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Flaws, Fallacies and Facts: Reviewing the Early History of the Lipid and Diet/Heart Hypotheses

J. Elliott*

Independent, Bega, Australia
Email: jennifer_lpe@hotmail.com

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Abstract

The lipid hypothesis of coronary heart disease proposes that a high total cholesterol level has a causative role in coronary heart disease (CHD), specifically in the development of atherosclerosis. It forms the basis for formulating target levels of serum cholesterol and hence the widespread use of statins for lowering cholesterol. An extension of the lipid hypothesis is the diet/heart hypothesis of coronary heart disease. This theory combines two ideas—that saturated fat raises cholesterol levels, and that a reduced saturated fat intake will lower cholesterol levels, thereby inhibiting the development of atherosclerosis and manifestations of CHD. Those who make diet recommendations or prescribe medication to reduce cholesterol may be unaware of the underpinning science. The original research behind these recommendations has given us "healthy heart" guidelines and preventive measures we assume to be true. While the lipid and diet/heart hypotheses are often presented as fact, they remain inadequately proven theories that have little agreement from experts. Historical perspectives can help us understand the basis of current-day beliefs. In the lipid hypothesis case, research from the 1950s and 60s was instrumental in its formation. This early work should not be considered irrelevant, outdated or obsolete because current recommendations from national heart associations in many countries continue to be shaped by these studies. This paper examines evidence used to formulate the lipid hypothesis and, subsequently, the diet/heart hypothesis. By critically evaluating steps in the formation of the theory, inconsistencies, mistakes and alternate explanations become apparent and cast doubt on its validity.

Keywords

Heart, Cholesterol, Fat

*Jennifer Elliott is a dietitian and the author of *Baby Boomers, Bellies and Blood Sugars* (2011).

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1. Introduction

The advice to reduce the amount of saturated fat we eat by trimming fat off meat, choosing reduced fat dairy products and not eating the skin on chicken is commonly recommended by nutrition advisory organisations [1]-[3]. It is thought that making such changes will reduce a person's total cholesterol (TC) level, thereby reducing their risk of developing coronary heart disease (CHD).

This reasoning is based on the belief that saturated fat increases the level of cholesterol in the blood, predisposing to cholesterol deposition in arteries and ultimately CHD. Unknown to many people, this lipid hypothesis of coronary heart disease is a theory that has never been proven nor agreed upon by scientists and researchers, even though it is often presented as fact.

The hypothesis was developed from five lines of evidence:

1) Diet experiments which reported that saturated fat increased total cholesterol (TC) levels;

2) A reported positive association between the saturated fat intake of a country and mortality from CHD;

3) Presence of cholesterol in human atherosclerotic plaques;

4) Population surveys where higher TC levels were found to be associated with a higher incidence of CHD;

5) Experiments where animals, usually rabbits, when fed large amounts of cholesterol developed high serum cholesterol levels and deposits of cholesterol in their arteries.

The lipid hypothesis was formulated to fit the above observations. Evidence from such observations is, however, circumstantial and those proposing the theory 50 years ago were aware of and acknowledged this fact. Even so, the theory was further expanded to propose that if TC levels were lowered by reducing saturated fat intake, protection against or delay in the development of atherosclerosis or manifestations of CHD will result. Commonly known as the diet/heart hypothesis of coronary heart disease, this theory has had widespread acceptance for many years and is the basis for advice designed to influence the diet of much of the world's population.

Anyone who thinks they are making healthy choices by using margarine or polyunsaturated oil instead of butter, low fat instead of whole milk and conscientiously removes the crispy skin from chicken has been influenced by the diet/heart hypothesis. They believe they are reducing their risk of heart disease and are generally not aware that making such choices has never been proven to be beneficial. In fact, the theory has never been adequately tested. The choices about what to eat for a "healthy heart" that people make today have been shaped by events and decisions made 50 years ago. Understanding this history is necessary to evaluate whether, for example, crispy chicken skin should go in the bin.

2. Early Research

The diet/heart hypothesis had its inception in the US. The idea that diet, particularly its fat and cholesterol content, may be related to the development of atherosclerosis and manifestations of CHD were discussed in the scientific literature in the 1940s and 50s. Despite the lack of evidence of any benefit, a low fat/low cholesterol diet had some support at that time. In reference to such a diet, it was stated in 1954 [4], "There is by no means agreement as to the value of the diet or its quantitative definition. ...Nevertheless, increasing numbers of physicians are prescribing the diet..."

A report to the American Heart Association (AHA) in 1957 stated, "The aim of this discussion is to summarize and evaluate evidence for and against the concept that the fat content of the average present-day North American or north European diet is a significant factor in the genesis of cerebral, myocardial, renal, or peripheral atherosclerosis" [5]. The conclusion stated, "Thus, the evidence at present does not convey any specific implications for drastic dietary changes, specifically in the quantity or type of fat in the diet of the general population, on the premise that such changes will definitely lessen the incidence of coronary or cerebral artery disease".

However four years later, without any additional research evidence but following changes to the committee membership, the A.H.A. advocated a reduced intake of saturated and total fat and an increased intake of polyunsaturated fat. In its 1961 report the A.H.A reported these changes "…as a possible means of preventing atherosclerosis and decreasing the risk of heart attacks and strokes" [6].

3. Diet/Heart Controversy

Neither the diet/heart hypothesis nor the recommendation for the population to reduce the intake of saturated fat had universal approval from scientists in 1961, nor does it today. Supporters of dietary change, both at the time

of the first proposal as well as later, believed the evidence was strong enough to implicate saturated fat and high total cholesterol as culprits in causation of CHD [7]-[11]. In addition, many felt that the high CHD mortality required preventive action and that waiting for definitive evidence would cost lives that may otherwise be saved.

An example of the belief that action was warranted before definitive evidence was established is indicated when the president of the American Heart Association in 1975 stated in his presidential address [11], "We still do not understand the basic etiology of the arterial lesion," but, "…don't ask that everything be known about the disease before implementing preventive programs". Many have opposed the hypothesis and large-scale diet change [12]-[25], pointing out flaws in dietary data, problems in extrapolating from animal studies, lack of agreement as to the causation of atherosclerosis and CHD, pitfalls in interpreting population studies and urged caution in recommending changes in diet with potentially unknown consequences.

Taubes [26] reports lipid researcher E.H. Ahrens as testifying, in reference to the 1977 US Dietary Goals proposal for Americans to reduce total and saturated fat, that this advice "…on the strength of such marginal evidence was equivalent to conducting a nutritional experiment with the American public as subjects". The controversy that continued to surround the diet/heart issue during the 1980s was considered undesirable and summed up by D. B. Zilversmit in a 1982 editorial as, "The argument about the 'diet-heart disease question' has left the scientific community exhausted and the public confused" [10].

The debate may have been settled after the US Surgeon General's Office in 1988 commissioned a committee to look at all the published evidence and decide whether it supported the diet/heart hypothesis or not. Unfortunately, the question was not resolved. Committee members could not say that the theory was correct, nor would they say that it was incorrect or at least make it clear to the public that such differences of opinion existed. After working on the project for 11 years, the committee of investigators was disbanded with the unsatisfactory explanation that the project required more expertise and staff resources than expected [27].

In reference to this reported explanation and in view of the not insurmountable difficulty of conducting a literature review on the subject, Dr. Malcom Kendrick commented [27], "After eleven years, they needed additional expertise and staff resources? What had they been doing up to then? Using a million monkeys bashing away randomly at type-writers in an attempt to produce a report?" However, the hypothesis, still without proof and more clearly without consensus, remained.

The efficacy of diet changes based on the diet/heart hypothesis came into further question after the publication of a report in 1991 titled, "What if Americans ate less fat? [28]" The authors calculated changes in life expectancy if all Americans followed the low saturated fat guideline for a lifetime and, theoretically, resulted in the expected lowering of serum cholesterol. The result was that after a lifetime of skinless chicken, low fat cheese and skim milk, an increase in average life expectancy of 3 to 4 months was proposed. This was based on statistical modelling and therefore its relevance to real life could be questioned. Similarly, however, the advice to eat less saturated fat in order to lower TC to prevent CHD is also based on statistical modelling; its relevance to real life could also be questioned on the same basis.

It is quite a responsibility to advise an individual, let alone an entire population, to change their eating habits. Those doing so need to be confident that the advice is correct, effective, will achieve its aims and not cause harm. A question that may be asked today is whether we should put our faith in the theory that reducing saturated fat will reduce our risk of CHD. It can be difficult to disprove a theory once it has taken hold in the collective psyche. One way to assess a theory is to look at the evidence on which it is based. In the case of the diet/heart hypothesis, the five lines of evidence on which it was based can be considered separately.

4. Diet Experiments

Most of the diet experiments that led to the belief that saturated fat increased TC were carried out in the 1950s and 60s, a time when many researchers were collecting data on what affected TC. It had been demonstrated that, at least in the short-term, TC was affected by variables such as stress [29] [30], plant sterols [31], dietary cholesterol [32]-[34], and carbohydrate [35]. Mechanisms of change or control of TC were unknown; however, two findings were well documented.

4.1. Individual Variability

Firstly, the variability of TC in some individuals, based on repeated tests, had been demonstrated [36] [37]. In one study multiple TC levels were determined among fasting individuals over a 5 hour period [34]. Levels var-

ied by as little as 25 mg% (0.65 mmol/l) in some individuals to as much as 84 mg% (2.17 mmol/l) in others. The authors reported, "Such variations render single or haphazard studies of blood cholesterol of doubtful value".

4.2. Short-Term Nature of TC Changes

The second well-documented finding in studies where diet changes were continued for a longer duration was that an initial change in TC was often followed by a return to the pre-test level [38]. Changes in TC occurring in the first 1 - 2 weeks of a new diet regimen would often return to pretreatment levels after some months. As Pomeranze noted, "The serum cholesterol of normal and hypercholesterolemic subjects was lowered by a rigidly fat restricted eucaloric diet. However, when these studies were continued for longer periods, serum cholesterol rose toward pretreatment levels despite the continuance of the low fat diet" [39]. Based on this knowledge, it was important that any experiments looking at TC changes firstly ascertained the normal variation of the individuals involved and maintained the test conditions long enough for equilibrium to be reached.

Ancel Keys, past head of the Department of Hygiene at Minnesota University conducted a series of dietary experiments in the 1950s on long-term hospitalised male patients [40]. The results of these experiments, showing changes in TC of groups of men on "controlled" diets, have been influential in the current belief that saturated fat increases TC so these will now be discussed in some detail. In Keys's diet studies, small groups of 6 - 12 men were fed diets containing different types of fat, generally for 2 - 4 weeks each. TC levels for the men in each group were usually measured near the end of each diet period and the mean level for the group calculated. Keys then compared the means to each other, attributing any differences in TC entirely to the fat in question. An example of one series from these experiments may help to illustrate Keys's study design.

Eight men on a "normal" hospital diet each had their TC level measured at the end of four weeks and the TC for the group calculated. The men were then changed to a low fat/higher carbohydrate diet for four weeks and the mean TC calculated at the end of this period. An immediate change to another four-week period followed, where olive oil replaced a proportion of sugar, jelly, rice, potato and bread in their diets. For the last four-week period the diet contained cotton-seed oil instead of olive oil.

The mean TC levels for each diet group were:

normal diet: 229 mg/dl (5.92 mmol/l);

low fat: 198.2 mg/dl (5.13 mmol/l);

olive oil: 213.6 mg/ dl (5.52 mmol/l);

cotton-seed oil: 208.8 mg/dl (5.4 mmol/l).

Keys compared these figures by subtracting one mean from another, concluding that the results were due to differences in the fatty acids of each oil. These studies had design flaws that preclude any useful conclusions being made.

Keys did not determine the degree of individual variation first, therefore it is unknown if any variation during a diet period is due to the test fat or a reflection of normal variation for any individual.

Hegsted [41] reports that when Keys's data was re-examined 20 years later, "The mean intra-individual standard deviation in these studies was 17.8 mg/dl, and it ranged from 6.0 to 44.9 mg/dl". The majority of differences in mean TC measured on the experimental diets was 0.4 - 30 mg/dl (0.01 - 0.8 mmol/l). Such relatively small differences could be partly due to normal individual variation and therefore not to dietary changes.

Time was not allowed for equilibrium to be reached during the diet periods.

As the diet periods were limited to 2 - 4 weeks duration, Keys measured only initial and probably short-term changes. As Epstein stated "Short-term feeding experiments may be misleading in this field, since it appears that serum lipids may rebound to their starting level after some weeks, even though the experimental regime is continued" [42].

Keys's data collection is therefore flawed in respect to study design. In addition, the way in which he interpreted his data is also questionable [43]. Firstly, Keys compared the average TC levels of men in different groups. This was despite the fact that, as his results show, even on the same diet different groups had different mean TC levels. For example, average TC of one group on the low fat diet was 181 and 203 mg/dl (4.73 and 5.25 mmol/l) for a second group. Secondly, and of major importance, Keys wrongly maintained that changes in TC levels were due only to the fatty acid composition of the test fats.

However, as pointed out by Reiser in his review of diet experiments up until 1973, including those by Keys, alternate explanations for observed changes in TC could be put forward [19]. For instance, in Keys's experiment a reduction in the mean TC of the seven men taking 100 ml sunflower oil for 2 weeks was interpreted by Keys

to be due to the polyunsaturated (PUS) nature of the oil. It could equally be due to this oil's sterol content.

Likewise, when the TC level increased following the men's dietary change from 100 ml sunflower oil to 100 g butter, Keys attributed this to the saturated fats in the butter. However, simply eliminating the cholesterol-suppressing effect of sterols when the sunflower oil was stopped could result in a rise in TC. In addition, the extra (approximate) 200 mg of cholesterol in the butter could also cause a temporary rise in TC. Even though it had been documented at the time that TC was influenced by plant sterols and dietary cholesterol, Keys erroneously attributed TC changes entirely to the respective fatty acids of the test fats. This point alone invalidates his conclusions of the effects of fatty acids, including his conclusion that saturated fat increases TC.

Although any conclusions about the effects of fat on TC based on this data are highly questionable, it is interesting to look at the method Keys used to further interpret his data. This is of interest because many subsequent researchers, as well as the National Heart Foundation of Australia [44] and American Heart Association [45] appear to have accepted his interpretation and continue to use this type of statistical methodology as a basis for dietary recommendations.

Following Keys's incorrect line of thinking that fatty acids were the only dietary variables affecting TC, he then formulated an equation that he believed represented their effect. He wrote the equation as:

$$\text{chol } 1 = a + b\text{S}1 + c\text{M}1 + d\text{P}1$$

where S, M and P represent the percentage of total calories supplied by saturated, monounsaturated and polyunsaturated fats respectively [43]. In addition, *b*, *c* and *d* are coefficients that supposedly express the influence of the respective fatty acids on TC.

In recognition that there are other factors likely to affect TC, Keys designated *a* to cover these. He gives no information about *a* other than, "…*a* is a characteristic of the particular group of men, independent of the diet fat" [43]. By failing to explain his logic about how it is possible to characterise a group of humans (e.g. in terms of cholesterol homeostasis), this is left to the reader's imagination. However, Keys designated *a* with the numerical value of −1.68. The equation represents Keys's belief that the TC level of a group of people is determined by the types of fats they eat plus an undefined "group characteristic".

Following this reasoning, Keys then entered flawed data from poorly designed dietary experiments into his equation and came up with numerical values that reflected the *probability* of S, M, P or the ubiquitous *a* influencing the TC level. Statistically it looked like the group characteristic and monounsaturated fats did not have much effect because their coefficients were small, so both were subsequently deleted from the equation. (This was the origin of the idea that monounsaturated fats do not affect the TC level). Keys made another couple of statistical alterations for the new equation that now contained only figures for S and P. The final result is, unsurprisingly, known as the Keys equation. It states that saturated fat has twice the ability to raise TC as polyunsaturated fat has to lower it.

This equation, formed from both flawed design and flawed interpretation of dietary studies, and indicating only statistical *probabilities* of the effects on TC of various fats, has been largely accepted as meaning that saturated fat increases and polyunsaturated fat decreases TC in real life. This error in thinking that probabilities derived from statistical equations equate with real life effects appears to have been, and remains, overlooked by many researchers.

Once the erroneous idea that statistical equations of probabilities could give useful information about real life cause and effect was accepted, such equations were employed to determine which individual saturated fatty acids were the culprits. Non-statisticians may think that each fatty acid would have to be extracted and tested separately for an effect on TC to be determined. This was not done, however, and statistical modelling was used instead, with the same erroneous conclusions that *probabilities* of association equate with actual cause and effect.

There has been some ongoing debate about which are the rogue saturated fatty acids because different researchers used different equations to supposedly determine this and therefore get different results. Generally, the current agreement that was confidently stated by the National Heart Foundation of Australia (NHFA) in 1992 is, "Palmitic and myristic acids, and to a lesser extent lauric acid, are the major cholesterol raising saturated fatty acids" [46].

Although the misuse of statistical equations appears to go unchallenged today, this was not the case when they were first developed. In 1965 Hegsted expressed caution in their use [47]. As Professor of Nutrition at Harvard University, Hegsted conducted experiments on the effects of diet on TC and also formulated equations that 'fitted' his observations. He, however, was aware of the limitations in their use when he stated,

> In the discussion of this paper, we indicated the rather severe limitations of multiple regression analysis as a means of determining the specific effects of the individual variables. Presumably, the effects of each fatty acid can only be determined with security by changing each fatty acid independently and keeping the rest of the diet constant. This cannot be done with natural oils.

He went on to say,

> Regression equations are simply descriptive and the equations we have developed appear to give the best description of the results in terms of the fatty acid composition that we can obtain. This is to say that the dietary oils act as though the specific fatty acids they contain had the activity proportional to their regression coefficients. The equations do not prove that the fatty acids have these effects.

Hegsted also pointed out that,

> These equations are primarily descriptive of the information from which they are derived. Should new variables be included, such as a carbohydrate component, the regression coefficients for the fatty acids might be expected to change. It is somewhat hazardous to attach as much functional significance to the regression equations as Keys *et al*. have done.

It is therefore likely that had variables such as dietary cholesterol and plant sterols been included, the supposed effect of the fats would be quite different. Hegsted was clearly trying to educate others that these equations do not ***predict*** what can happen to cholesterol levels, they merely ***describe***, in a purely statistical sense what has been observed.

This is an important distinction because the National Heart Foundation of Australia's advice for the Australian population to reduce saturated fat is based on the belief that the Keys, or similar equations, are predictive. This was stated clearly in a 1994 NHFA report [44].

> In the 1950s and early 1960s the studies of Keys *et al*. (1) and Hegsted *et al*. (2) provided the first quantitative estimates of the effects of different fatty acids on blood cholesterol. The results of both groups were similar. Compared with carbohydrate, saturated fats were found to have a potent cholesterol raising effect while polyunsaturated fats reduced blood cholesterol levels. Monounsaturated fats had a neutral effect. Based on these studies Keys and Hegsted developed equations to predict the changes in blood cholesterol with changes in dietary fat. Policies for the dietary prevention of coronary heart disease are based on these equations.

The Hegsted article cited by the NHFA as supporting evidence, is the article *cautioning* the use of, and questioning the validity of, such equations in predicting changes. Flawed data aside, it should also be kept in mind that information collected from Keys's experiments, which the NHFA uses as the basis for their diet recommendations to the Australian population, was collected from only 68 hospitalized men in the US.

Understanding this background on how saturated fat came to be thought of as "bad" should be enough to cast doubt on the conclusion. In addition, one could look at diet experiments to ascertain whether the Keys equation correctly predicts observed changes. One such example is reported by Conner *et al.* [48]. In this study, diets of different fat composition were given to volunteers and changes in TC recorded. Keys's equation was then applied and its accuracy in predicting TC change determined. The authors report,

> From Periods 1 to II, the use of the formula would predict no change in the serum cholesterol level. As noted, the mean serum cholesterol actually declined 38 mg per 100 ml. From Periods II to III, the application of the formula would predict a decline of 32 mg per 100 ml. The actual change was only 2 mg per 100 ml. Finally, from Periods III to IV, with the formula, no change in serum cholesterol would be expected, whereas there was actually an increase of 28 mg per 100 ml. Apparently, the cholesterol content of the diet was an important factor not considered by this prediction formula.

If more evidence is required, one could look at dietary experiments that failed to find that saturated fat increased TC. One example concerns a group of seven men who, because of multiple food allergies, were required to follow a diet in which most of the calories came from beef fat. The author states, "While eating an average, varied American diet, a group of seven patients had an average serum cholesterol level of 263 mmol/dl. After eating a diet high in beef fat for three to 18 months, the average serum cholesterol level fell to 189 mg/dl" [49].

(These figures equate to a reduction in TC from 6.8 to 4.9 mmol/l)

5. Reported Association between Countries with a High Fat Intake and High Incidence of CHD

An influential epidemiological study that suggested a connection between saturated fat intake, TC, and heart disease is the Seven Countries Study (7CS) published by Keys [50]. The NHFA gives this study prominence when it states, "The Seven Countries Study provides the strongest epidemiological evidence that diets high in saturated fatty acids increase the risk of CHD" [46]. The evolution of Keys's research culminating in the publication of this study is interesting and gives insight to his experimental approach and degree of scientific rigour. In 1953 Keys published a paper showing a correlation between fat intake and heart disease in six countries [51]. He reported that as national fat consumption increased, death from CHD increased in tandem. The correlation between the two factors in these six countries was striking: lowest fat intake = lowest incidence (Japan) and highest fat intake= highest incidence (US), with a straight line connecting the rest. Keys firstly reported this correlation cautiously as, "…it must be concluded that dietary fat somehow is associated with cardiac disease mortality at least in middle age" [51].

Yerushalmy, statistician at the National Institutes of Health and Hilleboe, past president of the American Public Health Association, report [52] that Key's interpretation of the same data in a second publication [53] was in much stronger terms when Keys stated,

> The analysis of international vital statistics shows a striking feature when the national food consumption statistics are studied in parallel. Then it appears that for men aged forty to sixty or seventy, that is, at the ages when the fatal results of atherosclerosis are most prominent, there is a remarkable relationship between the death rate from degenerative heart disease and the proportion of fat calories in the national diet... No other variable in the mode of life besides the fat calories in the diet is known which shows anything like such a consistent relationship to the mortality rate from coronary or degenerative heart disease.

The escalation of importance of this reported association was a concern to Yerushalmy and Hilleboe, who addressed a number of considerations about extracting information from epidemiological studies in general, and Keys's report in particular [52]. They began their paper by urging caution in interpreting epidemiological data.

> It is well known that the indirect method merely suggests that there is an association between the characteristics studied and mortality rates and, further, that no matter how plausible such an association may appear, it is not in itself proof of a cause-effect relationship. But quotation and repetition of the suggestive association soon creates the impression that the relationship is truly valid, and ultimately it acquires status as a supporting link in a chain of presumed proof.

Yerushalmy and Hilleboe noted that there was data available for over 20 countries at the time and that Keys gave no reason as to why he chose only six, nor the particular six he reported on. When the association was re-examined by including all countries with available data, the 'consistent relationship' noted by Keys was not apparent.

> Thus, we see that in countries with approximately the same proportion of the diet available as fat, the heart disease mortality ranges from 220 to 739 per 100,000. Hence, the selection of the original six countries, for whatever reason, greatly exaggerated the importance of the association [52].

Yerushalmy and Hilleboe also looked at whether the association suggested by Keys was specific to fat or reflected a relationship with other factors. Under the heading, "Tests for 'Specificity' of an association" the authors state:

> Thus, in the association considered in this paper it is necessary to test, first, whether the association between heart disease mortality and diet holds true only for fat or whether one may find a similar association between heart disease and other components of the diet, such as protein or carbohydrate.

They found that the association between dietary protein and heart disease was at least as strong as that between fat and heart disease. They state, "This clearly suggests that the dietary fat-heart disease association is not unique or specific." In addition, Yudkin [54] reported a similarly strong relationship between sugar consumption

and heart disease. Yerushalmy and Hilleboe also commented that there were severe limitations in the method of food data collection on which Keys based his theory. Dietary intake was estimated from national food balance sheets. Such data estimates the food *available* for consumption for an entire country by adding together imports, estimated carry-over stocks and domestic production, and subtracting exports, grain used for seed or feeding animals, edible crops used industrially and an estimate for spoilage. Balance sheets give a rough estimate of food available for consumption by the population and has little application for individuals.

Professor Trulson highlighted the deficiency of using the method employed by Keys as a guide to dietary intake for individuals, when commenting on a study of food purchased and served to army recruits [55]. She stated, "Fat loss was 50 per cent from food purchased to food served (this did not take into consideration plate waste). The food as purchased provided 216 g of fat per day, and as served provided 108 g of fat. This changed the percentage of calories available from fat from 43 percent to 34 percent".

Keys developed the idea for the Seven Countries Study after experiences in Italy and Spain in the early 1950s. He published a paper in 1954 on his observations in Spain, where he reported his belief that degenerative heart disease was much less common among Spanish men than among American men of equal age [56]. This study again highlights Keys' approach to his research. Keys reports, "Studies were made of bodily dimensions and serum cholesterol concentration in 181 clinically healthy adults in Madrid comprising men of the professional class and men and women in a poor sector (Vallecas) of the city."

Even though Keys reports the adults were clinically healthy, he adds, "Among the adults studied here, there were no real clinical signs of malnutrition except for the frequent appearance of calorie under-nutrition in the men; it was noted that the children were thin and appeared 'stunted'". Dietary information for the Vallecas men was obtained from household inventories and purchases recorded for a week. Keys comments, "The method of dietary survey by families in Vallecas did not allow precise estimation of the diets of the individuals." In addition he stated: 'It was not possible to get an accurate survey of dietary intake in the group of professional men'. However, Keys believed he was able to ascertain the usual diet of this group largely by observation. "From interviews and personal observation of the eating habits of this group it was clear that the diet was both rich and varied and roughly would correspond in the nutritive elements to upper middle-class diets in the United States."

Men from Minnesota were used as this US comparison. Keys reports, "The diets of the average Minnesota subjects are not precisely known, but it is certain that, on the average, they were calorically abundant and provided about 40% of calories in the form of fats." For information on heart disease, Keys states, "With regard to heart disease, no definitive data were secured." Without detailed vital statistics, Keys however made an "estimate" of mortality from heart disease in Spain, noting that it was "subject to some error". His conclusion was, "… certain that male mortality from heart disease in Spain is very much less than in the United States" at least for the poorer of the population. In the case of the more wealthy, Keys ascertained that CHD was more common by, "Repeated questioning of many physicians in Madrid and in Cardia" who apparently told him that it was.

On the basis of this somewhat incomplete data and aided by guesswork and personal beliefs, Keys concluded that the relationship between dietary fat and serum cholesterol was a major factor in the difference in heart disease mortality between Spain and the US. "It is concluded that a major factor in this difference lies in the relationship among dietary fats, serum cholesterol and atherosclerosis." Keys went on to plan and execute the Seven Countries Study. Begun in 1958, it was designed to gather information about healthy men in seven countries, follow their progress for a number of years and then determine factors associated with health outcomes. Many interesting findings were reported after ten years [57].

Higher TC levels were associated with a higher incidence of CHD in the US, Finland and Greece. This relationship was apparent at only the highest recorded decile level in Greece (above 260 mg/dl/ 6.7 mmol/l), above approximately 260 mg/dl in the US and 280 mg/dl/ 7.2 mmol/l in Finland. Countries where CHD mortality did not appear to be related to the level of TC were Yugoslavia, Italy and Croatia. In addition, the lowest recorded levels of TC were associated with increased mortality from all causes in Yugoslavia, Finland, Italy and Greece. This study was a huge undertaking and the information collected was valuable. However, the National Heart Foundation of Australia's assertion that, "The Seven Countries Study provides the strongest epidemiological evidence that diets high in saturated fatty acids increase the risk of CHD" could be debated. The ability to form accurate conclusions from any study depends on the quality of data on which it draws and the competency of the researcher in interpreting such data. The relationship between saturated fat and CHD as reported in the Seven Countries Study rests on both these criteria.

5.1. Dietary Data Collection in the Seven Countries Study

In Keys's previous studies he estimated diet intake from foods available for consumption, household inventories, questionnaires and observation. As these methods lack accuracy in estimating food intake for individuals, Keys included other dietary survey methods in the Seven Countries Study, including seven-day records of all foods eaten plus chemical analyses of duplicate sample meals. These more detailed diet surveys were conducted on smaller groups of men and the results of estimates of protein, fat, and carbohydrate intakes were extrapolated to the whole group. It is important to know how the dietary data were collected and interpreted in order to assess Keys's reported finding of an association between saturated fat intake and CHD. As an example, diet information for the US cohort is examined here.

Keys gives little detail about the diet surveys in the Seven Countries book [50]. He instead refers to an earlier publication [57] where he says, "The methods have been published in detail." This, however, is not the case, and contrary to Keys's statement there is little information on the collection of dietary data in this source. In this report Keys again states, "The dietary findings have been reported elsewhere", referring to an even earlier source [58]. Later, as co-author of a paper on food consumption patterns in the Seven Countries Study published in 1989, Keys admitted, "Detailed data on food consumption patterns have been published for only 9 of the 16 cohorts" [59]. Unfortunately the US cohort was not one of these.

Without published food intake data for the US, we have to rely on Keys to supply this information and he provides some inconsistent and conflicting information on how the dietary data for the US men was collected. In one source Keys reports, "The diets of the railroad men in the United States and Italy were estimated at the time of the entry examinations from a short questionnaire" [50]. More detail about data collection from the US men was given in the 1970 report when Keys stated, "The men were asked to recall in detail everything they had consumed on the previous day." They were also asked to estimate how often they ate some specific food items such as milk, eggs, butter, cheese and so forth. As Keys said, this check list was, "…aimed particularly at sources of fat…" [57].

Keys was aware of the limitations of this method as he noted, "It was realized that this simple short-cut method would not produce reliable quantitative estimates of the diets of individuals." Special studies were then made on a subsample of 50 men by visits to their homes and "prolonged examination of details with the wives". As a result, "Average correction factors were thus derived, and the dietary data for the US railroad men presented here are the result of this adjustment".

In 1989 Keys reported the collection of dietary data for the US men somewhat differently [59]. "In the United States, employees of the US Railroad in the State of Minnesota cooperated in the dietary survey of the study. This survey was carried out during 1960-1962…A total of 30 men weighed and recorded their food consumption for 1 day." As the original data were not published, it is difficult to ascertain how accurately the dietary habits of the 2571 US men enrolled in the study are characterised. And importantly, which of the two very different methods of dietary data collection reported by Keys was actually used.

H. Blackburn worked with Keys on this and other projects; in an overview of the Seven Countries Study he drew attention to the fact that criticisms had been made of the small sampling sizes on which the dietary data relied [60]. This would appear to be a valid criticism. For example, the Japanese cohorts consisted of a total of 1010 men; however, the dietary data characterising the Japanese was gathered from 4-day food records of 24 men in a village in the interior of Japan and eight men in a coastal village [59].

5.2. Interpretation of Results

Using dietary data collected from less than 4% of the 12,763 men enrolled in the study, Keys estimated the average intakes of protein, fat and carbohydrate for each cohort. The resulting data were then statistically analysed for correlations between diet constituents, serum cholesterol and incidence of heart disease in each cohort. Keys reports three findings of incidence rates of CHD after 10 years.

1) The coronary death rate was correlated strongly with saturated fat intake.

2) "The ten-year incidence rate of coronary heart disease was significantly correlated with the percentage of calories supplied by sucrose in the average diets of the cohorts."

3) "Incidence was significantly related to the percentage of dietary calories from animal proteins."

These results indicate that the relationship between saturated fat and CHD is not specific. As saturated fat, sucrose and animal protein intakes were all strongly associated with CHD, no single variable can be said to be

more important than another. The NHFA assertion that, "The Seven Countries Study provides the strongest epidemiological evidence that diets high in saturated fatty acids increase the risk of CHD" [46] is therefore highly questionable.

Notwithstanding the inconsistencies in the reporting and collection of dietary data, small sample numbers and the finding that CHD was correlated with *three* dietary variables rather than just saturated fat, the NHFA's reporting shows a lack of understanding of the type of conclusions that may be made from epidemiological data. The study did not, and in fact such indirect methods of study *cannot*, show that a variable such as saturated fat *increases risk* of disease, only that it is *associated*, in a statistical sense, with increased incidence. The former implies cause and effect, and statistical associations do not constitute proof of a cause and effect relationship. A "strong" or "significant" correlation in this context refers to an estimate, based on statistical probabilities, of the likelihood of the association being due to chance.

6. The Presence of Cholesterol in Human Atherosclerotic Plaques

Human atheromatous plaques have been found to contain a combination of cholesterol, neutral fat and phospholipids but it is the presence of cholesterol that has gained the most attention. The NHFA states,

> The underlying cause of coronary heart disease is a slow build up of fatty deposits on the inner wall of the blood vessels that supply the heart muscle with blood (the coronary arteries). These fatty deposits gradually clog the arteries and reduce the flow of blood to the heart. This process, called atherosclerosis, begins when people are young and can be well advanced by middle age [61].

The A.H.A. admits that not all the facts about atherosclerosis are known when it states,

> It's a complex process. Exactly how atherosclerosis begins or what causes it isn't known, but some theories have been proposed. Many scientists think atherosclerosis starts because the innermost layer of the artery becomes damaged… Because of the damage, over time fats, cholesterol, platelets, cellular debris and calcium are deposited in the artery wall [62].

The Heart and Stroke Foundation South Africa describes the process of atheroma formation as,

> Atherosclerosis is the gradual build-up of deposits, initially cholesterol … under the inner lining of the artery walls. It is a slow progressive disease which may start in childhood. The aetherosclerotic plaques progressively narrow the artery and decrease the blood flow. The process may be compared to the gradual build-up of lime deposits in a pipe which ultimately block the pipe completely [63].

The idea that cholesterol is *deposited* in arteries is crucial to the diet/heart hypothesis favoured by these and other heart advisory organisations, as a high TC level is thought to predispose to cholesterol deposition and atherosclerosis. However, alternate theories of atheroma formation have been suggested. An atheromatous plaque has been likened to a scar and, according to pathologists Saphir and Gore, "At a late stage of arteriosclerosis it is as impossible to ascertain its pathogenesis as it is to establish the nature of an injury by examining the scar long after the initial insult" [64]. Despite the obvious difficulties in understanding a process active through life by observations made at autopsy, theories of atheroma formation, and in particular the presence of cholesterol, have been put forward.

A forerunner of today's "cholesterol deposition" theory suggested that the artery wall, possibly through areas of endothelial damage, imbibed blood lipids. These lipids were thought to cause a local inflammatory reaction resulting in hyaline changes and fibrous thickening of the intima. It was believed that for this process to occur endothelial damage and high TC were required. This imbibition of lipids was referred to in early studies as the filtration theory of atherosclerosis and it offered an explanation for the presence of cholesterol in the artery wall and intimal thickening.

This theory has some support today. In reference to LDL infiltration, Professor Hamilton-Craig states, "This was proposed indirectly in the 1800s by the German pathologist Virchow, who suggested that cholesterol in the blood filters across the inner endothelial lining into the vessel wall, where it accumulates. This concept is still valid, although not quite as simple [65]. Pathologists who presented other theories explaining the presence of cholesterol in atherosclerotic plaques, as well as the observed thickened intima, argued against the infiltration theory. Based on observations of sections from a thrombosed artery examined during his pathology classes,

Professor Duguid reported,

> We have been taught to regard atherosclerosis as an overgrowth of and degenerative change in the intima of arteries. This view is so firmly established that we have come to regard it as a ruling principle, yet evidence may be found in the coronary arteries that the lesions we classify as atherosclerosis can arise by quite a different process, namely the organization of thrombus [66].

Duguid suggested that thrombi adhering to the artery wall may be overgrown with endothelium. Contents of the thrombus, platelets, red blood cells and fibrin, would then become incorporated into the sub-endothelial layer and the breakdown of cholesterol-rich platelet cell membranes could be the source of cholesterol observed in atheroma. As an explanation for the observed thickening of the intima Duguid states [67]:

> Mural thrombi when recently formed are easily recognized by the fibrin or blood they contain…or by the signs of organization, but when the organization is complete these distinctive features are lost and there remains only a fibrous thickening which looks like an overgrowth of the intima.

Duguid proposed that this theory better explained the observed narrowing of arteries [67]. He argued against the common interpretation of atherosclerosis as being a fibrous overgrowth of the intima resulting from cellular proliferation as, theoretically, this would result in less elasticity in the arteries. As it is the elasticity that allows the artery to recoil after dilation, less elasticity would result in a dilated vessel rather than a narrowed one. Duguid suggested that repeated thrombus formation and organisation after endothelial overgrowth better explained the layered appearance of the thickened intima, as well as the resultant arterial narrowing.

Current theories concerning the development of atherosclerosis acknowledge the role of thrombosis, however, opinions differ as to the extent of this role. Hamilton-Craig states, “Thrombosis is now recognized as one of the factors leading to plaque formation, but is probably more important in the later stages of plaque evolution” [65]. Dr. Kendrick offers an alternate view that “…thrombus formation is key to the growth of the plaque” [68]. In discussing the basic process of plaque formation, he suggests that two interrelated processes, endothelial damage/dysfunction and increased blood coagulability, are operating.

If both processes are happening at the same time, endothelial damage occurs, then a thrombus forms over the area of endothelial damage. The endothelium then re-grows over the top of the thrombus “drawing” it into the arterial wall. After this, other repair processes take over to “heal” the damaged area and remove the remnants of the thrombus. These healing processes are what we are looking at with inflammation/migration of monocytes, growth of smooth muscle cells.

If the area of damage and thrombus formation is imperfectly healed this will act as an (sic) focus for repeated endothelial damage/thrombus formation, and a plaque will grow at the point as a result of repeated thrombotic episodes. The reason why cholesterol is found in plaques is primarily because LDL and VLDL are both incorporated into blood clots as they form (they are integral to the clotting process).

Another theory that could explain the presence of cholesterol in atheromatous plaques was suggested by Paterson. He proposed that a collateral circulation from the lumen of the artery into the artery wall posed a potential threat [69]. Paterson states,

> The intima of a normal human artery has therefore no true blood supply; but it develops one as a result of disease. Apparently by a compensatory mechanism, to supply the extra nutritional requirements of an abnormally thickened intima in the atherosclerotic process, capillaries grow into the intima and ramify within its substance.

Paterson continues, “The peculiar position of these newly formed capillaries, in direct communication with the lumen of a major artery in which the pressure of blood is high, seems to be a major factor in determining their rupture” and “…with their rupture disruptive hemorrhages within atherosclerotic plaques are produced”. Paterson concludes that following such a haemorrhage, the fluid portion of the blood is reabsorbed but that the solid elements such as cholesterol remain.

Acceptance of the imbibition theory is implied by national heart organisations when they refer to *deposition* of cholesterol in arteries, even though alternate theories explaining both the presence of cholesterol in atheroma and intimal thickening, according to some pathologists, may better explain these observations. As the filtration theory presupposes that a higher cholesterol level is integral to the process (*i.e.* the more cholesterol in the blood, the more will be “deposited”), it would follow that people with the highest TC levels would also have the most

extensive atherosclerosis.

A study by Sperry and Lande sought to determine if a correlation existed between TC and the degree of atherosclerosis of the aorta at autopsy [70]. They reported that at the same TC level, the degree of atherosclerosis as measured by the lipid content of the aorta varied by as much as threefold. In addition, the same degree of atherosclerosis was found with both low (182 mg/dl/ 4.7 mmol/l) and high (331 mg/dl/ 8.6 mmol/l) TC levels. After examining 97 cases the authors concluded,

> …and since it has been shown in the present investigation that there is no correlation between the lipid content of the aorta and the cholesterol content of the blood serum, it follows that there is no direct relationship between the concentration of cholesterol in the blood serum and the degree of atherosclerosis in the aorta.

In reference to the imbibition/filtration theory of atherosclerosis Paterson and Dyer commented, "…it is important to note that no direct evidence has yet been obtained that high serum lipid levels are associated with more atherosclerosis than are low levels" [71]. They investigated the association by comparing TC levels through life with the degree of atherosclerosis in four different arteries at autopsy and reported, "Taken at their face value, these results offer little or no evidence of a relationship between the severity of atherosclerosis or the incidence of its complications and levels of serum cholesterol that lie in the range of 150 - 300 mg%".

7. Animal Experiments

Rabbits have been used frequently to gather information about the human atherosclerotic process. "The role of cholesterol in the production of atherosclerosis has been a matter of contention since 1911, when Anitschkow produced lesions resembling atherosclerosis in rabbits by feeding them cholesterol dissolved in oil" [72]. When fed fat and cholesterol, rabbits develop high TC levels and subsequent fatty deposits in their blood vessels. When cholesterol is taken out of their diet, TC levels generally reduce and the fatty deposits may regress. If not used as conclusive evidence as to the process in humans, such experiments are said to be *supportive* of the theory that under conditions of high TC, cholesterol is more likely to be deposited in human arteries. Such evidence has influenced the thinking in regard to the role of diet in the atherosclerotic process in humans.

Medical researchers who have examined lesions in both animals and humans have reported differences that indicate different processes were involved. Professor Pickering states,

> There is no doubt that fatty deposits can be produced in the intima of rabbits and other animals by feeding cholesterol. But the lesions in man are not at all similar. Dr. Louis Katz at my request once showed me the coronary arteries of the chickens in which he had produced what he called atherosclerosis (literally hardening through the agency of porridge or grits) by feeding them cholesterol. The intima and adventitia were literally stuffed with cholesterol, as was the liver. I had never seen anything like it in human atheroma. In the human lesion the fat is not superficial in the intima: it is deep. Moreover, the outstanding clinical feature of the human disease is thrombosis, and thrombi are not prominent in cholesterol-fed animals [73].

W. J. Cliff reports, "Duguid in his cholesterol feeding experiments in rabbits found that their arteries became severely dilated when they were loaded with cholesterol deposits. This, of course, is quite different to the pathological effect of atherosclerosis occurring in human beings" [74]. Professor Stehbens also commented that the lipid-containing lesions of rabbits fed high cholesterol diets differed markedly, "…topographically, macroscopically and microscopically…" to those seen in human atherosclerosis. He believed that the lesions in rabbits were xanthomatous in nature and indicative of a fat storage disease [75]. Cholesterol and fat feeding has not been the only type of procedure to produce experimental arterial lesions in animals.

Pathologist N. G. B. McLetchie was interested in testing Duguid's theory that human atheroma may result from recurrent episodes of mural thrombosis and subsequent organisation, and that the presence of lipid and cholesterol in the intima is explained by the breakdown of incorporated blood products. He devised a non-dietary procedure that produced lesions in rabbits [76]. Rabbits were injected with diluted viper venom, a powerful coagulant, resulting in a thin coating of thrombus over varying areas of endothelial surface of main branches of the pulmonary artery. Repeated injections of the venom were given to some animals. McLetchie followed the progression of arterial changes and reported that endothelium grew over the coagulum, thereby incorporating the contents, which degenerated into a mass of fatty material, in the sub-endothelial layer.

In agreement with Duguid's theory of atherogenesis, McLetchie further commented,

> The diffuse and focal lesions completed their organization within 6 to 8 weeks. He continued, "The prominent fatty lesions…resolved to produce fibro-fatty intimal thickenings… The latter lesions were indistinguishable from human atheroma in that they presented as focal fibrous thickenings confined to the intima, with a deposit of lipid on the juxtamedial aspect" [76].

McLetchie demonstrated that atheroma could be produced in rabbits by a non-dietary mechanism not involving an increase in the animals' TC levels. In addition, A. C. Ross *et al*. provided evidence that a low saturated fat/low cholesterol diet enriched with sucrose produced hypercholesterolemia and atherosclerosis in rabbits [77]. "We have shown that severe hypercholesterolemia (averaging 200 - 700 mg cholesterol per dl over a 26 - 36-week period) and severe atheroma can be produced in rabbits fed a diet lacking cholesterol and low in saturated fat."

To some early researchers it appeared that a straightforward connection existed between dietary saturated fat and cholesterol, hypercholesterolaemia and atherosclerosis in animals, which may lend support to such connections in humans. The situation, however, appears to be more complicated.

Not only can sucrose added to the diet of rabbits cause hypercholesterolaemia and fatty arterial deposits, it is possible that two types of lesions have been produced in animals by two different methods. It is also possible that these lesions have their counterparts in two different conditions seen in humans. The high TC levels produced in rabbits fed a high fat and cholesterol diet resulted in fatty arterial deposits, said to be xanthomatous in nature and indicative of a fat storage disorder. This may have its counterpart in familial hypercholesterolaemia, where the lesions are also widespread and xanthomatous. In this case the high TC levels result from a genetic defect of cholesterol receptors. The atherosclerotic process, possibly initiated as a response to injury, is likely to involve thrombosis. TC may not have a causative role in this process as it occurs in the presence of a wide range of cholesterol levels in humans and a normal TC in rabbits.

8. Population Surveys

An early large-scale population survey to report a correlation between higher TC levels and the incidence of CHD was conducted in the town of Framingham, Massachusetts. This study began in 1948 when health and lifestyle data was collected from Framingham residents. A 1957 publication reported that men aged 45 - 62 years could be separated into groups with different probabilities of developing angina pectoris, myocardial infarction, ECG changes possibly indicating myocardial infarction, sudden death or myocardial fibrosis (collectively termed atherosclerotic heart disease or ASHD early in this study, and later coronary heart disease or CHD), based on their TC level, blood pressure and relative weight [78].

It was reported that, "Hypercholesterolemia was strongly associated with the development of new ASHD in men 45 - 62." This could also be reported as, "Statistically, men with higher TC levels developed signs attributable to ASHD more often when compared to men with lower levels." In a follow-up report four years later, high TC, hypertension and smoking were described as "risk factors". The choice of the term "risk factor" may have been unfortunate, the danger being a subtle impression of a causative relationship between high TC and the development of atherosclerosis or signs of CHD.

In an overview of the history of the Framingham study, G. Oppenheimer comments, "The language of risk, specifically, the probability of developing CHD…permeated early Framingham publications" [79]. "In their 1957 report, Dawber and co-authors used the term 'risk' at least 18 times, generally to mean 'probability', as in 'there is an increased risk of [CHD] in persons with elevated cholesterol'." According to Oppenheimer, over the next couple of years Framingham authors referred to hypercholesterolemia as a "factor" possibly associated with the development of CHD and in a 1961 publication as a "factor of risk", contracted in the same article to "risk factor". This was apparently done without much thought. "Kannel, the lead author, claims to have soon forgotten that they had coined the term, or at least first applied it to epidemiology."

However, the legacy of this word choice may have had important consequences in influencing perception of the role of TC in the aetiology of CHD. As Oppenheimer states, "the very ambiguity of 'risk factor', resonating 'cause' while denoting 'correlation', gave it added imaginative power." He adds that even though the cause of CHD was unknown and that epidemiological "links" were correlations only, "This did not prevent 'risk factors' from being known as causal agents" [79].

In 1962, not long after the associations were first reported and one year after the term "risk factor" was introduced by Framingham authors, these same researchers were suggesting therapeutic interventions to reduce blood pressure, weight and dietary fats [80]. Likewise, in 1964 a committee of the Surgeon General's Office in the US

"…called for action against CHD risk factors despite the lack of demonstrated causal association" [81]. It is a pity that instead of following an early path of supposed causation, researchers at Framingham and elsewhere did not ask the simple question, "What else may explain the association between higher TC and CHD?" One possibility is that a higher TC level may be a marker for a metabolic abnormality or a disease state that is causative. Familial hypercholesterolaemia, thyroid deficiency and endogenous hypertriglyceridaemia are conditions associated with high TC levels plus a higher incidence of CHD.

8.1. Familial Hypercholesterolaemia (FH)

As an inherited disorder of cholesterol metabolism where the body lacks the normal mechanisms for cholesterol removal, TC levels in people with this disorder are often raised. It is a specific disease entity where defects in or reduced numbers of cholesterol receptors may result in excessively high levels of TC and deposition of cholesterol in organs and blood vessels. Cholesterol may be deposited in many sites including liver, heart and blood vessels and early death from heart disease is common in severe forms of the disease.

The high TC and higher incidence of cardiac deaths in FH have been thought to offer evidence of a causal association between high TC and CHD in the normal population. Professor Stehbens states, "Homozygous FH is regarded as the strongest evidence that hypercholesterolemia is the cause or prime factor in atherogenesis", and "…crucial to the validity of the lipid/cholesterol controversy" [75]. However, he points out that the lesions seen in FH are different to those of atherosclerosis. In his opinion they are xanthomatous in nature and "The vascular changes indicate a fat storage disease rather than atherosclerosis."

Stehbens states that the underlying causes of cardiac events in atherosclerotic HD and FH are due to different mechanisms, further differentiating between the two conditions. He states, "Myocardial ischemia in homozygotes is the consequence of xanthomatous narrowing of the ostia of the coronary artery, and not of intimal disruption and secondary thrombosis."… "The two disorders are fundamentally different." Commenting on results after 30 years of follow-up, Framingham researchers stated, "…there is an association between CVD death and serum cholesterol levels at age 50 years in both men and women" and "There is no association between cholesterol levels at age 60 years and CVD death" [82]. No mention was made in this report about the possible influence of the inclusion of people with FH.

In a critique of the Framingham report, H. Okuyama *et al*. comment, "Correlation between TC values and CHD mortality was clear in the younger generations but not in the generations over around 50 years old. Those with high TC values at 30 and 40 years of age are likely to include groups with genetic disorders such as familial hypercholesterolemia (FH), who develop CHD at 10 times higher rates and die younger" [83]. Therefore, part of the relationship between higher TC and incidence of CHD may be explained by the inclusion of unknown numbers of people with FH in the Framingham study.

8.2. Thyroid Deficiency

High TC levels are often seen in association with thyroid hormone deficiency and thyroid deficiency states may be associated with signs of CHD. As angina pectoris was part of the criteria used by Framingham researchers in assessing the incidence of CHD, angina from thyrotoxic anaemia may have contributed to this association.

In the 1957 report from Framingham [78] it was found that angina represented 43/84 new occurrences of ASHD in subjects aged 45 - 62. It was noted that in women the diagnosis shifted from definite to questionable angina pectoris and back in successive examinations, highlighting the difficulty in diagnosing CHD by their criteria. In addition, the authors reported, "Furthermore, there were persons in this study group who at some time showed definitive evidence of a myocardial infarction but who later lost all residual evidence of ECG changes." They concluded, "The difficulty of diagnosing ASHD…will no doubt continue to obscure the evaluation of causal relationships." Part of the relationship between higher TC and incidence of CHD may be due to cases of undiagnosed thyroid deficiency contributing to the diagnosis of angina pectoris.

8.3. Endogenous Hypertriglyceridaemia

Studies by Margar *et al.* brink in the 1960s demonstrated that the TC level could be raised by feeding volunteers more carbohydrate [84]. Those who responded in this way generally had an increased insulin response to carbohydrate. It was proposed that the conversion of carbohydrate to triglyceride (Tg) via de novo lipogenesis was

increased in these people, and resulted in higher production and release of very low-density lipoproteins (VLDL) by the liver. Even though VLDL is rich in Tg and proportionally poorer in cholesterol, the large numbers of VLDL produced could increase TC by 40 - 60 mg% (1 - 1.55 mmol/l), thereby contributing to higher TC levels.

Two metabolic conditions commonly associated with high triglycerides are insulin resistance and metabolic syndrome. Features of metabolic syndrome include overweight, hypertension, high triglycerides, low HDL and blood glucose dysregulation and the condition is associated with a higher incidence of CHD. Blood glucose, triglycerides and HDL were not measured in the early days of the Framingham study. However, it is possible that the correlation reported between CHD and three of the markers measured—overweight, hypertension and TC—identified those with insulin resistance/metabolic syndrome. In such cases, high TC may have resulted from hypertriglyceridaemia and influenced by primarily by carbohydrate intake [85]. The Framingham study has been seen to provide support for the lipid hypothesis on the basis of finding a positive association between higher TC and higher incidence of CHD.

In view of the examples mentioned above, it is incorrect to assume that high TC has a causative role in CHD. The possibility exists that it is a "marker" of other underlying abnormal metabolic or disease processes. "Risk marker" may have been a better choice than "risk factor", representing the relationship more accurately without any unintended bias.

9. Conclusion

In past decades the causation, prevention and treatment of CHD were hotly debated, without a satisfactory resolution ever being reached in the scientific community. The controversy surrounding the issue has been reignited recently, with the opportunity to re-examine the science on which beliefs about the connection between diet and CHD are based. This article has presented some of these conflicting studies that provide background to the diet/heart hypothesis. To more fully understand this relationship, we must also look more closely at other aspects of the connection including diet studies, classification of CHD, basis of recommendations of target ranges for TC, functions of cholesterol and alternate theories of atherosclerosis and CHD causation. My hope is that this article will trigger further informed discussion on this topic.

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Conflict of Interest

There are no conflicts of interest.

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