Chapter 12
Nutritional Aspect of Sex-Dependent Difference in Heart Disease

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Abstract According to a 2019 report by the American Heart Association, cardiovascular disease is the leading cause of death worldwide, accounting for more than 17.6 million deaths per year in 2016; this number is expected to grow to more than 23.6 million by 2030. Body composition, which is influenced by nutritional status, is considered to have a great significance in determining the incidence of chronic diseases including heart disease. The difference in body composition is not only affected by age, race, and heredity, but also to a greater extent by sex. Biological males and females show variable body fat at different locations as males tend to accumulate more fat in the abdominal area and females in the thighs and buttocks. This sex difference in fat distribution is considered to determine the difference in the susceptibility of males and females to various pathological stimuli for the development of heart disease. Because males have higher muscle mass as compared to females of the same age, difference in the metabolic status of the body has also been shown to determine sex difference for the occurrence of heart disease. Furthermore, in view of the role of different sex hormones in determining the incidence of heart disease, the interaction of sex hormones with some nutritional factors and metabolic pathways has been explored to explain gender difference for the development of cardiovascular abnormalities.

Keywords Sex differences · Cardiovascular disease · Calorie restriction · Sex hormones · Cholesterol metabolic pathway

Introduction

Over the past decade, there has been increasing awareness about the leading cause of cardiovascular disease, and a considerable effort has been made to better understand

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its implication with respect to sex differences. It is now well known that cardiovascular disease is the underlying cause of 1 in every 3 deaths in the United States (Table 12.1). Prior to the enactment of the National Institutes of Health (NIH) Revitalization Act of 1993, subjects of randomized controlled trials predominantly consisted of males, and the outcomes of early clinical studies were often founded on limited female representation [1]. However, it has now become evident that there are marked differences between males and females with respect to the incidences of heart disease and thus issues for both sexes are now handled separately. Furthermore, special attention is being paid to determine the role of nutrition in sex difference for the development of heart disease. It may be noted that some of the benefit of diet lie in the activation of various metabolic pathways including reduced inflammation, quick repair, less occurrence of cardiovascular problems and ultimately increased longevity [2]. Of these various metabolic pathways involved in longevity and sexual dimorphism are the nutrient—responsive mTOR [3], growth hormones [4, 5], Sirtuins [6], IGF-1 [7] and cholesterol metabolic pathway. Accordingly, this article will be focused on discussions of these aspects with respect to sex differences for the occurrence of heart disease. In addition, it is intended to discuss the sex differences in response to nutritional manipulation by calorie restriction, sex hormones, and cholesterol biosynthetic pathway. It should be noted that cholesterol is an integral part of the cell membrane of all organs and is considered to play a critical role in maintaining their functional status.

Table 12.1 Cardiovascular Disease (CVD) and death rate in US

Type of CVD	Death rate (%)	Remarks
Coronary heart disease	43.2	Average age of first **heart** attack: Male—65.6 years Female—72.0 years
Stroke	16.9	Disability following by stroke: Male—3% Female—2%
Peripheral artery disease	3.0	Individual with total cholesterol levels of 200 mg/dL or higher: Male—35.4% Female—41.8%
Hypertension	9.8	–
Heart failure	9.3	–
Other CVDs	17.7	–

Taken from the report by American Heart Association, 2019 (https://www.heart.org/en/about-us/heart-and-stroke-association-statistics)

Sex Differences in Response to Calorie Restriction and Cardiovascular Disease

After nearly a century of research observing the role of reduced energy intake on life extension in model organisms, calorie restriction and related diets continue to draw serious interest not only for their roles as mitigators of aging but also of age-related disease and pathogenesis [8, 9]. Clinical trials and observational studies have also revealed that calorie restriction (CR) has ameliorating effects on metabolic and hormonal homeostasis in both humans and experimental models, and is a promising, inexpensive mode of intervention for the treatment and prevention of cardiovascular disease [9–12]. Furthermore, there is ample evidence from animal and human studies to suggest that there are notable sex-dependent responses to these interventions, possibly influenced by a variety of sex differences including fat distribution in the body and the interaction of steroid hormones [12–15]. The lack of comprehensive analysis of possible sexual dimorphism in response to calorie restriction warrants the need for more rigorous research that considers sex and age as major variables.

Prior to discussing the sex-based differences in the effects of calorie restriction, it is necessary to introduce its implications on cardiovascular disease risk. It should be noted that calorie restriction, as practiced chronically or intermittently, is often defined as a physiological state of reduced energy intake while avoiding malnutrition [11]. Common protocols in rodent studies is 25–40% calorie reduction from usual ad libitum daily intake, with effects dependent not solely on sex but also on dose, age, strain, and nutrient composition of the diet [13, 15]. However, in a pilot study of the CALERIE (Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy) randomized controlled trials, weight loss induced by an average calorie reduction of just 11.5 (±2)% for 1 year in overweight and middle-aged individuals contributed to significant reductions in plasma concentrations of LDL-cholesterol, triglycerides, and C-reactive protein, along with reductions in total cholesterol:HDL ratio and insulin resistance which are the major risk factors of coronary heart disease [16–18]. The phase-2 multicenter trial, CALERIE-2, spanning two years, also demonstrated that prolonged mild calorie restriction significantly improved blood lipid profiles and insulin sensitivity while significantly reducing intramyocellular lipids as well as visceral and subcutaneous abdominal adipose tissue [18–22]. Another 6-month calorie restriction study found a reduction in oxidative stress, as measured by reduced ROS production, higher glutathione levels, and increased catalase activity [23]. Short-term interventions spanning 10 weeks with 20% calorie reduction have also been proven to be effective in reducing systolic and diastolic blood pressure, glucose concentration, as well as reversing fibrinolytic dysfunction, which has been associated with increased cardiovascular disease risk [18, 21, 22].

A cross-sectional look at members of the Calorie Restriction Optimal Nutrition (CRON) Society, who have been practicing, on average, 30% calorie restriction for 3–15 years while maintaining a highly nutrient-dense and low glycemic diet, demonstrated very low risk factors for atherosclerosis. Average serum total cholesterol

and LDL-cholesterol concentrations were in the bottom 10% of their age bracket and triglyceride concentrations were comparable to values at the 5th percentile for average 20-year-olds [19]. Carotid intima-media thickness (IMT) in CR practitioners also measured 40% less than age-matched controls on a typical Western diet. Lastly, heart rate variability in the calorie restriction group was comparable to healthy males and females at ages 20 years younger [19]. A considerable number of these studies did not observe or quantify the effects of calorie restriction in relation to sex. Despite limited analysis of sex differences in long-term clinical trials, substantial evidence exists in animal models, short-term calorie restriction and related interventions that may explain some fundamental physiological differences between males and females beyond the context of reproduction.

Effects of Calorie Restriction and Sex Differences on Endothelial Function

Maintaining healthy endothelial function is essential for the management of cardiovascular disease risk in males and females as endothelial impairment has been shown to precede hypertension, atherosclerosis, and other acute disease manifestations [12, 27]. One distinguishing characteristic of healthy endothelial function is estradiol (E_2) -mediated increase in nitric oxide (NO) bioavailability in relation to decreased oxidative stress [11, 27]. Estradiol (E_2) binding to estrogen receptors (ER) expressed on endothelial cells and vascular smooth muscle cells has been shown to stimulate production of NO through the activation of endothelial nitric oxide synthase (eNOS) [27]. And with accelerated NO production comes the need to suppress oxidative stress, particularly superoxide (O_2^-) whose reactivity with NO reduces its bioavailability [24]. In a 2-week study observing rats subjected to 40% calorie reduction (compared to controls) and vascular reactivity tests, caloric restriction significantly increased NO concentrations in males only and reduced NADPH-sensitive O_2^- production with greater effect in males than females [24]. While caloric restriction proved to be more effective towards males in this experiment, it is important to note that female controls had higher relative NO production and markedly lower O_2^- production than their male counterparts. This discrepancy in sex reveals high levels of E_2 in young female rats serves a cardioprotective advantage to which caloric restriction showed no significant effect [24].

Further testing to see if caloric restriction altered serum levels of estrogen and testosterone in animals demonstrated no change in serum level as well as mRNA expression of ERs. This observation indicates that calorie restriction benefits noted in males were not primarily the result of estrogens and ER activity but its mechanisms still remains to be investigated [25]. One known means of activating eNOS, noted when binding to subtypes ERα and ERβ, involves the induction of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway [28, 29]. Independent of ERs, calorie restriction—induced AMPK activation also triggers a downstream cascade of the

PI3K/Akt/eNOS pathway. Conversely, overconsumption of food has been shown to dysregulate this pathway, further demonstrating the potential benefits of calorie restriction in males and postmenopausal females in maintaining healthy vasculature [11, 28].

Effect of Calorie Restriction and Sex Differences in Fat Loss

Sex differences in visceral and subcutaneous fat distribution have largely been investigated in association with sex steroid characteristics. Analysis of participants from the Multi-Ethnic Study of Atherosclerosis revealed that higher levels of bioavailable testosterone was associated with lower visceral and subcutaneous fat composition in males while the reverse was seen in females. Other androgens at high levels, such as dehydroepiandrosterone, were again associated with higher visceral fat content in females but not in males [30]. Another study looking at the role of sex steroid profiles on fat loss from lifestyle modifications showed the amount of visceral and subcutaneous fat was inversely associated with high testosterone and sex-hormones binding globulin (SHBG) levels in males, while females only found correlation with high SHBG levels [31]. Considering the protective functions provided by high testosterone levels in males only, a 12-week 800 kcal/day caloric restriction intervention has been shown to significantly increase testosterone and SHBG levels in obese male subjects through improved testicular function and reduced aromatase conversion of testosterone to estradiol in adipose tissue [32]. Postmenopausal females in the SHAPE-2 weight reduction trial demonstrated that calorie restriction did not elevate serum testosterone and androstenedione levels in females and maintained cardioprotective testosterone to estradiol ratios [33]. These findings demonstrate the role of calorie restriction in ameliorating age-associated decline in cardioprotective sex steroid profiles.

As shown in Fig. 12.1, the observation of calorie restriction effects on fat loss in mice studies reveals male mice tend to lose both visceral and subcutaneous fat while females predominantly lose more visceral fat [34]. A direct comparison of visceral adipose tissue loss after 8-weeks of 800–1000 kcal/day consumption in adult human males and females, revealed males experienced greater loss than females after adjusting for relative changes in fat mass [35]. However, when sample sizes were reduced to compare males and females with matched baseline ratios of visceral-to-subcutaneous fat, the significant difference was no longer evident [35]. Despite evident sexually dimorphic patterns of fat loss between sexes in mice studies, human studies still require further examination with larger sample sizes matched in baseline characteristics. Lastly, an analysis of adipose tissue genes from males and females subjected to 12 weeks of a high protein-low calorie-intermittent fasting protocol showed higher expressions of 158 sex-biased genes, except one, in female adipose tissue. Even after controlling for fat mass (which is generally higher in females), the discrepancy remained consistent in 80% of the genes [36]. While it is difficult to draw conclusions relative to cardiovascular disease risk from these findings, the

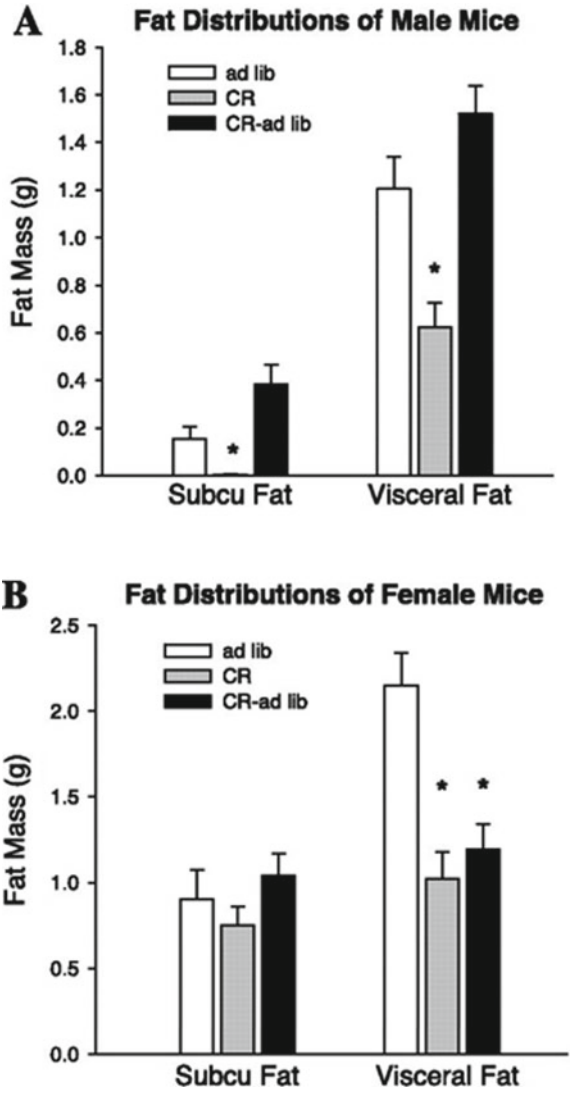

Fig. 1 Fat Distribution of male and female mice subjected to Ad-lib feeding (sham control) and caloric restriction (CR) Reprinted from Shi H, Strader AD, Woods SC, Seeley R. Am J Physiol Endocrinol Metab 293, E316–E326, 2007. Subcu—subcutaneous

evident sexual dimorphism in adipose tissue gene expression further implicates the possibly greater role of sex on metabolic health [36].

Sex Differences in Response to Hormones and Predisposition to Cardiovascular Diseases

It is now well documented that heart disease is the primary cause of morbidity and mortality in both males and females. While males are at higher risk than females during early adulthood, risk appears to increase in females after menopause. The connection between lower levels of estrogen and higher levels of androgens and their role in cardiac health has been widely studied, although results remain conflicting [37]. Androgens and estrogens are typically thought of, respectively, as the "male" and "female" sex hormones. However, both classes of hormones are found in each sex and proper regulation may be necessary in order to prevent health risk. In addition to the development of reproductive organs and secondary sex characteristics, sex hormones participate in the regulation of a variety of other systems within the body. Testosterone assists in regulation of muscle mass and function, bone mass, and fat distribution [38, 39]. As for estrogen, there are three primary types; Estrone (E1), Estradiol (E2), and Estriol (E3). 17β-Estradiol is the primary estrogen found in the human body. It has been shown to impact cardiac health through stimulating vasodilation, cell differentiation, and regulation of cholesterol production in the liver [40–42].

Obesity and Metabolic Syndrome

Obesity has been significantly linked to increased risk of health-related conditions such as insulin resistance, diabetes, and cardiovascular disease [43, 44]. Measurements of abdominal obesity such as waist circumference, which measure abdominal obesity, verses body mass index which measures general obesity, has shown to indicate greater health risk especially when considering cardiovascular disease [45]. In addition to individual risk, obesity is part of the collection of conditions that make up metabolic syndrome which, together, increase risk for development of cardiovascular disease, diabetes, or stroke. Metabolic syndrome is diagnosed when a person has three or more of the following conditions: abdominal obesity/40 inch waist measurement (male) and 35 inch (female), blood pressure of >/=130/85 mmHg, triglycerides of >150 mg/dL, fasting blood glucose > 100 mg/dL, and HDL < 40 mg/dL (male) or <50 mg/dL (female) [44–46]. It had been largely understood that males had a higher rate of risk of cardiovascular disease than females of the same age. However, continuing research has shown that cardiovascular disease risk increases for peri and post-menopausal females [46]. Understanding the role of sex hormones in obesity, diabetes, cardiovascular disease, and its risk is imperative for treatment and prevention of such conditions [47–54].

Several studies have shown a link between diminished testosterone levels/elevated estrogen levels in males and elevated testosterone/diminished estrogen in females and metabolic syndrome [55, 56]. There are a variety of factors that could affect

circulating hormone levels. Both the aging process and increased adiposity have been shown to have a negative association in relation to testosterone and estrogen levels [39, 57]. In addition to serving as a storage site for energy, adipose tissue is metabolically active. Free testosterone is metabolized by adipose tissue into estradiol. Therefore, excess adipose tissue could result in depleted levels of circulating testosterone. In males, this increase in estrogen exasperates testosterone levels further by suppressing testosterone production from the testes [40]. In addition to metabolic factors, adipose tissue could affect circulation and delivery of testosterone to target tissues. While some testosterone circulates in free form, most is bound to either albumin or SHBG. Studies are not clear on the relationship between obesity and SHBG levels, however, there could be a relationship between the effect of visceral fat on SHBG synthesis in the liver [41]. Adipose tissue distribution is also affected by estrogen as it has shown to promote subcutaneous fat accumulation in premenopausal females with normal estrogen levels. During and post-menopause, the decline in estrogen has been linked to a decrease in subcutaneous fat accumulation and an increase in abdominal visceral fat [57, 58]. It is pointed out that sex hormones can increase risk of metabolic syndrome in obese and normal weight persons alike. In males, regardless of weight, low testosterone and androgen receptor deficiency have been linked to hyperinsulinemia and type II diabetes risk. In females, risk is also increased when free testosterone levels are elevated, and SHBG and estrogen levels are diminished [57, 59]. This is of concern as type II diabetes increases risk for cardiovascular disease by contributing to arterial stiffness, hypertension, endothelial dysfunction, inflammation, and atherosclerosis 60].

Hypertension

Males have higher rates of hypertension in relation to age-matched females up until menopause. After menopause, both systolic and diastolic blood pressure increase in females beyond that of comparative aged males [37]. This noted change could point to a connection with decreased estrogen levels after menopause and the effect estrogen has on the regulation of overall blood pressure. Considering estrogen is found in both males and females, it has shown to have cardioprotective effects in both sexes. Estrogen affects blood pressure directly by impacting vascular tone and endothelial function through receptor dependent genomic and non-genomic actions [47]. Endothelial function is primarily regulated by endothelial nitric oxide synthases (eNOS) for the secretion of nitric oxide (NO). Estrogen has also been shown to have a positive effect on blood pressure through rapid non-genomic action of NO. Through genetic transcription, estrogen binding to estrogen receptors (ER-α and ER-β) is integral in the synthesis of eNOS. While this process takes time, endothelial production of nitric oxide appears to be stimulated by estrogen action [37, 53]. In addition to regulating NO production, ER-α and ER-β are observed to protect against vascular injury and atherosclerosis as well as control blood pressure and arterial tone, respectively [47]. Testosterone has also shown to have a possible relationship

with NO and eNOS. While reports on this relationship have been conflicting, many studies have reported that deficiency in testosterone is connected to decreased eNOS expression and low NO production [48].

While appropriate endothelial function and vascular tone is important in cardiovascular health, the primary regulator of arterial blood pressure is the renin-angiotensin-aldosterone system (RAAS). Continuing research has examined how sex hormones impact the regulation of RAAS. Estrogen appears to participate in all aspects of RAAS. Ways in which estrogen participates in reduction of blood pressure may include decreasing the synthesis of renin and ACE, decreasing the expression of angiotensin II receptor type 1 (AT_1R) and attenuating the expression of angiotensin II receptor type 2 (AT_2R) [50]. AT_1R is the primary receptor that participates in the regulation of blood pressure. AT_2R is upregulated after vascular injury and appears to assist in cardioprotective activities [49]. While estrogen's role in the RAAS appears to be cardioprotective in nature, testosterone has shown to have an impact on increased arterial pressure by participating in the expression of angiotensinogen, renin, and AT_1R system [50].

Hormone Replacement Therapy

Considering the noted increase of cardiovascular disease risk as associated with sex hormone imbalance, hormone replacement therapy has been used as the primary way to minimize risk of cardiovascular disease development. While estrogen and testosterone hormone replacement therapy are common treatments for the reduction of risk, studies remain conflicting in results [43, 51, 52]. Regarding post-menopausal hormone replacement therapy (HRT), treatment became controversial after the Women's Health Initiative released research findings that conflicted with research that showed the benefits of estrogen HRT. It was found that HRT increased risk of coronary heart disease (CHD) and stroke [61]. In a follow-up study, published in 2013, it was observed that timing of initiation of HRT could play a role in risk of CHD. Starting HRT closer to the point of menopausal onset, in association with decreased independent biomarkers for CHD risk, has shown to reduce HRT CHD risk. However, more research needs to be conducted to determine increased stroke risk and timing of HRT [61]. For testosterone replacement therapy, while a long-term study comparable to the Women's Health Initiative has not been conducted, many studies have pointed to improvement in cardiovascular risk following testosterone replacement [38].

Sex Differences in Response to Genes Involving Cholesterol Biosynthesis

The cholesterol biosynthetic pathway also known as the mevalonate pathway is an important mechanism involved in a vast array of cellular functions. This pathway has been very closely observed by various researchers to study genes affecting heart function. HMG-Coa Reductase (HMGCR) is the rate limiting enzyme of this pathway and catalyze the conversion of HMG-CoA to mevalonate. This pathway provides sterols for membrane structure and non-sterol intermediates for the post-translational modifications and membrane anchorage of growth-related proteins, including Ras, Rac, and Rho GTPase families. In the past years, the regulation of HMGCR has been thoroughly investigated because of its prime involvement in cholesterol and isoprenoid biosynthesis. As all cells require a steady supply of mevalonate, both the sterol (i.e. cholesterol) and non-sterol (i.e. isoprenoid) as products of mevalonate metabolism exert coordinated feedback regulation on HMGCR through different mechanisms. The right functioning of HMGCR as the rate limiting enzyme in the mevalonate pathway is of prime importance under both normal physiologic conditions and in many diseases. HMGCR has diverse roles in various cellular pathways involving cell proliferation, cellular metabolism and cholesterol biosynthesis, in maintaining cytoskeletal stability and dynamics, cellular structure, it's fluidity, mitochondrial function and eventually in regulating overall fate of the cell [62]. Due its implication with endogenous cholesterol metabolism and heart diseases, this pathway has been studied very closely to determine the effect of vitamins and micronutrients targeting the regulation of HMGCR and its downstream effectors. Several studies suggested vitamin isomers mimic the role of statins, a class of drugs used in patients to lower their cholesterol by targeting HMGCR [63]. Earlier studies have substantially found the sex difference in metabolism of mevalonate in rats [64]. The two known pathways of mevalonate metabolism—the shunt pathway and the cholesterol synthesis pathway, both have been demonstrated to exhibit the sex difference. Firstly, the shunt pathway in female rats has shown to exhibit twice the ability to metabolize circulating mevalonate accounted by greater ability of the female kidney to convert mevalonate to CO_2. Secondly, male rats have significantly greater ability to convert circulating mevalonate to cholesterol as compared to the females [64, 65]. However, there is no significant data to extrapolate these findings in humans and further research is needed to justify these findings. It may be a little premature to relate these findings to lower cholesterol levels and less occurrence of atherosclerosis in females in spite of the major findings reported in the studies indicated above.

Conclusion

From the foregoing discussion, it is evident that nutrition and incidence of CV disease go hand in hand. Although, calorie restriction, sex hormones and cholesterol metabolism play a significant role, there is still a void in concise understanding of the exact role of sex with respect to diet and heart disease. There is a lower risk of heart disease, hypertension and atherosclerosis in females before menopause then that in the male; however, this sex-dependent difference is not evident after menopause. Such a switch in the susceptibility of females may be attributed to alterations in the levels of estrogen hormones and/or the sensitivity of its receptors. Differences in the lipid profile, particularly in the cholesterol biosynthetic pathway has also been suggested to explain the differences in the development of cardiovascular disease in male and female population. Nutritional status is considered to have a profound impact on the sex-specific differences in the sensitivity of various factors involved in the induction of heart disease. Caloric restriction has been observed to modify the sex-dependent difference in the incidence of cardiovascular disease by changing the distribution of body fats, altering the difference in the endothelial function, reducing the oxidative stress and regulating the levels of sex hormones. Thus, it is suggested that the nutrition plays a critical role in modifying the incidence of heart disease in both males and females.

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