilitating influences of starvation.

Starvation and high carbohydrate refeeding caused some significant alterations of plasma sodium and potassium concentrations but all were well within the physiological range. These normal values are suggestive of renal conservation mechanisms. Significant increases ($P<0.01$) in blood urea nitrogen concentrations of all treatment dogs were produced during starvation. Adequate blood glucose concentrations during the experiment indicate the reason for high blood urea nitrogen concentrations is catabolism of proteins and gluconeogenesis. Inhibition or retardation of protein synthesis during starvation was substantiated by the faint or absent alpha band in electrophoresis.

Starvation I produced significantly ($P<0.01$) increased plasma cholesterol concentrations in males. Females, however, showed a significant decrease ($P<0.01$) in plasma cholesterol concentration. Females showed significantly decreased ($P<0.01$) plasma cholesterol concentrations at the end of the experiment (end of Refeeding II). The cholesterol-bearing HDL

fraction of both female and male treatment dogs showed a similar significant decrease ($P<0.01$) in its concentration.

Plasma triglycerides for both sexes were significantly increased ($P<0.01$) during Starvation I and both refeedings. The VLDL triglycerides were significantly elevated ($P<0.01$) in both sexes during Refeeding I. Both HDL and VHDL triglycerides in females and males were significantly increased ($P<0.01$) by both dietary treatments. The LDL triglycerides were increased significantly ($P<0.01$) in both sexes during refeedings, and in males during starvation.

Triglyceride-to-cholesterol ratios for each fraction demonstrated a constancy between available triglycerides and cholesterol concentration. The triglyceride response to the high carbohydrate diet seems to control the amount of cholesterol-bearing capacity of each lipoprotein fraction.

The lipid in the vesicles, vacuoles, cisternae, and inclusions of the endothelial and subendothelial spaces in the artery could be derived from the increased availability of triglycerides. Associated increases in ribosomes in the endothelial cells suggest increased metabolic activity of the granular endoplasmic reticulum due to the arterial intima's response to the high carbohydrate diet. Since the LDL fraction carried the majority of the triglycerides during carbohydrate refeedings and LDL is known to penetrate the intima, it may be that triglycerides are the lipids in the electron-dense cisternae of the agranular endoplasmic reticulum, vesicles, vacuoles, and inclusions in the femoral arteries of treatment dogs. The increased intimal lipids and the metabolism possibly associated with them may induce reparation to damaged tissues. In the case of the male

treatment dog (no. 29), reparations were in the form of collagen fibrils and other fibrillar materials.

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ACKNOWLEDGMENTS

The author wishes to express his appreciation to his major professor, Dr. Frederick B. Hembrough, for the counsel, guidance, and encouragement given throughout the course of these studies; to Dr. Melvin J. Swenson for the opportunity to pursue graduate study and for his constructive criticisms; to Dr. Marvin H. Stromer for technical assistance, suggestions, and use of the Food Research electron microscope; and to Dr. William O. Reece and Dr. Harry T. Horner for suggestions and constructive criticisms.

Appreciation is, also, expressed to Dr. Donald C. Beitz for suggestions and the use of the Nutritional Physiology laboratory; to Dr. Robert D. Glock for the use of the ultramicrotome in Veterinary Pathology; to Dr. Roger A. Ball at the Veterinary Medical Research Institute for help in the interpretation of histological preparations so ably done by Mrs. Ione S. Vold; to Mr. Arthur R. Anderson and Mrs. Ann Zimmerli for technical assistance in blood chemistry analyses; to Dr. David F. Cox for his assistance with the statistical analysis of the data; and to Dr. Dean H. Riedesel for his able assistance throughout the course of the project.

Special appreciation is expressed to my wife, Gail, for making everything worthwhile and providing her able assistance in the typing of the manuscript.

APPENDIX A

Table A-1. Stock solutions for lipoprotein ultracentrifugation

(A)	NaCl	0.15 M	density = 1.005
	8.775 g NaCl[a] [8.77 g/liter]		
(B)	density = 1.346		
	153 g NaCl + 354 g KBr/liter		
(C)	density = 1.177		
	500 ml (A) + 508.9 ml (B)		
(D)	density = 1.063		
	250 ml (A) + 51.2 ml (B)		
(E)	density = 1.21		
	165.9 ml (A) + 250 ml (B)		

[a]Chemicals from J. T. Baker Co., Phillipsburg, New Jersey.

The determination of a centrifugal force value[a] in lipoprotein ultracentrifugation

Speed (rpm)	$(rpm)^2$	Centrifugal force: Number (R) x gravity		
		R_{min}=3.8 cm	R_{av}=5.9 cm	R_{max}=8.1 cm
37,500	1406×10^6	59,726	92,739	127.313
40,000	1600×10^6	67,968	105,536	144,880

Relative Centrifugal Force (RCF) = $\frac{w^2}{g} r$, where

w = speed in rpm and

r = distance in cm from axis of rotation that particles are affected minimally or maximally by force, or acceleration of gravity (g). An average value (R_{av}) will be used here.
Type 40 Rotor operational values were used.

To convert w in rpm to radians per second,

$$w = rpm \times \frac{2\pi}{60}.$$

Since g = 980 cm per second per second; then

$$RCF = \frac{w^2}{g} r = \left(\frac{rpm \times 2\pi}{60}\right)^2 \times \frac{r}{980}$$

$$= \left[\left(\frac{2\pi}{60}\right)^2 \times \frac{1}{980}\right] \times r \times (rpm)^2$$

$$= \left[1118 \times 10^{-8}\right] \times r \times (rpm)^2$$

In that a speed of 39,800 rpm was used, then

$$1118 \times 10^{-8} \times 5.9 \times (39.8 \times 10^3)^2 = 104,486 \times g$$

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[a] The Models L2-50 and L2-HV Preparative Ultracentrifuge Instruction Manual L250-IM-2. 1966. Spinco Division Technical Publications, Beckman Instruments, Inc., Palo Alto, California.

Cholesterol determination of plasma and lipoprotein fractions (Mann, 1961)

Reagents

1. Alcohol-KOH mixture. Prepare each day, as needed, a mixture of a stock solution of 33.0% KOH[a] and 94 parts of absolute ethyl alcohol.

2. Petroleum ether. Redistill reagent grade solvent in a glass still, collecting the fraction that boils between 30 and 60 C. Store in a brown bottle.

3. Iron stock solution. Dissolve 2.50 g $FeCl_3 \cdot 6H_2O$[b] in 2.5 ml of glacial acetic acid.[a] Store this in the cold room. Do not use if a precipitate is observed in the bottle. This is stable for many months.

4. Working iron-sulfuric acid reagent (FeSac). Measure exactly 1 ml of the iron stock solution into a dry 100 ml volumetric flask. Add reagent grade concentrated H_2SO_4[a] cautiously and make to volume. Discard this if a precipitate is evident.

5. Cholesterol standard. Dissolve 100.0 mg of recrystallized cholesterol,[c] with a melting point of 148-149 C, in 100.0 ml of glacial acetic acid.

This stock solution of standard contains 1000 µg/ml. The working standard is made by transferring 10.0 ml of the stock solution to a 100 ml volumetric flask and bringing to volume with glacial acetic acid. This solution contains 100 µg/ml. The standards should be stored in 23 C for no

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[a] KOH, glacial acetic acid, H_2SO_4 were purchased from J. T. Baker Co., Phillipsburg, New Jersey.

[b] The $FeCl_3 \cdot 6H_2O$ was purchased from Mallinckrodt Chemical Works, St. Louis, Missouri.

[c] Cholesterol standard was purchased from the Hormel Institute, University of Minnesota, Austin, Minnesota.

longer than 3 months and for a shorter period of time if much of the standard is used.

Procedure

1. Transfer 0.1 ml of well-mixed plasma to the bottom of a tube. Sometimes 0.2 ml was used for some fractions that were known to have little lipid. The amount is critical; a clear Levy-Lang constriction pipet is used for each replicate of a sample.

2. Add 5.0 ml of freshly prepared alcoholic KOH and mix by swirling.

3. Place the tubes in a 65 C water bath for 60 minutes with stoppers loose.

4. Remove the tubes and cool them to room temperature. Add exactly 10.0 ml of petroleum ether with a pipet, replace the stopper, and mix on Vortex mixer for 30 seconds. Add 5.0 ml of distilled water to each tube, stopper, and mix again for 30 seconds.

5. When the petroleum ether layer has separated, remove exactly 2.0 ml of the ether layer. It was necessary sometimes to remove 4.0 ml when analyzing lipoprotein fractions, so as to obtain optical densities in the most sensitive range. Transfer this to the bottom of a 22 x 175 mm test tube. Place the tubes in a 65 C water bath for about 30 minutes, or until all the solvent evaporates. If aeration is used, it must be with nitrogen. Smell the tubes to be sure all traces of solvent have been removed. Cool the tubes to room temperature.

6. Add 4.0 ml of glacial acetic acid to each tube. Prepare four additional tubes, one for a blank and three for standard solutions, containing 20 µg, 50 µg, and 100 µg of cholesterol, respectively. Add 3.8, 3.5, and

3.0 ml of glacial acetic acid to each standard as appropriate.

7. Add exactly 2.0 ml of FeSac reagent to each tube. Mix. The tubes will become warm and a transitory brown color will change to violet. Gas bubbles will form. If a petroleum ether residue is present, an emulsion will result and these tubes should be discarded.

8. After 20-30 minutes at room temperature, the tubes should be equilibrated with room temperature.

9. Determine the optical densities of each sample and standards, reading at 560 nm and then correcting for the reagent blank.

10. Plot the standards on rectilinear paper. A linear relationship exists between optical density and concentration. Determine the cholesterol concentration of samples from this graph.

Triglyceride determinations of plasma and lipoprotein fractions (Fletcher, 1968)

Reagents

1. KOH.[a] Five percent KOH in isopropanol[a]-water (40:60, v:v).

2. Sodium metaperiodate.[a] Make a stock solution containing 0.025 M sodium metaperiodate in 1.0 N acetic acid.[a] Working solution: dilute 12.0 ml of sodium metaperiodate and 20.0 ml isopropanol to 100.0 ml with 1.0 N acetic acid. This solution must be prepared fresh daily.

3. Acetylacetone. 0.75 ml 2, 4-pentanedione[a] and 2.5 ml isopropanol are dissolved in 100.0 ml 2.0 M ammonium acetate,[a] pH 6.0. This reagent must be stored in the refrigerator in a dark bottle and remains stable for a month.

4. Ammonium acetate.[a] Dissolve 15.42 g in 60.0 ml water. Adjust to pH 6.0 with 5.0 N HCl and dilute to 100.0 ml with distilled water.

5. Silicic acid mixture. Silicic acid (100.0 g), $CuSO_4$ (5.0 g), and $Ca(OH)_2$ (10 g) are mixed together.

6. Triolein[b] standard solution. 300.0 mg triolein are dissolved in 100.0 ml of isopropanol.

Procedure

1. Preparation of plasma samples. Plasma (0.2 ml) is added slowly to 9.8

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[a]KOH, isopropanol, acetic acid, sodium metaperiodate, ammonium acetate, and 2, 4-pentanedione were purchased from the J. T. Baker Co., Phillipsburg, New Jersey.

[b]Triolein was purchased from the Hormel Institute, University of Minnesota, Austin Minnesota.

ml of isopropanol. After the addition of 2.0 g silicic acid mixture, the tubes are shaken on a Vortex mixer for 1 minute and then centrifuged at 1478 x g for 5 minutes to sediment the silicic acid mixture. At this point the supernatants, as well as the standard solution, may be stored in the refrigerator. Aliquots (2.0 ml) are pipetted from each sample for the assay. It was necessary sometimes in this experiment to use 0.4 ml of a lipoprotein sample so as to have enough glycerides to form the fluorescent product and give an optical density with enough sensitivity.

2. Preparation of calibration curve. Triolein standard solution (0.2 ml) is added to 8.8 ml isopropanol and 1.0 ml of water. The solutions are mixed well with the 2.0 g silicic acid mixture. The tubes are centrifuged for 5 minutes to sediment the silicic acid mixture. From the supernatant, which contains 0.06 mg triolein/ml, a standard curve to match whole plasma and lipoprotein fractions with 30-300.0 mg triolein/100 ml is prepared. Aliquots of the supernatant (0.2, 0.4, 0.8, 1.0, 1.4, 1.6, and 2.0 ml) are diluted to 2.0 ml with isopropanol where necessary. These solutions correspond to concentrations of 30, 60, 120, 150, 210, 240, and 300 mg triolein/100 ml.

3. Two ml of prepared samples, standards, and blank are mixed with 0.6 ml of 5.0% KOH and stoppered.

4. Incubate contents at 60-70 C for 15 minutes. Cool.

5. Add 1.0 ml of sodium metaperiodate and mix.

6. Add 0.5 ml of acetylacetone and mix. Stopper.

7. Incubate at 50 C for 30 minutes. Cool.

8. Read the samples and standards against a reagent blank at 405 nm.

9. Plot the standards on rectilinear paper. A linear relationship exists between optical density and concentration. Determine the triglyceride concentration of the samples from this graph.

APPENDIX B

Table B-1. Dog body weights

Regimen	Control[a]			Starvation I				Refeeding I			Starvation II			Refeeding II	
Week	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
							(kg)								
Females															
Control															
01	9.8	9.3	9.5	9.1	10.0	10.5	10.0	10.5	10.0	10.2	10.0	10.0	9.5	10.0	10.5
02	10.5	10.0	10.2	10.0	10.0	10.5	10.0	10.0	10.5	10.5	10.9	10.5	10.0	10.7	10.2
04	10.5	10.0	9.8	10.0	10.5	10.2	10.5	10.5	10.5	10.0	10.0	10.2	10.0	10.9	10.9
06	10.2	10.2	10.5	10.0	10.0	10.5	10.0	10.0	10.5	10.2	10.5	10.0	10.2	10.0	10.5
10	11.8	11.4	11.8	12.3	12.3	11.8	12.3	11.8	11.8	11.8	11.8	12.3	12.0	11.8	11.8
Treatment															
03	8.9	8.9	8.6	8.0	7.3	6.8	6.1	5.7	7.0	—[b]					
05	9.1	8.9	9.1	8.2	7.3	6.1	5.9	5.0	8.0	9.1	7.7	6.6	6.6	8.0	9.1
07	8.9	9.1	9.1	7.7	6.8	6.8	5.9	5.5	7.0	7.5	6.8	5.7	5.2	6.4	7.3
08	10.9	11.4	10.9	10.0	9.1	8.4	8.0	7.5	9.5	10.5	9.5	8.2	8.2	9.9	10.9
09	10.9	10.2	10.5	9.1	8.2	7.7	6.4	5.2	6.6	—[b]					
Males															
Control															
22	13.6	13.4	13.6	13.6	13.4	13.6	14.1	13.6	13.6	13.4	13.6	13.6	13.4	14.1	13.6
23	13.9	13.9	14.5	13.6	14.1	13.9	14.1	14.5	13.9	13.9	14.1	13.9	13.6	13.6	14.1
24	14.1	14.1	13.9	13.6	14.1	14.1	14.1	15.0	14.8	14.5	14.5	15.0	14.3	14.5	14.5
27	13.6	13.9	14.1	14.1	14.1	14.3	14.5	14.8	14.5	14.5	14.5	14.5	13.6	14.3	14.5
28	12.3	12.3	12.7	12.3	12.3	12.7	12.7	12.3	13.2	12.7	12.7	12.7	12.7	12.3	12.7
Treatment															
21	10.5	10.5	10.5	9.1	8.2	7.7	7.0	5.5	5.9	7.5	6.4	5.5	4.1	5.5	6.4
25	13.0	12.3	12.3	11.8	10.0	9.3	8.2	8.4	9.5	10.9	10.0	8.6	8.6	9.8	10.9
26	13.9	13.9	14.1	12.5	11.4	10.9	9.3	9.1	10.7	11.4	10.5	9.3	8.4	10.0	10.9
29	13.2	12.3	12.3	11.4	10.5	10.0	8.9	9.1	10.7	11.4	10.0	9.5	8.6	10.7	11.6

[a]Control diet, consisting of Friskies dog food, was used for weeks 0 to 2. Control dogs were fed this control diet for weeks 3 to 14. Treatment dogs were fed during weeks 3 to 14 according to the regimens listed at the top of the table.

[b]Dogs 03 and 09 died during Refeeding I.

Table B-2. Mean body weights of dogs with respect to sex, group (control or treatment) and regimen

Regimen	Control[a]	Starvation I	Refeeding I	Starvation II	Refeeding II
Week	0-2	3-6	7-9	10-12	13-14
			(kg)		
Females					
Control	10.3±0.3[b](5)[c]	10.5±0.3(5)	10.6±0.3(5)	10.5±0.3(5)	10.8±0.3(5)
Treatment	9.7±0.3(5)	7.5±0.3(5)	7.2±0.4(3)[d]	7.2±0.4(3)	9.1±0.4(3)
Males					
Control	13.5±0.3(5)	13.7±0.3(5)	14.0±0.3(5)	13.8±0.3(5)	13.8±0.3(5)
Treatment	12.3±0.3(4)	9.8±0.3(4)	9.2±0.3(4)[d]	8.3±0.3(4)	10.1±0.3(4)

[a]A control diet, consisting of Friskies dog food, was used by all dogs for weeks 0 to 2. Control dogs were fed this control diet for weeks 3 to 14. Treatment dogs were fed during weeks 3 to 14 according to the regimens listed at the top of the table.

[b]Standard Error of the Mean (SEM) $= \sqrt{\frac{S^2}{n}}$, where S^2 is the pooled variance or error mean squares (EMS) and n is the number of dogs sampled. EMS here is equivalent to 0.42.

[c]The values in parentheses indicate the number(n) of dogs available for sampling.

[d]The apparent decrease in mean body weight of treatment dogs from Starvation I to Refeeding I is not a comparison of final weights for each of the two regimens, but is a comparison of overall means of body weights for each of the two regimens.

Table B-3. Plasma sodium concentrations in the dog

Regimen	Control	Starvation I	Refeeding I	Starvation II	Refeeding II
			(meq/liter)		
Females					
Control					
01	162.0[a]	166.0	160.0	160.0	157.0
02	165.0	184.0	159.0	191.0	158.0
04	164.0	164.0	157.0	162.0	158.0
06	163.0	164.0	160.0	162.0	159.0
10	166.0	165.0	156.0	158.0	167.0
Mean	164.0±2.8[b](5)[c]	168.6±2.8(5)	158.4±2.8(5)	166.6±2.8(5)	159.8±2.8(5)
Treatment					
03	161.0	179.0	—[d]		
05	160.0	163.0	154.0	162.0	156.0
07	164.0	163.0	157.0	183.0	164.0
08	166.0	179.0	158.0	164.0	157.0
09	171.0	192.0	—[d]		
Mean	164.4±2.8(5)	175.2±2.8(5)[e,**]	156.3±3.6(3)	169.7±3.6(3)	159.0±3.6(3)
Males					
Control					
22	165.0	180.0	155.0	159.0	157.0
23	166.0	180.0	158.0	163.0	156.0
24	167.0	179.0	157.0	162.0	163.0
27	167.0	164.0	155.0	163.0	163.0
28	165.0	165.0	159.0	183.0	169.0
Mean	166.0±2.8(5)	173.6±2.8(5)	156.8±2.8(5)	166.0±2.8(5)	161.6±2.8(5)

Treatment					
21	166.0	183.0	157.0	181.0	169.0
25	166.0	178.0	154.0	157.0	155.0
26	170.0	181.0	157.0	166.0	162.0
29	169.0	180.0	158.0	165.0	165.0
Mean	167.8±3.1(4)	180.5±3.1(4)[f,**]	156.5±3.1(4)	167.2±3.1(4)	162.8±3.1(4)
Overall control mean	165.0±2.0(10)	171.1±2.0(10)	157.6±2.0(10)	166.3±2.0(10)	160.7±2.0(10)
Overall treatment mean	165.9±2.1(9)	177.6±2.1(9)[g,**]	156.4±2.4(7)[g,**]	168.3±2.4(7)	161.1±2.4(7)

[a]Values represent plasma concentrations at the end of each regimen.

[b,c]SEM was derived from the pooled variance, EMS (39.51), and n, the number of dogs.

[d]Dogs 03 and 09 died during Refeeding I.

[e]The Control regimen mean for the female treatment group (164.4±2.8 meq/liter) was significantly less than the female treatment mean at Starvation I.

[f]The Control regimen mean for the male treatment group (167.8±3.1 meq/liter) was significantly less than the male treatment mean at Starvation I.

[g]The Control regimen mean for all treatment dogs was significantly less than overall treatment mean at Starvation I and significantly greater than the overall treatment mean at Refeeding I.

**Difference at Starvation I was significant at $P<0.01$.

Table B-4. Plasma potassium concentrations in the dog

Regimen	Control	Starvation I	Refeeding I	Starvation II	Refeeding II
			(meq/liter)		
Females					
Control					
01	4.4[a]	4.3	3.9	3.9	4.4
02	4.6	4.6	3.9	4.2	4.4
04	4.4	4.1	3.7	3.9	4.2
06	4.6	4.4	4.1	4.1	4.2
10	4.5	4.6	4.8	4.3	4.4
Mean	4.5±0.1[b](5)[c]	4.4±0.1(5)	4.1±0.1(5)	4.1±0.1(5)	4.3±0.1(5)
Treatment					
03	4.4	4.0	—[d]		
05	4.2	3.9	4.5	4.1	3.9
07	4.0	4.7	2.9	4.7	2.8
08	4.5	4.5	4.2[d]	4.3	4.7
09	4.9	4.8	—[d]		
Mean	4.4±0.1(5)	4.4±0.1(5)	3.9±0.2(3)	4.4±0.2(3)	3.8±0.2(3)
Males					
Control					
22	4.7	4.5	4.2	4.2	4.3
23	4.7	4.3	4.1	4.2	4.2
24	5.1	4.6	4.1	4.3	4.7
27	4.5	4.5	4.2	4.4	4.4
28	4.5	4.3	4.3	4.4	4.1
Mean	4.7±0.1(5)	4.4±0.1(5)	4.2±0.1(5)	4.3±0.1(5)	4.3±0.1(5)

Treatment					
21	4.6	4.7	3.1	4.2	3.1
25	4.8	4.5	3.3	4.4	3.8
26	4.6	4.4	3.4	4.3	3.1
29	4.3	4.1	4.1	4.0	3.9
Mean	4.6±0.2(4)	4.4±0.2(4)	3.5±0.2(4)	4.2±0.2(4)	3.5±0.2(4)
Overall control mean	4.6±0.1(10)	4.4±0.1(10)	4.1±0.1(10)[e,**]	4.2±0.1(10)[e,**]	4.3±0.1(10)
Overall treatment mean	4.5±0.1(9)	4.4±0.1(9)	3.6±0.1(7)[f,**]	4.3±0.1(7)	3.6±0.1(7)[f,g,**]

[a]Values represent plasma concentrations at the end of each regimen.

[b,c]SEM was derived from the pooled variance, EMS (0.1), and n, the number of dogs.

[d]Dogs 03 and 09 died during Refeeding I.

[e]The Control regimen mean for all control dogs (4.6±0.1 meq/liter) was significantly greater than overall control group means at Refeeding I and Starvation II.

[f]The Control regimen mean for all treatment dogs (4.5±0.1 meq/liter) was significantly greater than overall treatment group means at Refeedings I and II.

[g]The overall treatment group mean at Refeeding II was significantly less than overall control mean at the same regimen.

**Difference was significant at $P<0.01$.

Table B-5. Analysis of variance plan and observed mean squares for plasma sodium concentrations in dogs that were periodically starved and refed a high carbohydrate diet

Source of variation	d.f.	Mean squares
Group	1	69.29
Sex	1	60.34
Sex · group	1	2.71
Dog (sex · group) error	15	81.97
Treatment regimen	4	671.45**
Group · treatment regimen	4	27.61
Sex · treatment regimen	4	53.99
Sex · group · treatment regimen	4	7.73
Error	54	39.51
Total	88	

**Statistical significance is at $P<0.01$.

Table B-6. Analysis of variance plan and observed mean squares for plasma potassium concentrations in dogs that were periodically starved and refed a high carbohydrate diet

Source of variation	d.f.	Mean squares
Group	1	1.19*
Sex	1	0.0007
Sex · group	1	0.32
Dog (sex · group) error	15	0.16
Treatment regimen	4	1.13**
Group · treatment regimen	4	0.42
Sex · treatment regimen	4	0.11
Sex · group · treatment regimen	4	0.07
Error	54	0.10
Total	88	

*Statistical significance is at $P<0.05$.

**Statistical significance is at $P<0.01$.

Table B-7. Plasma glucose concentrations in the dog

Regimen	Control	Starvation I	Refeeding I	Starvation II	Refeeding II
			(mg/100 ml)		
Females					
Control					
01	115.0[a]	103.0	100.0	92.0	100.0
02	110.0	103.0	82.0	90.0	98.0
04	112.0	101.0	96.0	88.0	102.0
06	108.0	105.0	96.0	90.0	106.0
10	115.0	97.0	104.0	98.0	100.0
Mean	112.0±3.1[b](5)[c]	101.8±3.1(5)	95.6±3.1(5)[d,**]	91.6±3.1(5)[d,**]	101.2±3.1(5)
Treatment					
03	112.0	99.0	—[e]		
05	122.0	133.0	116.0	108.0	112.0
07	104.0	132.0	98.0	90.0	104.0
08	105.0	86.0	98.0	82.0	104.0
09	104.0	75.0	—[e]		
Mean	109.4±3.1(5)	105.0±3.1(5)	104.0±4.0(3)	93.3±4.0(3)[f,**]	106.7±4.0(3)
Males					
Control					
22	114.0	112.0	108.0	110.0	104.0
23	116.0	112.0	110.0	110.0	108.0
24	107.0	106.0	106.0	110.0	108.0
27	112.0	110.0	100.0	90.0	110.0
28	110.0	102.0	98.0	94.0	104.0
Mean	111.8±3.1(5)	108.4±3.1(5)	104.4±3.1(5)	102.8±3.1(5)	106.8±3.1(5)

Treatment					
21	107.0	107.0	104.0	78.0	98.0
25	114.0	99.0	102.0	82.0	100.0
26	106.0	103.0	104.0	78.0	110.0
29	114.0	109.0	104.0	106.0	106.0
Mean	110.2±3.5(4)	104.5±3.5(4)	103.5±3.5(4)	86.0±3.5(4)[g,h,**]	103.5±3.5(4)
Overall control mean	111.9±2.2(10)	105.1±2.2(10)	100.0±2.2(10)[d,**]	97.2±2.2(10)[d,**]	104.0±2.2(10)
Overall treatment mean	109.8±2.3(9)	104.8±2.3(9)	103.7±2.6(7)	89.1±2.6(7)[g,**]	104.9±2.6(7)

[a]Values represent plasma concentrations at the end of each regimen.

[b,c]SEM was derived from the pooled variance, EMS (48.27), and n, the number of dogs.

[d]Control regimen means for female control group and overall control group were significantly greater than their respective group means at Refeeding I and Starvation II.

[e]Dogs 03 and 09 died during Refeeding I.

[f]The Control regimen mean for female treatment group (109.4±3.1 mg/100 ml) was significantly greater than female treatment group mean at Starvation II.

[g]The Control regimen means for male treatment group and overall treatment group were significantly greater than their respective group means at Starvation II.

[h]Male treatment group mean was significantly less than the respective control group at Starvation II.

[**]Difference was significant at $P<0.01$.

Table B-8. Blood plasma urea nitrogen concentrations in the dog

Regimen	Control	Starvation I	Refeeding I	Starvation II	Refeeding II
			(mg/100 ml)		
Females					
Control					
01	13.0[a]	13.0	15.0	16.0	14.0
02	11.0	11.0	11.0	11.0	12.0
04	12.0	13.0	12.0	15.0	13.0
06	14.0	9.5	10.0	13.0	14.0
10	14.0	11.5	14.0	13.0	11.0
Mean	12.8±1.9[b] (5)[c]	11.6±1.9(5)	12.4±1.9(5)	13.6±1.9(5)	12.8±1.9(5)
Treatment					
03	12.0	17.5	—[d]		
05	13.0	13.5	11.0	19.0	12.0
07	15.5	41.0	15.0	26.0	16.0
08	13.5	14.5	13.0	13.0	15.0
09	13.0	45.0	—[d]		
Mean	13.4±1.9(5)	26.3±1.9(5)	13.0±2.4(3)	19.3±2.4(3)	14.3±2.4(3)

Males					
Control					
22	12.0	10.5	12.0	12.0	11.0
23	12.0	12.5	12.0	14.0	12.0
24	13.0	13.0	14.0	13.0	12.0
27	12.0	12.5	11.0	15.0	13.0
28	12.5	14.5	16.0	15.0	16.0
Mean	12.3±1.9(5)	12.6±1.9(5)	13.0±1.9(5)	13.8±1.9(5)	12.8±1.9(5)
Treatment					
21	12.0	23.0	14.0	37.0	22.0
25	11.5	31.0	10.0	18.0	12.0
26	17.0	29.5	14.0	28.0	12.0
29	12.5	14.0	13.0	19.0	15.0
Mean	13.2±2.1(4)	24.4±2.1(4)	12.8±2.1(4)	25.5±2.1(4)	15.2±2.1(4)
Overall control mean	12.6±1.3(10)	12.1±1.3(10)	12.7±1.3(10)	13.7±1.3(10)	12.8±1.3(10)
Overall treatment mean	13.3±1.4(9)	25.4±1.4(9)[e,f,**]	12.9±1.6(7)	22.9±1.6(7)[e,f,**]	14.9±1.6(7)

[a]Values represent plasma concentrations at the end of each regimen.

[b,c]SEM was derived from the pooled variance, EMS (17.90), and n, the number of dogs.

[d]Dogs 03 and 09 died during Refeeding I.

[e]Control regimen mean for all treatment dogs (13.3±1.4 mg/100 ml) was significantly less than means at both starvations.

[f]Overall treatment group means for both starvation regimens were significantly greater than corresponding regimen means of the overall control group.

[**]Difference was significant at $P<0.01$.

Table B-9. Analysis of variance plan and observed mean squares for plasma glucose concentrations in dogs that were periodically starved and refed a high carbohydrate diet

Source of variation	d.f.	Mean squares
Group	1	22.59
Sex	1	98.03
Sex · group	1	391.19
Dog (sex · group) error	15	194.44**
Treatment regimen	4	802.70
Group · treatment regimen	4	82.90
Sex · treatment regimen	4	28.45
Sex · group · treatment regimen	4	27.34
Error	54	48.27
Total	88	

**Statistical significance is at P<0.01.

Table B-10. Analysis of variance plan and observed mean squares for blood urea nitrogen concentrations in dogs that were periodically starved and refed a high carbohydrate diet

Source of variation	d.f.	Mean squares
Group	1	533.16**
Sex	1	7.89
Sex · group	1	2.57
Dog (sex · group) error	15	46.84**
Treatment regimen	4	144.14**
Group · treatment regimen	4	141.46
Sex · treatment regimen	4	6.82
Sex · group · treatment regimen	4	9.10
Error	54	17.90
Total	88	

**Statistical significance is at P<0.01.

Table B-11. Correlation coefficients, positive and negative, for some plasma constituents in dogs periodically starved and refed a high carbohydrate diet

Plasma constituent	Sodium	Potassium	Glucose	Urea nitrogen
Sodium	1.0000	0.3153	-0.2156	0.4406
	0.0000[a]	0.0030	0.0399	0.0001
Potassium	0.3153	1.0000	0.0998	0.1010
	0.0030	0.0000	0.6455	0.3065
Glucose	-0.2156	0.0998	1.0000	-0.2802
	0.0399	0.6455	0.0000	0.0078
Urea nitrogen	0.4406	0.1010	-0.2802	1.0000
	0.0001	0.3065	0.0078	0.0000

[a]Value indicates the level at which the correlation coefficient is significant.

Table B-12. Plasma cholesterol concentrations in the dog

Regimen	Control	Starvation I	Refeeding I	Starvation II	Refeeding II
			(mg/100 ml)		
Females					
Control					
01	175.3[a]	130.7	136.0	124.3	130.4
02	164.7	151.0	144.0	132.3	147.2
04	140.0	112.0	119.0	114.0	124.7
06	204.0	125.0	115.3	101.7	127.3
10	140.0	202.7	264.3	209.3	179.3
Mean	164.8±9.5[b](5)[c]	144.3±9.5(5)	155.7±9.5(5)	136.3±9.5(5)	141.8±9.5(5)
Treatment					
03	237.0	142.3	—[d]		
05	182.3	157.3	198.0	170.3	145.7
07	218.0	110.0	198.3	159.2	197.3
08	221.3	182.0[d]	180.7[d]	179.7	162.3
09	159.0	—	—		
Mean	203.5±9.5(5)[e,**]	147.9±10.6(4)[f,**]	192.3±12.2(3)	169.7±12.2(3)[f,**]	168.4±12.2(3)[f,**]
Males					
Control					
22	156.7	141.0	148.3	140.0	140.3
23	132.0	115.0	121.7	117.7	117.3
24	221.4	181.0	195.7	172.7	200.0
27	150.7	140.0	146.3	124.7	130.3
28	153.7	167.7	169.0	142.7	159.0
Mean	162.9±9.5(5)	148.9±9.5(5)	156.2±9.5(5)	139.6±9.5(5)	149.4±9.5(5)

Treatment					
21	129.3	173.3	129.0	155.3	113.0
25	147.7	227.0	179.3	183.7	140.3
26	126.0	197.7	141.7	176.0	125.7
29	132.7	138.3	111.7	139.7	118.0
Mean	133.9±10.6(4)	184.1±10.6(4)[g,**]	140.4±10.6(4)	163.7±10.6(4)	124.2±10.6(4)
Overall control mean	163.8±6.7(10)	146.6±6.7(10)	156.0±6.7(10)	137.9±6.7(10)	145.6±6.7(10)
Overall treatment mean	172.6±7.1(9)	166.0±7.5(8)	162.7±8.0(7)	166.3±8.0(7)	143.2±8.0(7)

[a]Values represent plasma concentrations at end of each regimen.

[b,c]SEM was derived from EMS, the pooled variance (448.3), and n, the number of dogs.

[d]Dogs 03 and 09 died during Refeeding I. Lack of datum in Starvation I was due to insufficient sample.

[e]Control regimen plasma cholesterol concentration (203.5±9.5 mg/100 ml) for female treatment group was significantly different from female control group Control regimen value (164.8±9.5 mg/100 ml).

[f]Female treatment group showed significant decreases in whole plasma cholesterol concentrations at Starvations I and II and Refeeding II with respect to the Control regimen value.

[g]Male treatment group showed a significant increase in whole plasma cholesterol concentration at Starvation I.

[**]Difference was significant at $P<0.01$.

Table B-13. Plasma triglyceride concentrations in the dog

Regimen	Control	Starvation I	Refeeding I	Starvation II	Refeeding II
			(mg/100 ml)		
Females					
Control					
01	44.7[a]	27.7	49.0	42.7	43.2
02	66.7	51.3	60.3	54.7	57.2
04	59.3	41.7	59.3	47.7	60.7
06	62.7	41.7	52.7	37.7	54.7
10	64.0	49.0	65.0	54.3	54.3
Mean	59.5±3.2[b](5)[c]	42.3±3.2(5)	57.3±3.2(5)	47.4±3.2(5)	54.0±3.2(5)
Treatment					
03	52.0	59.3	—[d]		
05	58.7	63.0	84.3	56.7	85.3
07	62.0	32.7	91.0	48.0	73.0
08	60.0	65.0	79.3	47.7	62.3
09	46.0	—[d]			
Mean	55.7±3.2(5)	55.0±3.6(4)	84.9±4.2(3)	50.8±4.2(3)	73.5±4.2(3)
Males					
Control					
22	77.3	42.3	64.7	47.7	49.0
23	73.0	39.7	61.3	47.0	45.0
24	62.7	47.7	68.0	53.0	51.3
27	60.7	41.0	62.3	47.3	54.8
28	60.3	39.0	55.0	50.3	46.0
Mean	66.8±3.2(5)	41.9±3.2(5)	62.3±3.2(5)	49.1±3.2(5)	49.2±3.2(5)

Treatment					
21	54.0	44.7	60.3	35.3	45.3
25	58.3	66.3	103.7	76.7	77.7
26	58.0	54.0	92.0	68.7	86.0
29	72.0	52.0	67.3	62.0	69.0
Mean	60.6±3.6(4)	54.2±3.6(4)	80.8±3.6(4)	60.7±3.6(4)	69.5±3.6(4)
Overall control mean	63.1±2.3(10)	42.1±2.3(10)[e,**]	59.8±2.3(10)	48.2±2.3(10)[e,**]	51.6±2.3(10)[e,**]
Overall treatment mean	57.9±2.4(9)	54.6±2.6(8)[f,**]	82.6±2.7(7)[f,g,**]	56.4±2.7(7)	71.2±2.7(7)[f,g,**]

[a]Values represent plasma concentrations at end of each regimen.

[b,c]SEM was derived from EMS, the pooled variance (52.08), and n, the number of dogs.

[d]Dogs 03 and 09 died during Refeeding I. Lack of datum in Starvation I was due to insufficient sample.

[e]Overall control group means at Starvations I and II and Refeeding II were significantly less than the overall control group mean at Control regimen.

[f]Overall treatment group means at Starvation I and Refeeding I and II were significantly greater than respective regimen means of overall control group.

[g]Overall treatment group means at Refeedings I and II were significantly greater than the overall treatment group mean at Control regimen.

[**]Difference was significant at $P<0.01$.

Table B-14. Analysis of variance plan and observed mean squares for whole plasma cholesterol concentrations in dogs that were periodically starved and refed a high carbohydrate diet

Source of variation	d.f.	Mean squares
Group	1	3507.23
Sex	1	3140.39
Sex · group	1	4764.92
Dog (sex · group) error	15	2817.66
Treatment regimen	4	1218.05*
Group · treatment regimen	4	455.96
Sex · treatment regimen	4	2477.75**
Sex · group · treatment regimen	4	2089.06**
Error	53	448.30
Total	87	

*Statistical significance is at $P<0.05$.

**Statistical significance is at $P<0.01$.

Table B-15. Analysis of variance plan and observed mean squares for whole plasma triglyceride concentrations in dogs that were periodically starved and refed a high carbohydrate diet

Source of variation	d.f.	Mean squares
Group	1	2864.21**
Sex	1	46.01
Sex · group	1	1.83
Dog (sex · group)	15	284.04
Treatment regimen	4	1349.01**
Group · treatment regimen	4	469.66**
Sex · treatment regimen	4	71.26
Sex · group · treatment regimen	4	43.68
Error	53	52.08
Total	87	

**Statistical significance is at $P<0.01$.

Table B-16. Micromethod determined cholesterol and triglyceride concentrations in dog plasma[a]

Regimen / Dog	Control	Starvation I	Refeeding I[b]	Refeeding II
		Cholesterol (mg/100 ml)		
Females				
05	181.8	165.2	136.3	127.2
07	225.0	103.1	105.0	176.9
09	158.8	50.4	81.5	—[c]
Mean	188.5±15.5[d](3)[e]	106.2±15.5(3)[f,**]	107.6±15.5(3)[f,**]	152.0±19.0(2)
Males				
25	139.0	217.9	171.0	109.4
26	132.5	212.9	188.7	99.4
29	123.5	134.9	123.5	93.4
Mean	131.7±15.5(3)	188.6±15.5(3)[g,**]	161.1±15.5(3)	100.7±15.5(3)
		Triglycerides (mg/100 ml)		
Females				
05	35.4	56.7	79.6	84.2
07	38.2	54.8	85.8	74.8
09	34.4	54.5	93.3	—[c]
Mean	36.0±2.6(3)	55.3±2.6(3)[h,**]	86.2±2.6(3)[h,**]	79.5±3.1(2)[h,**]

Males				
25	31.1	78.1	101.0	79.8
26	30.2	71.1	97.9	74.5
29	27.5	66.7	84.0	62.7
Mean	29.6±2.6(3)	72.0±2.6(3)[i,j,**]	94.3±2.6(3)[i,**]	72.3±2.6(3)[i,**]

[a]Each value is the mean of two individual samples with two replicates each, or three individual samples.

[b]Values below represent plasma concentration at 3 days refeeding. Control, Starvation I, and Refeeding II values represent plasma concentrations at end of regimen.

[c]Dog died during Refeeding I.

[d,e]SEM was derived from EMS, the pooled variance (723.6 for cholesterol and 19.56 for triglycerides), and n, the number of dogs.

[f]Female mean cholesterol concentrations of the plasma at Starvation I and Refeeding I were significantly lower than the plasma mean cholesterol value at Control regimen.

[g]Male mean cholesterol concentrations of the plasma at Starvation I was significantly higher than female mean plasma cholesterol at the same regimen.

[h]Female mean plasma triglyceride concentrations at Starvation I and Refeedings I and II were significantly higher than the plasma mean triglyceride value at Control regimen.

[i]Male mean plasma triglyceride concentrations at Starvation I and Refeedings I and II were significantly higher than the plasma mean triglyceride value at Control regimen.

[j]Male mean plasma triglyceride concentration at Starvation I was significantly higher than female mean plasma triglyceride concentration at the same regimen.

[**]Difference was significant at $P<0.01$.

Table B-17. Analysis of variance plan and observed mean squares for micromethod determined whole plasma cholesterol concentrations in dogs that were periodically starved and refed a high carbohydrate diet

Source of variation	d.f.	Mean squares
Sex	1	269.18
Dog (sex)	4	2627.14
Treatment regimen	3	1757.81**
Sex · treatment regimen	3	6442.12
Error	11	723.60
Total	22	

**Statistical significance is at P<0.01.

Table B-18. Analysis of variance plan and observed mean squares for micromethod determined whole plasma triglyceride concentrations in dogs that were periodically starved and refed a high carbohydrate diet

Source of variation	d.f.	Mean squares
Sex	1	43.75
Dog (sex)	4	81.02**
Treatment regimen	3	3545.74**
Sex · treatment regimen	3	194.66**
Error	11	19.56
Total	22	

**Statistical significance is at P<0.01.

Table B-19. Cholesterol and triglyceride concentrations of the plasma chylomicra and VLDL fraction[a] in the dog

Regimen / Dog	Control	Starvation I	Refeeding I[b]	Refeeding II
		Cholesterol (mg/100 ml)		
Females				
05	0.0	1.4	1.7	0.7
07	0.0	1.6	2.1	0.4[c]
09	0.0	1.9	1.8	—
Mean	0.0±0.3[d](3)[e]	1.6±0.3(3)	1.9±0.3(3)	0.6±0.4(2)
Males				
25	2.2	1.7	1.6	1.2
26	0.9	1.7	2.0	0.0
29	1.6	3.3	2.4	0.0
Mean	1.6±0.3(3)	2.2±0.3(3)	2.0±0.3(3)	0.4±0.3(3)
Overall mean	0.8±0.2(6)	1.9±0.2(6)[f,**]	1.9±0.2(6)[f,**]	0.5±0.2(5)
		Triglycerides (mg/100 ml)		
Females				
05	4.0	4.1	9.4	10.2
07	8.5	6.5	27.3	9.7[c]
09	7.7	7.7	21.4	—
Mean	6.7±2.0(3)	6.1±2.0(3)	19.4±2.0(3)	10.0±2.4(2)

Males				
25	6.8	6.3	11.6	13.2
26	8.5	7.2	13.8	14.1
29	6.8	6.9	6.4	8.1
Mean	7.4±2.0(3)	6.8±2.0(3)	10.6±2.0(3)	11.8±2.0(3)
Overall mean	7.0±1.4(6)	6.4±1.4(6)	15.0±1.4(6)[g,**]	11.1±1.5(5)

[a]Each value is the mean of either individual samples with two replicates each or three individual samples.

[b]Values listed below represent plasma fraction concentration at 3 days refeeding. Control, Starvation I, and Refeeding II values represent plasma concentrations at end of regimen.

[c]Dog died during Refeeding I.

[d,e]SEM was derived from EMS, the pooled variance (0.28 for cholesterol and 11.11 for triglycerides) and n, the number of dogs.

[f]The Control regimen mean cholesterol concentration for all dogs (0.8±0.2 mg/100 ml) was significantly less than mean cholesterol concentrations at Starvation I and Refeeding I.

[g]The Control regimen mean triglyceride concentration for all dogs (7.0±1.4 mg/100 ml) was significantly less than mean triglyceride concentration at Refeeding I.

[**]Difference was significant at $P<0.01$.

Table B-20. Analysis of variance plan and observed mean squares for plasma cholesterol concentrations of chylomicra and VLDL fraction in dogs that were periodically starved and refed a high carbohydrate diet

Source of variation	d.f.	Mean squares
Sex	1	1.63
Dog (sex)	4	0.26**
Treatment regimen	3	3.11
Sex · treatment regimen	3	0.81
Error	11	0.28
Total	22	

** Statistical significance is at $P<0.01$.

Table B-21. Analysis of variance plan and observed mean squares for plasma triglyceride concentrations of chylomicra and VLDL fraction in dogs that were periodically starved and refed a high carbohydrate diet

Source of variation	d.f.	Mean squares
Sex	1	11.00
Dog (sex)	4	28.68**
Treatment regimen	3	94.48
Sex · treatment regimen	3	33.47
Error	11	11.11
Total	22	

** Statistical significance is at $P<0.01$.

Table B-22. Cholesterol and triglyceride concentrations of the plasma LDL fraction[a] in the dog

Regimen / Dog	Control	Starvation I	Refeeding I[b]	Refeeding II
		Cholesterol (mg/100 ml)		
Females				
05	18.6	15.6	21.0	25.3
07	36.6	11.5	14.2	33.0
09	11.0	4.6	21.8	—[c]
Mean	22.1±4.3[d](3)[e]	10.6±4.3(3)	19.0±4.3(3)	29.2±5.3(2)
Males				
25	33.4	57.1	56.3	16.9
26	30.0	53.2	43.7	16.5
29	24.6	33.8	24.2	13.3
Mean	29.3±4.3(3)	48.0±4.3(3)[f,**]	41.4±4.3(3)[f,**]	15.6±4.3(3)
		Triglycerides (mg/100 ml)		
Females				
05	13.0	7.5	30.6	27.6
07	15.7	8.6	15.1	24.2
09	14.2	8.4	30.5	—[c]
Mean	14.3±2.0(3)	8.2±2.0(3)	25.4±2.0(3)[g,**]	25.9±2.5(2)[g,**]

Males				
25	10.2	21.0	40.4	25.9
26	10.0	19.0	36.9	26.2
29	9.2	16.7	33.0	23.3
Mean	9.8±2.0(3)	18.9±2.0(3)[h,i,**]	36.8±2.0(3)[h,i,**]	25.1±2.0(3)[h,**]

[a]Each value is the mean of either two individual samples with two replicates each or three individual samples.

[b]Values listed represent plasma fraction concentration at 3 days refeeding. Control, Starvation I, and Refeeding II values represent plasma concentrations at end of regimen.

[c]Dog died during Refeeding I.

[d,e]SEM was derived from EMS, the pooled variance (56.34 for cholesterol and 12.66 for triglycerides) and n, the number of dogs.

[f]Male mean plasma LDL cholesterol concentrations were significantly greater than those of females at Starvation I and Refeeding I.

[g]Female Control regimen mean triglyceride concentration (14.3±2.0 mg/100 ml) was significantly less than mean triglyceride concentrations at both refeedings.

[h]Male Control regimen mean triglyceride concentration (9.8±2.0 mg/100 ml) was significantly less than mean triglyceride concentrations at Starvation I and both refeedings.

[i]Male mean plasma LDL triglyceride concentrations were significantly greater than those of females at Starvation I and Refeeding I.

[**]Difference was significant at $P<0.01$.

Table B-23. Analysis of variance plan and observed mean squares for plasma cholesterol concentrations of the LDL fraction in dogs that were periodically starved and refed a high carbohydrate diet

Source of variation	d.f.	Mean squares
Sex	1	1012.10
Dog (sex)	4	183.52
Treatment regimen	3	85.37**
Sex · treatment regimen	3	566.47
Error	11	56.34
Total	22	

**Statistical significance is at P<0.01.

Table B-24. Analysis of variance plan and observed mean squares for plasma triglyceride concentrations of the LDL fraction in dogs that were periodically starved and refed a high carbohydrate diet

Source of variation	d.f.	Mean squares
Sex	1	100.01
Dog (sex)	4	18.08**
Treatment regimen	3	508.19**
Sex · treatment regimen	3	97.60
Error	11	12.66
Total	22	

**Statistical significance is at P<0.01.

Table B-25. Cholesterol and triglyceride concentrations of the plasma HDL fraction[a] in the dog

Dog \ Regimen	Control	Starvation I	Refeeding I[b]	Refeeding II
		Cholesterol (mg/100 ml)		
Females				
05	161.0	133.6	101.0	78.2
07	185.1	86.9	84.8	120.7[c]
09	140.6	35.8	54.2	—
Mean	162.2±12.7[d](3)[e]	85.4±12.7(3)[f,**]	80.0±12.7(3)[f,**]	99.4±15.2(2)[f,**]
Males				
25	92.2	154.6	109.7	83.5
26	88.7	155.0	128.8	77.0
29	94.5	91.1	84.3	78.6
Mean	91.8±12.7(3)[g,**]	133.6±12.7(3)	107.6±12.7(3)	79.7±12.7(3)
		Triglycerides (mg/100 ml)		
Females				
05	9.1	24.6	19.5	19.6
07	7.6	21.8	20.6	17.3[c]
09	7.6	18.2	18.6	—
Mean	8.1±0.9(3)	21.5±0.9(3)[h,**]	19.6±0.9(3)[h,**]	18.4±1.1(2)[h,**]

Males				
25	5.0	27.6	18.8	18.5
26	4.0	26.4	22.8	17.5
29	4.2	27.0	20.7	14.2
Mean	4.4±0.9(3)	27.0±0.9(3)[h,i,**]	20.8±0.9(3)[h,**]	16.7±0.9(3)[h,**]

[a]Each value is the mean of either two individual samples with two replicates each or three individual samples.

[b]Values listed below represent plasma fraction concentration at 3 days refeeding. Control, Starvation I, and Refeeding II values represent plasma concentrations at end of regimen.

[c]Dog died during Refeeding I.

[d,e]SEM was derived from EMS, the pooled variance (490.54 for cholesterol and 2.53 for triglycerides), and n, the number of dogs.

[f]Female Control regimen mean cholesterol concentration (162.2±12.7 mg/100 ml) was significantly greater than mean cholesterol concentrations at Starvation I and both refeedings.

[g]Male Control regimen mean cholesterol concentration was significantly less than the female cholesterol concentration at the same regimen.

[h]Both female and male Control regimen mean triglyceride concentrations were significantly less than triglyceride values for each sex at Starvation I and both refeedings.

[i]Male mean triglyceride concentration at Starvation I was significantly greater than the female mean triglyceride value for the same regimen.

**Difference is significant at $P<0.01$.

Table B-26. Analysis of variance plan and observed mean squares for plasma cholesterol concentrations of the HDL fraction in dogs that were periodically starved and refed a high carbohydrate diet

Source of variation	d.f.	Mean squares
Sex	1	74.04
Dog (sex)	4	1567.78*
Treatment regimen	3	2086.82**
Sex · treatment regimen	3	4022.67
Error	11	480.54
Total	22	

*Statistical significance is at $P<0.05$.

**Statistical significance is at $P<0.01$.

Table B-27. Analysis of variance plan and observed mean squares for plasma triglyceride concentrations of the HDL fraction in dogs that were periodically starved and refed a high carbohydrate diet

Source of variation	d.f.	Mean squares
Sex	1	0.55
Dog (sex)	4	4.58**
Treatment regimen	3	356.83**
Sex · treatment regimen	3	22.13
Error	11	2.53
Total	22	

**Statistical significance is at $P<0.01$.

Table B-28. Cholesterol and triglyceride concentrations of the plasma VHDL fraction[a] in the dog

Regimen / Dog	Control	Starvation I	Refeeding I[b]	Refeeding II
		Cholesterol (mg/100 ml)		
Females				
05	0.0	1.2	3.6	0.7
07	0.0	0.9	2.9	2.3[c]
09	0.0	1.0	3.4	—
Mean	0.0±0.3[d](3)[e]	1.0±0.3(3)	3.3±0.3(3)[f,g,**]	1.5±0.4(2)[f,**]
Males				
25	0.0	1.6	0.0	0.6
26	1.2	0.6	0.0	0.6
29	1.0	1.8	0.0	0.6
Mean	0.7±0.3(3)	1.3±0.3(3)	0.0±0.3(3)	0.6±0.3(3)
		Triglycerides (mg/100 ml)		
Females				
05	4.0	15.8	17.0	16.3
07	3.2	17.2	19.6	14.5[c]
09	4.0	18.5	18.4	—
Mean	3.7±0.6(3)	17.2±0.6(3)	18.3±0.6(3)	15.4±0.7(2)

Males				
25	3.8	16.9	18.3	15.9
26	4.7	17.3	18.7	15.6
29	3.8	14.7	18.2	13.8
Mean	4.1±0.6(3)	16.3±0.6(3)	18.4±0.6(3)	15.1±0.6(3)
Overall mean	3.9±0.4(6)	16.7±0.4(6)[h,**]	18.4±0.4(6)[h,**]	15.2±0.4(5)[h,**]

[a]Each value is the mean of either two individual samples with two replicates each or three individual samples.

[b]Values listed below represent plasma fraction concentration at 3 days refeeding. Control, Starvation I, and Refeeding II values represent plasma concentrations at end of regimen.

[c]Dog died during Refeeding I.

[d,e]SEM was derived from EMS, the pooled variance (0.27 for cholesterol and 0.91 for triglycerides), and n, the number of dogs.

[f]Female Control regimen mean cholesterol concentration (0.0±0.3 mg/100 ml) was significantly less than the values at both refeedings.

[g]Female mean cholesterol concentration at Refeeding I was significantly greater than the male concentration of cholesterol at that regimen.

[h]The Control regimen mean triglyceride concentration for all dogs (3.9±0.4 mg/100 ml) was significantly less than mean triglyceride concentrations at Starvation I and both refeedings.

**Difference was significant at $P<0.01$.

Table B-29. Analysis of variance plan and observed mean squares for plasma cholesterol concentrations of the VHDL fraction in dogs that were periodically starved and refed a high carbohydrate diet

Source of variation	d.f.	Mean squares
Sex	1	3.54**
Dog (sex)	4	0.05**
Treatment regimen	3	1.69
Sex · treatment regimen	3	4.91
Error	11	0.27
Total	22	

**Statistical significance is at P<0.01.

Table B-30. Analysis of variance plan and observed mean squares for plasma triglyceride concentrations of the VHDL fraction in dogs that were periodically starved and refed a high carbohydrate diet

Source of variation	d.f.	Mean squares
Sex	1	0.19
Dog (sex)	4	1.55**
Treatment regimen	3	257.31
Sex · treatment regimen	3	0.47
Error	11	0.91
Total	22	

**Statistical significance is at P<0.01.

Table B-31. Percentage of the total plasma cholesterol and triglycerides in the chylomicra and VLDL fraction[a] in the dog

Dog \ Regimen	Control	Starvation I	Refeeding I	Refeeding II
		Cholesterol (%)		
Females				
05	0.0	0.9	1.3	0.7
07	0.0	1.6	2.0	0.2[b]
09	0.0	4.4	2.2	—[b]
Mean	0.0	1.6	1.8	0.5
Males				
25	1.7	0.8	1.0	1.2
26	0.8	0.8	1.2	0.0
29	1.3	2.5	2.2	0.0
Mean	1.3	1.2	1.3	0.4
		Triglycerides (%)		
Females				
05	13.3	7.9	12.3	13.8
07	24.3	12.0	33.1	14.8[b]
09	23.0	14.6	24.1	—[b]
Mean	20.4	11.5	23.5	14.3
Males				
25	26.4	8.8	13.0	18.0
26	31.2	10.3	15.0	19.2
29	28.4	10.6	8.2	13.6
Mean	28.8	9.8	12.3	17.2

[a]The percentage was determined by obtaining for each fraction the mean of two individual samples (two replicates each) or of three individual samples and dividing the mean of each fraction by the sum of the means of all four fractions.

[b]Dog died during Refeeding I.

Table B-32. Percentage of the total plasma cholesterol and triglycerides in the LDL fraction[a] in the dog

Dog \ Regimen	Control	Starvation I	Refeeding I	Refeeding II
		Cholesterol (%)		
Females				
05	10.4	10.3	16.5	24.1
07	16.5	11.4	13.7	21.1[b]
09	7.3	10.6	26.8	—[b]
Mean	12.0	10.8	18.2	22.3
Males				
25	26.1	26.6	33.6	16.5
26	24.8	25.3	25.0	17.6
29	20.2	26.0	21.8	14.4
Mean	23.7	25.9	27.4	16.2
		Triglycerides (%)		
Females				
05	43.2	14.4	40.0	37.5
07	44.9	15.9	18.3	36.8[b]
09	42.4	15.9	34.3	—[b]
Mean	43.6	15.5	30.7	37.2
Males				
25	39.5	29.2	45.4	35.2
26	36.8	27.2	40.0	35.7
29	38.3	25.6	42.2	39.2
Mean	38.1	27.4	42.5	36.5

[a]The percentage was determined by obtaining for each fraction the mean of two individual samples (two replicates each) or of three individual samples and dividing the mean of each fraction by the sum of the means of all four fractions.

[b]Dog died during Refeeding I.

Table B-33. Percentage of the total plasma cholesterol and triglycerides in the HDL fraction[a] in the dog

Dog \ Regimen	Control	Starvation I	Refeeding I	Refeeding II
		Cholesterol (%)		
Females				
05	89.6	88.0	79.4	74.5
07	83.5	86.1	81.5	77.2
09	92.7	82.7	66.8	—[b]
Mean	88.0	86.6	76.8	76.1
Males				
25	72.2	71.9	65.4	81.7
26	73.4	73.6	73.8	81.8
29	77.7	70.1	76.0	85.0
Mean	74.4	72.2	71.3	82.8
		Triglycerides (%)		
Females				
05	30.2	47.3	25.5	26.6
07	21.7	40.3	24.9	26.3
09	22.7	34.5	20.9	—[b]
Mean	24.7	40.6	23.7	26.4
Males				
25	19.4	38.5	21.1	25.2
26	14.7	37.8	24.7	23.8
29	17.5	41.3	26.4	23.9
Mean	17.1	39.2	24.0	24.3

[a]The percentage was determined by obtaining for each fraction the mean of two individual samples (two replicates each) or of three individual samples and dividing the mean of each fraction by the sum of the means of all four fractions.

[b]Dog died during Refeeding I.

Table B-34. Percentage of the total plasma cholesterol and triglycerides in the VHDL fraction[a] in the dog

Regimen / Dog	Control	Starvation I	Refeeding I	Refeeding II
		Cholesterol (%)		
Females				
05	0.0	0.8	2.8	0.7
07	0.0	0.9	2.8	1.5[b]
09	0.0	2.3	4.2	—
Mean	0.0	1.0	3.2	1.1
Males				
25	0.0	0.7	0.0	0.6
26	1.0	0.3	0.0	0.6
29	0.8	1.4	0.0	0.6
Mean	0.6	0.7	0.0	0.6
		Triglycerides (%)		
Females				
05	13.3	30.4	22.2	22.1
07	9.1	31.8	23.7	22.1[b]
09	11.9	35.0	20.7	—
Mean	11.3	32.4	22.1	22.1
Males				
25	14.7	23.5	20.5	21.6
26	17.3	24.7	20.3	21.3
29	15.8	22.5	23.2	23.3
Mean	16.0	23.6	21.2	22.0

[a]The percentage was determined by obtaining for each fraction the mean of the two individual samples (two replicates each) or of three individual samples and dividing the mean of each fraction by the sum of the means of all four fractions.

[b]Dog died during Refeeding I.

Table B-35. Correlation coefficients, positive and negative, for cholesterol and triglycerides in dog plasma and its lipoprotein fractions

Constituent	Plasma Chol.	Plasma Trig.	VLDL Chol.	VLDL Trig.	LDL Chol.	LDL Trig.	HDL Chol.	HDL Trig.	VHDL Chol.	VHDL Trig.
Plasma Cholesterol	1.0000 0.0000[a]	-0.0546 0.7999	-0.1614 0.5318	-0.3341 0.1158	0.7342 0.0002	0.0896 0.6866	0.9449 0.0001	0.0641 0.7683	-0.2781 .1962	-0.2612 0.2268
Plasma Triglycerides	-0.0546 0.7999	1.0000 0.0000	0.3154 0.1394	0.5421 0.0075	0.2879 0.1800	0.8290 0.0001	-0.2224 0.3084	0.7410 0.0002	0.3142 0.1410	0.8788 0.0001
VLDL Cholesterol	-0.1614 0.5318	0.3154 0.1394	1.0000 0.0000	0.1218 0.5860	0.2372 0.2754	0.0628 0.7724	-0.3225 0.1301	0.4783 0.0199	0.2867 0.1819	0.4381 0.0346
VLDL Triglycerides	-0.3341 0.1158	0.5421 0.0075	0.1218 0.5860	1.0000 0.0000	-0.1064 0.6341	0.3524 0.0958	-0.3604 0.0878	0.1426 0.5229	0.4809 0.0192	0.4089 0.0501
LDL Cholesterol	0.7342 0.0002	0.2879 0.1800	0.2372 0.2754	-0.1064 0.6341	1.0000 0.0000	0.3875 0.0648	0.5046 0.0135	0.2396 0.2702	-0.1458 0.5133	0.0631 0.7714
LDL Triglycerides	0.0896 0.6866	0.8290 0.0001	0.0628 0.7724	0.3524 0.0958	0.3875 0.0648	1.0000 0.0000	-0.0782 0.7227	0.3607 0.0876	0.0495 0.8171	0.5255 0.0098
HDL Cholesterol	0.9449 0.0001	-0.2224 0.3084	-0.3225 0.1301	-0.3604 0.0878	0.5046 0.0135	-0.0782 0.7227	1.0000 0.0000	-0.0435 0.8378	-0.3118 0.1442	-0.3835 0.0679
HDL Triglycerides	0.0641 0.7683	0.7410 0.0002	0.4783 0.0199	0.1426 0.5229	0.2396 0.2702	0.3607 0.0876	-0.0435 0.8378	1.0000 0.0000	0.3012 0.1595	0.8753 0.0001
VHDL Cholesterol	-0.2782 0.1962	0.3142 0.1410	0.2867 0.1819	0.4809 0.0192	-0.1458 0.5133	0.0495 0.8171	-0.3118 0.1442	0.3012 0.1595	1.0000 0.0000	0.3806 0.0701
VHDL Triglycerides	-0.2612 0.2268	0.8788 0.0001	0.4381 0.0346	0.4089 0.0501	0.0631 0.7714	0.5255 0.0098	-0.3835 0.0679	0.8758 0.0001	0.3806 0.0701	1.0000 0.0000

[a]Value represents the level at which the correlation coefficient above is significant.