We are IntechOpen,
the world's leading publisher of
Open Access books
Built by scientists, for scientists

6,900
Open access books available

186,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS
BOOK CITATION INDEX
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Importance of Dietary Fatty Acid Profile and Experimental Conditions in the Obese Insulin-Resistant Rodent Model of Metabolic Syndrome

Annadie Krygsman

Additional information is available at the end of the chapter
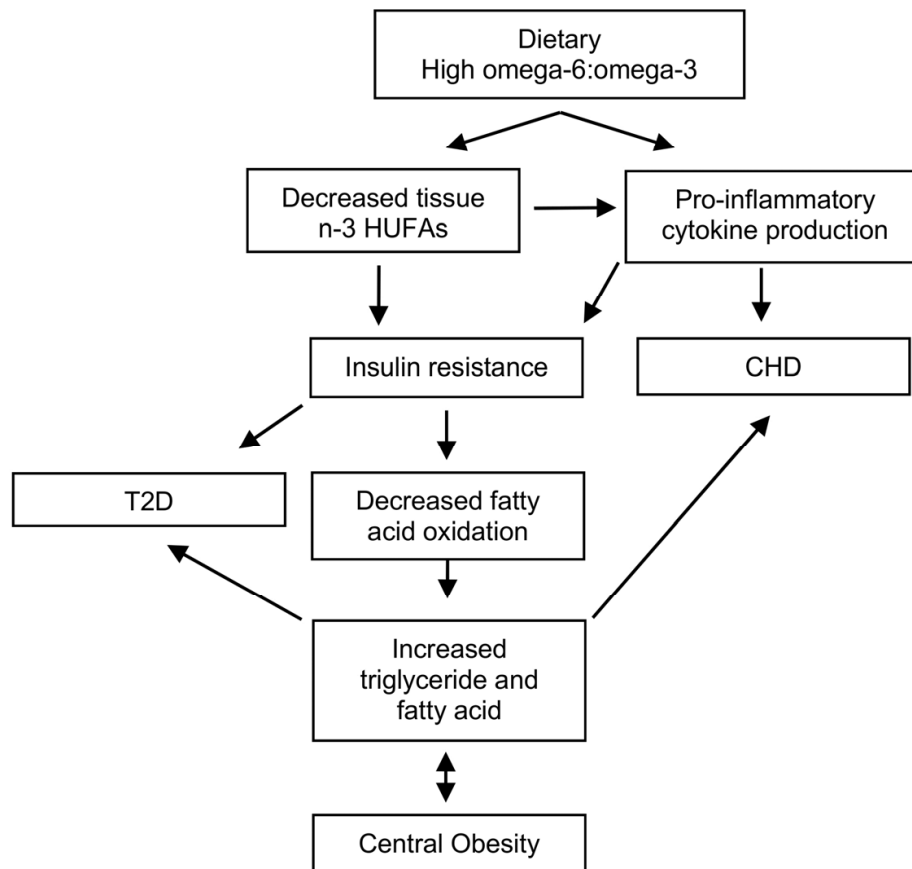
1. Introduction

Obesity is currently regarded as one of the most alarming pandemic diseases worldwide as it is closely related to, and in many cases causative of, Type 2 Diabetes (T2D), coronary heart disease (CHD), cancer and other pathophysiological disturbances. A cluster of these disorders are linked together under the term Metabolic Syndrome (MetS). These factors include impaired glucose tolerance and/or insulin resistance, dyslipidemia (high plasma levels of triglycerides and low density lipoproteins), central obesity and hypertension [1]. In the USA a recent Health and Nutrition Examination Survey (NHANES) reported that 68.3% of study subjects were considered as overweight (BMI $\geq$ 25) and 33.9% obese (BMI $\geq$ 30) [2]. The rising incidence of childhood obesity and T2D, high blood pressure, hyperinsulinemia and dyslipidemia are particularly worrisome as these children often mature to be obese adults [3]. This risk of developing obesity and T2D has largely been blamed on the increased consumption of energy dense foods and fat intake, particularly saturated fat, but it is interesting to know that the mean fat intake of the human population has not increased much in the past 50 years [4]. It is true that the vast advancement in technological developments has led to a reduction in physical activity worldwide, but as obesity now involves infants and the populations of developing countries [5], this obesity pandemic cannot be attributed to this alone. In addition, laboratory and other domesticated animals have also been subject to the increased prevalence of obesity, despite having largely unchanged living conditions for many years [5].

Obesity and MetS have nevertheless been blamed on excess intake of fat leading to excess energy intake exceeding energy expenditure, which causes the deposition of this energy in the format of greater numbers of excessively enlarged fat cells. When this fat deposition occurs in the central trunk (visceral fat accumulation) it is highly causative and suggestive of

the risk of developing MetS, due to associated high levels of blood cholesterol and triglycerides [6]. It is especially dietary saturated fat acid (SFA) consumption that has been thought to lead to the elevation of these blood factors which are highly indicative of CHD risk. Most recently, through meta-analyses of large international studies, the consumption of SFA has been de-vilified and the causal link between these parameters and CHD disproven [7-9]. The question thus arises which changes in the dietary composition in the past half-century is causing this obesogenic outcome? Although the total fat content of the diet has not changed significantly, a growing number of reports are making it quite clear that the dietary fatty acid quality may be responsible for the differential influence on obesity and pathophysiological outcome.

The main alteration within the fatty acid profile of the modern diet has been the increased use of vegetable oil, both as a cheaper and more accessible alternative to animal fats, but also as a substitute to animal fats to reduce the intake of SFAs. Vegetable oils, although higher in monounsaturated fatty acids, are very high in omega-6 fatty acids (100-fold larger) compared with animal fat [10]. These oils are also much lower in omega-3 fatty acids, leading to an increase in the ratio of dietary omega-6:omega-3 fatty acids. In the past few years a growing number of studies and meta-analyses have focused on the influence of dietary omega-6:omega-3 ratio, and the role of omega-3 fatty acids in the development of T2D and CHD [11-13] and a clear link between a high ratio of omega-6:omega-3 fatty acids and the development of these pathologies illustrated (Fig. 1). These effects are, to a large extent, believed to be due to the pro-inflammatory functions of the omega-6 arachidonic acid (AA) cascade which has deleterious effects in a variety of tissues due to chronic low-grade systemic inflammation. This inflammatory environment is now considered a key component of MetS, as the enlarged adipocytes are known to secrete many relevant pre-inflammatory adipokines [14]. These pro-inflammatory adipokines are to a great extent responsible for insulin resistance, hallmarked by decreased fatty acid oxidation and an increase in triglyceride and free fatty acid (FFA) synthesis. These FFAs are then deposited within the adipocytes, leading to and worsening obesity and the perpetual further effect on metabolic dysregulation of fat and glucose homeostasis. Conversely, the omega-3 fatty acids eicosapentanoic acid (EPA) and docohexanoic acid (DHA) have anti-inflammatory properties, and serve as protection against MetS and CHD [15]. In adipocytes, these omega-3 fatty acids prevent insulin resistance mediated by the AA-induced increase in pro-inflammatory eicosanoids, via modulation of adiponectin levels and function [16-18]. This positive effect can only occur when an excess of omega-6 fatty acids is not present, as high levels of omega-6 fatty acids prevent the synthesis of omega-3 fatty acids in tissues [19], and it is thus arguable whether supplementation or dietary increase of omega-3 fatty acids would effectively attenuate the deleterious effects of omega-6 mediated inflammatory mechanisms without a decrease in the intake of omega-6 fatty acids [20-22].

Close scrutiny of the modern Western diet in epidemiological studies have brought to attention the role of the fatty acid profile of certain native and regional diets in the prevention of the development of MetS and all of its associated symptoms [8, 9, 23, 24]. In populations consuming diets rich in omega-3 fatty acids, the prevalence of impaired glucose tolerance and CHD is much lower than those populations consuming the Western diet.

Figure 1. The role of dietary omega-6:omega-3 ratio on the development of obesity, T2D and CHD.

Japanese island-dwellers and Alaskan natives, who adopt the Western diets through migration, forsake the consumption of their native high omega-3 foods in lieu of high omega-6 Western foods leading to as much as an 80% increase in T2D. Icelandic natives have a very low incidence of T2D, despite their high prevalence of overweight and obesity [25], found to be due to an inverse association with the omega-3 content of reindeer milk, and thus positively associated with its omega-6:omega-3 fatty acid ratio. Furthermore, Icelandic animal fodder contains fish meal which greatly reduces the omega-6:omega-3 content of the milk, and this provides a protective factor against MetS and T2D. This is contrast to other Norwegian countries, as well the rest of the Western world, where animal fodder contains high amounts of omega-6 fatty acids.

Animal feeds were also subject to the change from a low to high omega-6 content with the incorporation of vegetable oils, and this has led to and increase in obesity in domestic and laboratory animals [5]. Laboratory animals, whose housing and husbandry practices have no changed much in the last 50 years, now have a much increased mid-life bodyweight.

This is proposed to be due to the substitution of dietary fat source from lard to soy bean oil (or soy meal) and hence the omega-6:omega-3 ratio has increased from 2:1 to 12.5:1 [26]. This is very concerning as these feeds serve as control and maintenance diets and are utilized in most rodent research colonies worldwide. Maternal programming is a well-described field,

especially in the development of T2D, and animals exposed to pro-diabetic diets during pregnancy give rise to offspring with a higher propensity of developing metabolic perturbations [27]. The 'control' diet used in the majority of studies thus in fact may represent a high omega-6 treatment diet, which casts a degree of doubt on the accuracy of results obtained from such experiments. More careful scrutiny and transparency of rodent dietary composition is needed in order to make valid comparisons with other dietary interventions in the study of MetS components.

Animal models of MetS are not only influenced by dietary fatty acid composition, and other husbandry-associated factors such as day/night cycle and time-of-day experimental procedures can also influence the outcome of such studies. Rodents, by nature, are nocturnally active animals, but sampling and experimental procedures commonly take place in the day time which is the inactive rest phase of these animals. The circadian clock, which is regulated by daylight exposure, plays a critical role in many physiological processes associated with insulin, fatty acid and glucose metabolism. In the rat, insulin levels are low during its inactive diurnal phase, and high during the nocturnal phase with high glucose utilization [28]. Sampling of blood and performance of glucose tolerance tests usually take place in the morning, as it represents the most convenient time for the scientist, following a 6-12 h overnight fast during the animal's active phase. This causes a rapid fall in blood glucose levels, whereas fasting during the day in the animal's rest phase causes a much less significant drop in blood glucose levels. In addition, stress induction due to this food deprivation, causes a rise in corticosteroid levels which mobilizes fatty acids from triglyceride stores [29] giving an inaccurate reflection of the blood levels of these compounds. This of course is not desirable in an experimental setting and undue bias is incorporated. A solution is to reverse the light-dark cycle, so that all animal procedures are carried out during the animal's active phase. This creates the most effective scenario, but is critically dependent on the absence of dark-phase light contamination, as even dim light during the dark phase causes the inhibition of the circadian rhythms in blood glucose, lactic acid, insulin and corticosteroid levels [29] which would compromise or alter the outcome of scientific investigations.
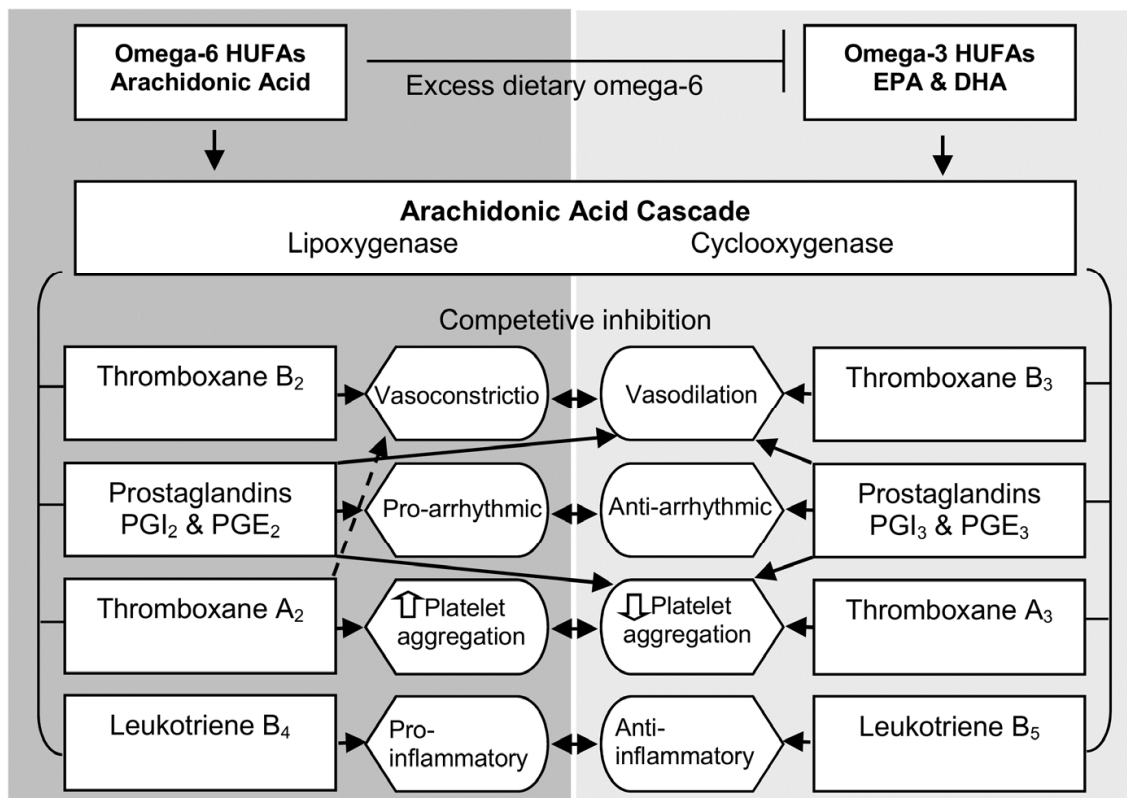
Creating and using accurate and comparable models for research into MetS and associated CHD is essential in providing relevant and applicable data that may be extended to human disease state research. Basic principles such as dietary composition and fatty acids profile, together with appropriate husbandry practices are thus of major importance in the design an execution of research studies using animal models. This chapter will endeavor to highlight some of the crucial aspects surrounding dietary fat composition and use of the rodent model of diet-induced obesity and metabolic syndrome.

2. Role of omega-6 and omega-3 fatty acids in insulin resistance and CHD

Fatty acids are important sources of fuel as they yield large quantities of ATP when metabolized and the heart and skeletal muscle prefer fatty acids to glucose as fuel [30]. Fatty acids consist of a long aliphatic chain with a carboxylic acid group at one end and may be

saturated or unsaturated (those that contain no double bonds are saturated, and those that have double bonds are unsaturated). The biological reactivity of these compounds are dependent on both the length of the fatty acid chains as well as the number and positional location of double bonds prevalent (n-3, n-6, n-7 and n-9). An 18-carbon fatty acid containing two or more double bonds is referred to as being polyunsaturated fatty acids (PUFA), whereas fatty acids consisting of 20 or 22 carbons are termed highly unsaturated fatty acids (HUFA). It is especially the n-3 and n-6 HUFA that are considered to be major role-players affecting diet-dependent disease. These fatty acids are responsible for maintaining correct cell membrane protein function and fluidity and cellular functioning. The more highly unsaturated fatty acids, such as the n-6 arachidonic acid, are converted to eicosanoid derivatives which have a hormone-like action and are involved inflammatory pathologies such as obesity, insulin resistance and atherosclerosis [31-33]. In contrast, the n-3 fatty acid-derived eicosanoids have anti-inflammatory and anti-thrombotic activities [34, 35]. It is this action of the n-6 derived eicosanoids that is of particular importance as they are major role-players in diet-associated pathologies through their pro-inflammatory actions (Fig. 2).
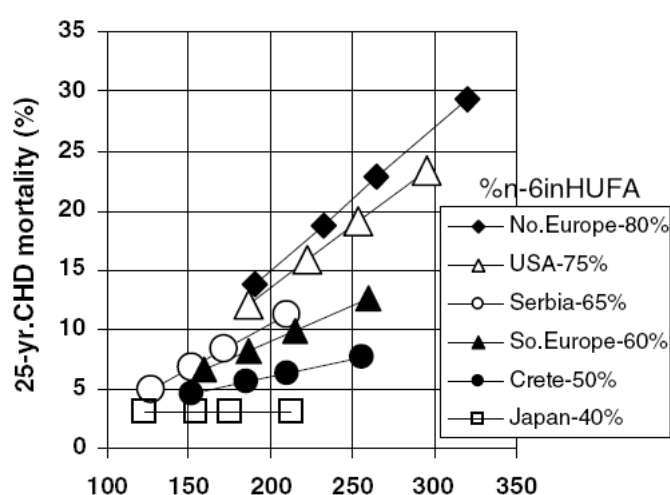
A number of studies, including the Seven Countries Study, have shown the Mediterranean Diet to be preventative of insulin resistance and CHD and protective against certain cancers [19, 36, 37]. This diet is characterized by a high intake of unsaturated fatty acids in the form of virgin olive oil containing a very low ratio of omega-6:omega-3 fatty acids (2:1). In these

Figure 2. The effects of omega-6 vs. omega-3 conversion pathways on inflammation in coronary heart disease (adapted from [45])

studies, Western diets of non-Mediterranean countries (USA, Western Europe and Scandinavia), and containing omega-6:omega-3 fatty acid ratios in excess of 15:1, was associated with significant higher incidences of insulin resistance and CHD. More importantly, the lack of detrimental effect of SFA and cholesterol intake, in the presence of optimal omega-3 intake, was simultaneously indicated [38, 39], as was the failure to document a relationship between dietary cholesterol and CHD (reviewed by [9]). Reducing SFA intake or replacing SFA with MUFA was found to not improve cardiovascular risk [40, 41]. Moreover, blood cholesterol levels alone do not predict absolute CHD mortality rates observed for different groups worldwide (Fig. 3) and these death rates differ at each cholesterol level. In fact, the elevations in blood cholesterol are fatal only to the magnitude that omega-6 exceeds omega-3 in tissue HUFA [19]. It is thus not surprising that it has recently been proposed that omega-6 HUFA blood levels are more indicative of heart disease risk than total cholesterol level, where high omega-6 HUFA levels inhibits omega-3 synthesis [42, 43]. The authors of the 25-year follow-up of the Seven Countries Study considered factors affecting inflammatory oxidative processes and thrombosis to be of great importance [44].

Figure 3. Dietary fatty acid imbalances which elevate blood cholesterol are fatal only to the magnitude that omega-6 exceeds omega-3 in tissue HUFA (From [19]).

Omega-6 fatty acids are further known to amplify post-prandial oxidative stress leading to these elevations in inflammatory vascular lesions and atherosclerosis. High percentages of HUFA in plasma and adipose tissue of obese children have been reported, associated with higher concentrations of inflammatory markers [46]. In contrast, omega-3 HUFAs prevent and reverse high fat diet-induced insulin resistance, in part via modulation of adipose tissue inflammatory product secretion patterns [11, 20, 47]. The omega-3 HUFAs act via different pathways to establish this protective effect by, amongst others, inhibiting the pro-inflammatory NFKB pathway due to its inhibition of SFA-activation of Toll like receptors (TLR) 2 and -4, with DHA being the most potent inhibitor of this pathway. The omega-3 HUFA EPA competes for cyclooxygenase and lipooxygenase enzymes in the AA cascade, and in this manner reduces the production of pro-inflammatory AA-derived eicosanoids.

Furthermore, EPA and DHA inhibits the release of AA by inhibiting phopholipase-A2, and thus also reduces activation of the pro-inflammatory AA-cascade [48]. Inflammation, mediated via AA-derived eicosanoids, also plays a critical role in the pathophysiology of atherosclerosis with coronary plaques being highly inflamed with a core rich in lipids [49]. Recently published studies reported that the blood plasma ratio of omega-6:omega-3 plays an important role in endothelial function and vascular tone, and that a high ratio is significantly associated with a high prevalence of coronary artery lesions [50]. The plasma EPA/AA ratio and high-sensitivity CRP levels were further found to be independent predictors of the presence of complex coronary lesion. In addition, the fact that omega-3 fatty acid supplementation improves endothelium-dependent vasodilation further supports the recommendation that modulation of dietary omega-6 and omega-3 fatty acids is a safe and necessary approach to improving vascular health and reducing the risk of CHD [51, 52].

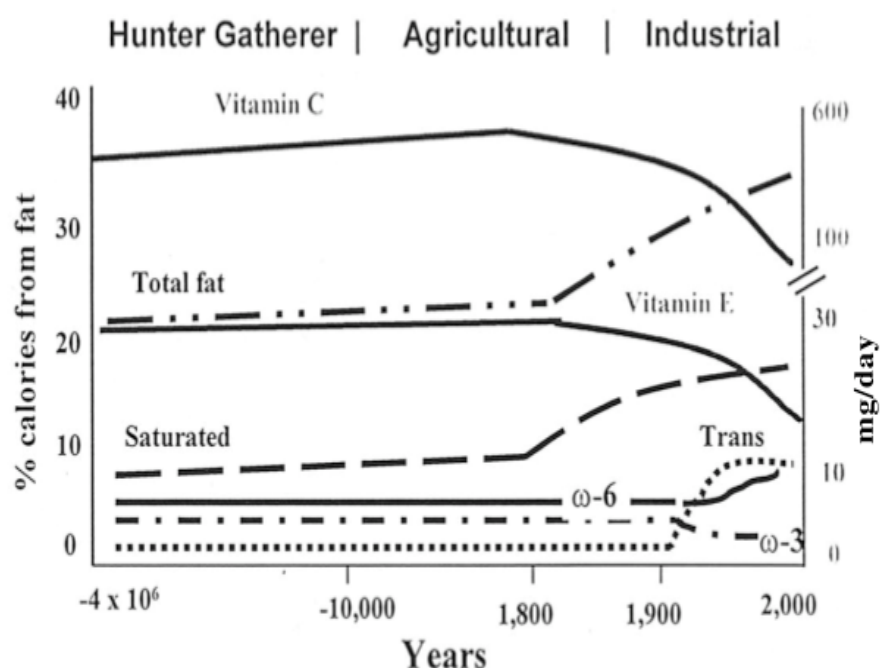## 3. Evolution of the omega-6:omega-3 fatty acid dietary ratio

Studies in Paleolithic nutrition and modern-day hunter-gatherer populations estimate that humans evolved on a diet that was much lower in saturated fatty acids than is today's diet, and contained small but roughly equal amounts of omega 6 and omega 3 fats [10]. The Paleolithic diet, also known as the Cave Man or Hunter-Gatherer diet, prevailed for about 2.5 million years and ended 10 000 years ago with the development of agriculture [53, 54]. These stone age humans centered their diet on foods that could be hunted and fished, such as meat, offal and seafood, and could be gathered, such as eggs, insects, fruit, nuts, seeds, vegetables, mushrooms, herbs and spices. The proportion of nutrients, although dependent on latitude and other influences, consisted of roughly 20-35% each protein and carbohydrates and 30-60% fat. Of the total food intake 45-65% came from animal products and 35-55% from plant products, but it is debatable whether any grains or tubers were consumed [54]. Omega-3 fatty acids were found in all of these foods, and a balance existed between omega-3 and omega-6 for this entire period. Hunters tended to target herbivores, and the total carcass fat of such animals averaged about 7% compared with domesticated beef cows 40%, and with 35% PUFA compared with the current 7% in grain-fed beef [55]. Carbohydrates nearly all came from fruit and vegetables, and a small proportion from honey [56], thus consisting overall of low glycemic load carbohydrates.

The modern human inherited characteristics by genetic adaptation accrued over millions of years of the Paleolithic Period, and the vast majority of our biochemistry and physiology are adapted to life conditions that existed prior to the advent of agriculture about 10 000 years ago. At this time humans began to cultivate plants and later to domesticate animals constituting the Agricultural Revolution leading to the Neolithic Period. The gradual spread of agriculture throughout the Old and New Worlds generally led to an increase in population, and demand for foodstuffs which soon led to difficulties in exploitation of game and wild plant food. The main dietary innovation that accompanied agriculture involved the introduction of cereal grains. Crop cultivation became necessary to supply in the increased demand for foodstuffs, leading to cereal grains becoming staple food – a departure from native nutrition unparalleled and unseen in other free-living primates of

that period [57]. Before that time, early humans did recognize that grains could be consumed in times of shortage, but the labor involved in milling the grains discouraged frequent use. The almost 60% intake of fruits and vegetables soon declined to an estimated 20% of daily caloric intake, with cereal grains consumed in place thereof. Grains are high in omega-6 fatty acids and low in omega-3, and this led to the increased dietary ratio of omega-6:omega-3 (Fig 4).

Since this major change in the human diet, natural selection has had too little time to make the optimal genetic adaptations to the new dietary composition, leading to physiological and metabolic maladaptation [58]. These incomplete genetic changes are not seen in modern hunter-gatherer populations it is thus reasonable to conclude that humans are maladapted to diets of domesticated and processed plant foods, contributing too many of the current diseases of civilization. What has compounded these maladaptive consequences of the agricultural age, is the fact that the demand for meat in the past 50 years has led to the implementation of large cattle and poultry feedlots in order to provide in the increased demand for meat. Prior to that time, animals were pasture-reared and consumed a diet of green leaves high in the omega-3 fatty acid α-linolenic acid (ALA) resulting in a meat ratio of omega-6:omega-3 of 2:1, vs. 4:1 in grain-fed meat in beef and poultry as well as eggs. Cattle moved from pasture to feedlots for fattening prior to slaughter lose this valuable ALA store, as well as EPA and DHA with the supply diminishing each day the animal spends in the feedlot (Fig. 5 [59]).
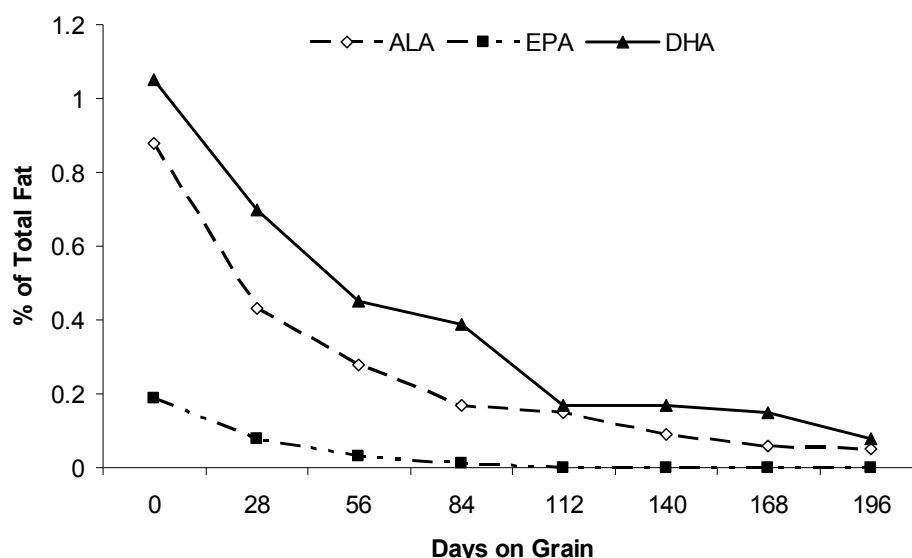

Figure 4. Change in dietary omega-6:omega-3 ratio from the Paleolithic Period to the current dietary composition [10].
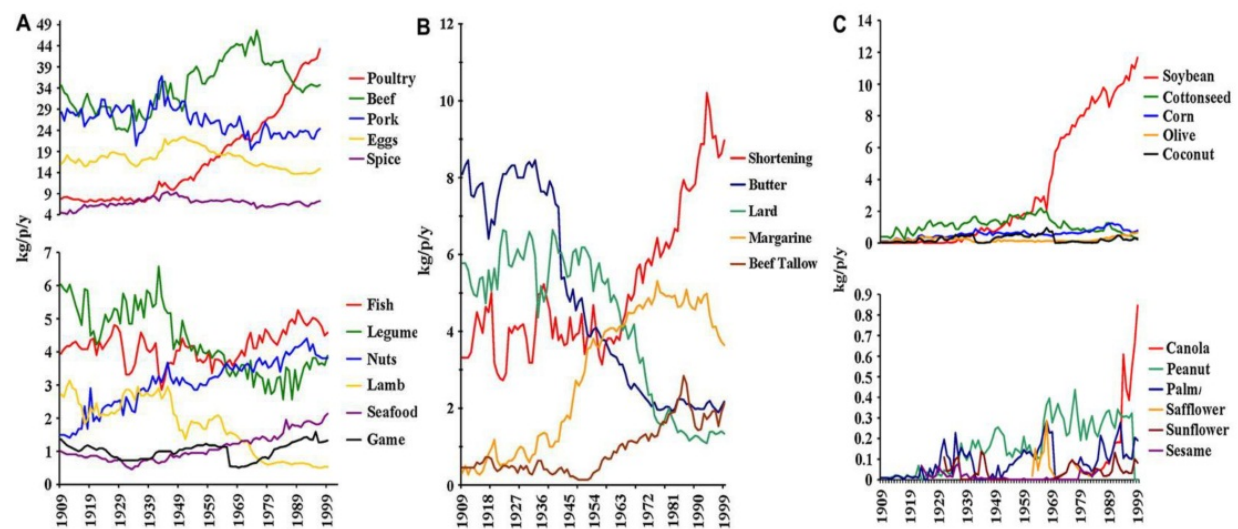
In addition to the new emphasis on grain feeds for domestic livestock, the first part of the 20th century marked the onset of the industrial vegetable oil industry [60]. Technological

advancements after World War I made large-scale production of vegetable oil more efficient and economic. With the advent of hydrogenation of oils to increase solidification, the ALA content was greatly reduced leaving a high concentration LA, and hence a high omega-6:omega-3 ratio, with similar changes in the production of other oils such as cottonseed oil, safflower oil and sunflower seed oil. Soy bean meal/oil forms the main constituent of livestock and poultry feed, shortening and margarine and it thus forms a large proportion of the average daily fat intake in most countries. These changes in the consumption of essential fatty acids throughout the 20th century was recently (2011) reported for the first time as a detailed quantitative analysis (Fig. 6 [61]). They found that the estimated consumption of soy bean oil increased in excess of 1000-fold from 1909 to 1999. This consequently led to a 3-fold increase in the intake of LA which was the primary determinant for the declining percentages of omega-3 HUFAs in tissue throughout the 20th century.

Animals have not been spared the fate of this deleterious dietary transformation, as presented in a very recent study which investigated the mid-life body weight of animal species living with or around humans in industrialized societies [5]. The authors found that the body weight of primates and rodents living in controlled laboratory environments, as well as feral rodents and domestic dogs and cats, has increased over the past few decades similarly to humans. Although in this study it was speculated that certain viral pathogens and epigenetic factors possibly could have contributed to this obesogenic effect, it is also highly likely that it could be due to the deleterious transformation to a high omega-6 intake in chow. As in the human diet, the fat content of chow has remained constant during the time period o.n which this study was focused.

Figure 5. Rapid decline in fat-derived omega-3 stores of beef cattle receiving grain after being moved from pasture to feedlot (from [59])

Figure 6. Changes in the estimated per capita consumption (kg/person/year) in the USA between 1909 and 1999 of A: of major food commodities; B: Major fat commodities; C: Vegetable and seed oils (from [61])

Indeed the fatty acid composition of standard rodent chow has changed similarly to that of humans, with comparable effects on the omega-6:omega-3 ratio [62-64]. Fifty years ago, laboratory chow fat content consisted of the lard of animals grazing on grass which had an omega-6:omega-3 of less than 6:1, but since then the omega6:omega-3 ratio in the control rodent diet has changed from 6:1 in 1998, to 9:1 in 2002, 12.5:1 in 2006 [26]. Standard high fat research diets now contain omega-6:omega-3 ratios in excess of 11:1 [51]. All maintenance diets contain soy bean oil as their main fat source with omega-6:omega-3 ratio of 8:1 which is much higher than lard produced from grass-reared beef at 2:1 used before the change to soy bean oil (Table 1). Hydrogenated soy bean oil produces severe insulin resistance [65], and is thus not suitable as a source of fat for rodent maintenance diets.

Fat/Oil	Omega-6:omega-3
Sunflower seed	Omega-6 only
Safflower	253:1
Corn	83:1
Lard	10:1
Soy Bean	8:1
Beef Tallow (Grain-fed)	8:1
Milk Fat (Grain-fed)	5:1
Beef Tallow (Grass-finished)	2:1
Canola	2:1
Milk Fat (Grass-finished)	1:1
Flax Seed	0.2:1

Table 1. Omega-6:omega-3 ratios of commonly-consumed fat sources in the USA [66, 67]

Although the evidence is now mounting and the clear link between excessive omega-6 intake and modern Western diseases of lifestyle established, formal dietary recommendations (Institute of Medicine, National Academies of Science), have not incorporated these alarming findings. Dietary Reference Intakes (Food and Nutrition Board) in 2006 recommended an adequate intake of omega-6:omega-3 of 10:1, a ratio now proven to be deleterious to especially cardiovascular health [67]. These recommended dietary intake guidelines are thus in urgent need of revision to include the levels and profiles based on the evidence now available in literature.

4. Rodent models for the study of insulin resistance and CHD

Model organisms have extensive value as sentinels informing us about environmental factors which may potentially have an impact on humans [68]. The severity of the obesity epidemic has created an urgent need to study the causes and progression of the pathologies associated therewith such as MetS. Animal models that sufficiently mimic all the aspects of this syndrome, including obesity, insulin resistance, dyslipidemia and hypertension are required for such studies.

A large variety of animal species have been used as models to study MetS, with varying degrees of success. The ideal model should be small and economical, but large enough to perform the required experimental procedures. Non-human primates are in many respects the ideal models as they are phylogenetically close to humans, consume a comparable omnivorous diet, and develop MetS and CHD as they age [69], but in addition to important ethical issues, they have an extended lifespan, are expensive to house and carry viral zoonoses dangerous to humans. Other animals, such as the domestic pig, the dog and the rabbit all provide potential as models for MetS and CHD research, but cost, cultural status and species-specific vulnerabilities to dietary modulation [70], place doubt on their efficacy for this research field. The rat, in particular, resembles the human both in physiology and metabolism, and follows an omnivorous diet similar to humans with whom the rat has spread worldwide [71]. Since the development of the first defined rat strain at the Wistar Institute in the 1920s, the rat has been used regularly and to great effectivity for the study of diet-induced obesity, insulin resistance and the disease states associated therewith, such as CHD [70, 72].

Standard laboratory rat species, such as the Wistar or Sprague-Dawley strains, are successfully utilized for diet-induced MetS models [73]. The diets aim at reproducing high fat diets (HFD) similar to what is ingested in the Westernized society, i.e. a high percentage total fat (of calories) with a high SFA content with or without high carbohydrate content. Varying combinations and amounts of carbohydrates and fats are used in different studies [72, 74, 75], with the carbohydrates fructose or sucrose standardly used, but the fat source varying. Long-term feeding of these diets induce obesity, increased abdominal fat deposition, hyperinsulinemia, hypertension and impaired glucose tolerance in most rat and mouse strains [76]. Fat fractions in these diets range between 20% and 60% of energy as fat as either animal-derived lard or beef tallow, or plant oils such as soy bean oil, coconut oil,

olive oil, safflower oil [73]. The problem is that in many cases, these constituents or profile of the fatty acid element in the diet are not available, not taken into account or not reported in published literature. In fact, standard chow prepared by commercial companies contains fats obtained from a variety of sources, both animal and vegetable, which, considering the variation in omega-6:omega-3 fatty acid ratio between such foodstuffs can have far-reaching implications for the outcomes of studies employing these diets. This complicates the study of the effects of dietary fats on obesity and insulin resistance and associated disease states, and makes comparison between studies almost impossible. A rodent high fat diet high in SFA content, in the presence of a low omega-6:omega-3 ratio would not give the same results as a diet similar in total fat and SFA content, but with a high omega-6:omega-3 ratio. Even with low to moderate fat percentages (20-30%) in the presence of the recommended SFA content (10%), high omega-6:omega-3 ratios can lead to glucose intolerance in rodents indicated by decreased insulin response to intravenous glucose tolerance tests [77].

Rodent models share many traits with human diabetic cardiomyopathy, such as left ventricular hypertrophy, increased ventricular stiffness, ventricular dilatation, decreased fatty acid uptake, cardiac inflammation and fibrosis, decreased cardiac function and endothelial dysfunction [72]. In the Wistar rat the onset of cardiomyopathy occurs within 7 weeks of receiving the diet, and leads to cardiac steatosis, impaired contractile function and mitochondrial degeneration with increased myocardial fatty acid uptake [78]. Simple high fat feeding with caloric excess is thus sufficient to induce metabolic defects that area associated with diabetic cardiomyopathy, but isocaloric high fat diets based on saturated fats alone do not induce insulin resistance and in fact improve cardiac function [79, 80]. This could point to both the deleterious role of hyperinsulinemia and impaired glucose homeostasis on the development of cardiac defects due to the consumption of the Western Diet, or could indicate the failure to take into account the PUFA profile of said diets used in these studies.

It is worthy to note the normal rat is resistant to the development of atherosclerosis, and simple Western high fat diets do not induce atherosclerosis in these animals. Induction of atherosclerosis is less easily achieved, and generally requires addition of cholesterol to the animal's diet. Atherosclerosis can be induced by increasing the oxidized form of LDL in the blood by either increasing the total LDL while maintaining the proportion of oxidized LDL, or by increasing the proportion of oxidized LDL. Linoleic acid may be a factor in the susceptibility of LDL to oxidation as LDL is rich in LA, but the dietary content of LA differs in its ability to cause LDL oxidation depending on its effect on the blood lipid profile which in turn varies according to species. Saturated fat feeding only exacerbates an increase in LDL when excessive cholesterol is consumed, and in the absence of cholesterol SFA does not cause a rise in LDL both in rats and humans and atherosclerosis does not result [81] (reviewed in [82]).

Although rodent models play a very important role in, and indeed have advanced the understanding of the underlying pathological mechanisms of human MetS and CHD, these models do have limitation and none exist that exactly phenocopies the human MetS disease condition. By adding dietary inconsistencies these models cannot be used to their full

potential and clear and transparent dietary formulations, which include the fatty acid profile, are crucial for providing comparable studies and thus results. Maintenance and Control diets should consist of the correct recommended balance of omega-6:omega-3 fatty acids to provide a 'healthy' model and control group as reference with which to compare dietary interventions.

5. The circadian clock in animal models of insulin resistance and fatty acid metabolism

Nutrient homeostasis in many species of vertebrates is intricately controlled by coordinated interactions of daily rhythms of activity and rest, feeding behavior and energy expenditure and storage across the daily 24h light-and-dark cycles [83]. This circadian clock, under the control of molecular mechanisms cycling in the suprachiasmatic nuclei (SCN) of the hypothalamus, plays a critical role in a diverse group of physiological processes in different cell types associated with insulin, glucose and fatty acid metabolism. The main factor responsible for synchronization of the circadian rhythm is sun light, which determines the precise length of the day and night in the 24 h period. This serves to orient the human or animal in relation to the point of day, and light is thus one of the most powerful synchronizers of the circadian rhythm. In the absence of pronounced SCN signals the circadian rhythm in peripheral tissues becomes uncoupled, resulting in aberrant cellular metabolism and disease risk [29, 83]. The changes in lifestyle during the past 50 years has, in addition to an increased intake of inappropriate, energy-dense and poor fat- and nutrient-quality foods, also led to increased time spent awake and hence disrupted pattern of eating, sleeping and physical activity. This causes an asynchrony with the circadian rhythm with profound effects on glucose and fatty acid metabolism, which is well-described in the human and linked to metabolic derangements associated with and leading to increased adiposity and BMI [84].

The main synchronizers of the peripheral circadian clocks are food restriction, glucocorticoids and melatonin [85]. Of the peripheral tissues, the adipose tissue circadian clock plays a fundamental role in glucose and lipid metabolism [86, 87]. Any disturbances of the adipocyte circadian clock van alter adipocyte responsiveness to different stimuli, i.e. levels of glucose, insulin and fatty acids, and can alter the level of lipogenesis and lipolysis. The adipokine, adiponectin, which plays an important role in insulin sensitivity, inflammation and fatty acid oxidation, presents a circadian rhythm similar to insulin, with peak levels in the early active phase and low levels in the inactive phase (Table 2). The early hours of the active phase are characterized by higher glucose levels and thus higher insulin demand in anticipation of the onset of stimulus [88-90]. At the end of the active phase and the start of the rest phase, insulin levels and sensitivity are low and oral or intravenous glucose (or consumption of a meal) leads to a significantly higher plasma glucose response. Accordingly, rodents respond differently by day and night to insulin and 2-deoxy-D-glucose with a rapid fall in blood glucose levels occurring at the end of the active phase after a 6h overnight fast in contrast to delayed and diminished hypoglycemia following a day time (inactive phase) fast [89]. Furthermore, the circulating FFA levels follow the same circadian

pattern as glucose and are low in the active phase and high in the inactive phase in both humans and rats, with an increased turnover present in humans with T2D [90, 91]. The role of adiponectin in FFA, insulin and glucose metabolism in context of the circadian rhythm is shown in Figure 7. Under normal condition, i.e. healthy diet and daily sleep-wake cycle, adiponectin levels are high in the early active phase which stimulates AMPK, resulting in increased fatty acid oxidation and energy yield, with resultant low triglyceride levels. Adiponectin also upregulates PI3K which further downstream contributes to the improvement and optimization of insulin action, thus counteracting any pro-obesogenic and insulin-resistance inducing effects.

	Active phase	Inactive phase
Glucose	Low	High
Insulin	High	Low
FFAs	Low	High
- Omega-6	Low	High
- Omega-3	High	Low
- Eicosanoids	High	Low
Adiponectin	High	Low
O-GlcNAc	High	Low

Table 2. Circadian pattern of compounds involved in the regulation metabolic processes linked with metabolic homeostasis related to the development of insulin resistance and CHD. [88, 92-94].

Both obesity and dysregulated circadian rhythms can profoundly modulate adiponectin from various perspectives, all which may have bearing on the application of the rodent model of insulin resistance. Peak levels of corticosteroids occur at the start of the active/dark phase, and then fall during the light/inactive phase, reaching the lowest level just before the dark/light transition. Any stressor experienced at this time, be it anxiety, starvation, fear or pain would cause an increase in corticosteroid levels and hence release of FFAs from fat stores.

The circadian rhythm of specific PUFAs is largely lacking in literature, and to our knowledge, only one study has been published in which the daily variation of omega-6 and omega-3 fatty acid levels was reported [94]. In this study only preliminary evidence is presented, but the fact that a differential circadian rhythm for these fatty acids was identified is of great possible importance and deserves further exploration. This also supports earlier findings that a circadian rhythm for eicosanoid production exists, with low levels during the inactive phase and high levels present during the active phase [92]. In light of the fact the plasma FFA profile represents the dietary FFA profile [77], it is likely that the levels omega-6 HUFAs will be increased when a diet high in omega-6 fatty acids is followed. As arachidonic acid, via NFkB and eicosanoid conversions products, causes a suppression of adiponectin, this would exaggerate and amplify the pro-diabetic, insulin resistance and hyperglycemic propensity of the hormonal profile of the inactive phase. This further suppression of adiponectin, already low at the active/inactive phase transition,

Figure 7. A simplified representation of the interrelation of obesity, circadian rhythm disruption and dietary fatty acid intake on development of metabolic perturbations related to T2D and CHD.

would lead to down regulation of AMPK and hence increased triglyceride levels due to decreased fatty acid oxidation and clearance. Furthermore, this would ultimately lead to hyperglycemia due to the increased fatty acid-induced flux through the hexosamine biosynthetic pathway (HBP) which further decreases adiponectin levels, and thus increases its stimulating effect on liver gluconeogenesis.

It is known that a glucose-fatty acid cycle operates in the heart and diaphragm muscle by decreasing the rate of oxidation of the alternative substrate in the presence of increased availability of the other substrate [95, 96]. In the presence of high circulating levels of FFAs, a decrease in the rate of carbohydrate oxidation is seen, and vice versa when high circulating levels of glucose prevail. It is in this context that the HBP serves as a nutrient-sensing mechanism and both excess FFA and glucose can lead to augmented flux through this pathway, which ultimately plays a role in causing insulin resistance in the adipocyte, liver, muscle and pancreatic β-cell via reduced recruitment and translocation of GLUT4 to the plasma membrane [97, 98]. In the presence of excess FFA, glycolysis is inhibited distal to fructose-6-phosphate by increased levels of acetyl-CoA, leading to inhibition of pyruvatedehydrogenase, which in turn increases fructose-6-phosphate availability and thus

increased flux via catalyzation of glutamine:fructose 6-phosphate amido transferase (GFAT). This ultimately leads to accumulation of uridine-5'-disphospho-N-acetylglucosamine (UDP-GlcNAc) levels in tissues and this is directly correlated with the degree of insulin resistance. UDP-GlcNAc is the end product of the HBP, and serves as a substrate for O-linked glycosylation (O-GlcNAc) of proteins. It is thus regarded as the mediator of the HBP flux and thus insulin resistance by posttranslational protein modification of the insulin receptor proteins (IRS-1 and IRS-2) as well as GLUT4 (reviewed in [99]). There have, however, been conflicting reports in literature in which the lipid supplement Intralipid (a fat emulsion used intravenously as source of calories for patients requiring extended periods of parenteral nutrition regarded as 'safe' by the FDA) was found to induce insulin resistance without increasing HBP products [100], but as Intralipid consists mainly of soy bean oil with a ratio of omega-6:omega-3 of 7:1 [101] it is likely that insulin resistance was mediated mainly through the pro-inflammatory effects of the arachidonic acid cycle (Fig. 7) and to a lesser extent via flux through the HBP. Circulating FFAs also invoke insulin resistance via upregulation of PKC/PTEN which has an inhibitory effect on IRS-1 and IRS-2, which then further downstream prevents translocation of GLUT4 to the plasma membrane due to downregulation of PI3K [102]. This places increased emphasis on the critical need of clear definition of the profile of fats used in research studies, as it is probable that high levels of omega-6 fatty acids have a differential influence on these metabolic pathways in addition to being intricately involved in the circadian time structure of organisms.

In the heart, the cardiomyocyte circadian clock, in a time-of-day-dependent manner, regulates metabolism and ischemic tolerance similar to the regulatory roles of that of protein O-GlcNAcylation [93]. These variations mediate the clock-dependent regulation of O-GlcNAc transferase and O-GlcNAcase levels and nutrient metabolism and uptake. This would involve the coordinated regulation of the hexosamine biosynthetic pathway and play an important role in not only insulin resistance [103] but also myocardial apoptosis in the diet-induced insulin resistant rat [104] which in turn conversely depends critically on an adequate dark-phase. Any stress could increase flux through the HBP, leading to detrimental effects on the level of the cardiomyocyte [29, 104]. As the O-GlcNAcylation of proteins show clear circadian patterns and is high during the active phase and low during the inactive phase, it is thus proposed to have major consequences in the rat model of insulin resistance [93]. Any stress in the rodents during the inactive phase or at the transition from the active to inactive phase, as frequently is the case due to overnight starvation and early morning sampling, would lead to an increase in glucocorticoid levels which not only causes increased lipolysis and FFA levels, but also leads to inappropriately increased O-GlcNAcylation via direct recruitment of OGT [105]. Peak levels of corticosteroids occur at the start of the dark phase, and then fall during the light phase reaching the lowest level just before dark-light transition. Early morning sampling would thus possibly induce bias, due to the stress associated with starving and experimental procedures imposed on the animal.

A remedy for the deleterious introduction of bias, due to researcher convenience-of-sampling time, is to reverse the light-dark cycle, so that all animal handling and procedures are carried out during the animal's active phase. This constitutes the most effective scenario,

but is exclusively dependent on the absence of dark-phase light contamination. Even dim light during the dark phase causes the inhibition of the circadian rhythms in blood glucose, lactic acid, insulin and corticosteroid levels [29] and would compromise or alter the outcome of scientific investigations.

6. Conclusions

In summary, this chapter described the importance of the dietary fatty acid profile, with particular reference to the ratio of omega-6:omega-3 essential fatty acids, and the role it plays in insulin-resistance with reference specifically to the utilization of animal research models. Fatty acids are well-known to play a cardinal role in lipid and glucose homeostasis, and there is now compelling evidence that a reduction in the intake of omega-6 fatty acids in the diet is crucial to prevent disease states such as insulin resistance, obesity and cardiovascular disease. The majority of studies in the field of diet-induced obesity are performed using the high fat rodent model, but consistency and comparability is severely lacking/compromised due to incomplete disclosure of dietary fat components, and in many cases uncontrolled variation in the diet due to rat chow manufacturers using different fat sources according to availability or personal preference. In addition, the importance of the circadian rhythm in fatty acid and glucose metabolism and metabolic disturbances in the rodent experimental model is underemphasized, and in many cases not taken into account. This too, is of concern in the accuracy of results expressed in such studies, especially those investigating dietary interventions in insulin resistance, as seen in the regulative role that the humoral factors and the hexosamine biosynthetic pathway plays the circadian clock of insulin, glucose and fatty acid metabolism. These points highlight some frequently overlooked aspects in the experimental use of the diet-induced rodent model for insulin resistance, T2D and CHD research. In order to utilize the model to its full potential, attention to the exact dietary composition and experimental conditions are of critical importance.

Author details

Annadie Krygsman
Department of Physiological Sciences, Stellenbosch University, Stellenbosch, South Africa

Acknowledgement

7. References

[1]   Alberti, KG, P Zimmet, and J Shaw, Metabolic syndrome--a new world-wide definition. A Consensus Statement from the International Diabetes Federation. Diabet Med, 2006; 23(5) p 469-80.

[2]   Flegal, KM, et al., Prevalence and trends in obesity among US adults, 1999-2008. JAMA, 2010; 303(3) p 235-41.

[3]   Grundy, SM, The changing face of cardiovascular risk. J Am Coll Cardiol, 2005; 46(1) p 173-5.

[4]   Nguyen, DM and HB El-Serag, The epidemiology of obesity. Gastroenterol Clin North Am, 2010; 39(1) p 1-7.

[5]   Klimentidis, YC, et al., Canaries in the coal mine: a cross-species analysis of the plurality of obesity epidemics. Proc Biol Sci, 2011; 278(1712) p 1626-32.

[6]   Bjorntorp, P, Metabolic implications of body fat distribution. Diabetes Care, 1991; 14(12) p 1132-43.

[7]   Jakobsen, MU, et al., Major types of dietary fat and risk of coronary heart disease: a pooled analysis of 11 cohort studies. Am J Clin Nutr, 2009; 89(5) p 1425-32.

[8]   Siri-Tarino, PW, et al., Saturated fat, carbohydrate, and cardiovascular disease. Am J Clin Nutr, 2010; 91(3) p 502-9.

[9]   McNamara, DJ, Dietary cholesterol and blood cholesterolemia: a healthy relationship. World Rev Nutr Diet, 2009; 100 p 55-62.

[10]   Simopoulos, AP, Evolutionary aspects of the dietary omega-6:omega-3 fatty acid ratio: medical implications. World Rev Nutr Diet, 2009; 100 p 1-21.

[11]   Griffin, BA, How relevant is the ratio of dietary n-6 to n-3 polyunsaturated fatty acids to cardiovascular disease risk? Evidence from the OPTILIP study. Curr Opin Lipidol, 2008; 19(1) p 57-62.

[12]   Ramsden, CE, et al., n-6 fatty acid-specific and mixed polyunsaturate dietary interventions have different effects on CHD risk: a meta-analysis of randomised controlled trials. Br J Nutr, 2010; 104(11) p 1586-600.

[13]   Wijendran, V and KC Hayes, Dietary n-6 and n-3 fatty acid balance and cardiovascular health. Annu Rev Nutr, 2004; 24 p 597-615.

[14]   Makowski, L and GS Hotamisligil, The role of fatty acid binding proteins in metabolic syndrome and atherosclerosis. Curr Opin Lipidol, 2005; 16(5) p 543-8.

[15]   Chapkin, RS, et al., Dietary docosahexaenoic and eicosapentaenoic acid: emerging mediators of inflammation. Prostaglandins Leukot Essent Fatty Acids, 2009; 81(2-3) p 187-91.

[16]   Storlien, LH, AJ Hulbert, and PL Else, Polyunsaturated fatty acids, membrane function and metabolic diseases such as diabetes and obesity. Curr Opin Clin Nutr Metab Care, 1998; 1(6) p 559-63.

[17]   Mori, Y, et al., Effect of highly purified eicosapentaenoic acid ethyl ester on insulin resistance and hypertension in Dahl salt-sensitive rats. Metabolism, 1999; 48(9) p 1089-95.

[18]   Kuda, O, et al., n-3 fatty acids and rosiglitazone improve insulin sensitivity through additive stimulatory effects on muscle glycogen synthesis in mice fed a high-fat diet. Diabetologia, 2009; 52(5) p 941-51.

[19]   Lands, B, A critique of paradoxes in current advice on dietary lipids. Prog Lipid Res, 2008; 47(2) p 77-106.

[20] Griffin, MD, et al., Effects of altering the ratio of dietary n-6 to n-3 fatty acids on insulin sensitivity, lipoprotein size, and postprandial lipemia in men and postmenopausal women aged 45-70 y: the OPTILIP Study. Am J Clin Nutr, 2006; 84(6) p 1290-8.

[21] Giacco, R, et al., Insulin sensitivity is increased and fat oxidation after a high-fat meal is reduced in normal-weight healthy men with strong familial predisposition to overweight. Int J Obes Relat Metab Disord, 2004; 28(2) p 342-8.

[22] Giacco, R, et al., Fish oil, insulin sensitivity, insulin secretion and glucose tolerance in healthy people: is there any effect of fish oil supplementation in relation to the type of background diet and habitual dietary intake of n-6 and n-3 fatty acids? Nutr Metab Cardiovasc Dis, 2007; 17(8) p 572-80.

[23] Kagawa, Y, et al., Eicosapolyenoic acids of serum lipids of Japanese islanders with low incidence of cardiovascular diseases. J Nutr Sci Vitaminol (Tokyo), 1982; 28(4) p 441-53.

[24] Schraer, CD, et al., The Alaska Native diabetes program. Int J Circumpolar Health, 2001; 60(4) p 487-94.

[25] Thorsdottir, I, J Hill, and A Ramel, Omega-3 fatty acid supply from milk associates with lower type 2 diabetes in men and coronary heart disease in women. Prev Med, 2004; 39(3) p 630-4.

[26] Strandvik, B, The omega-6/omega-3 ratio is of importance! Prostaglandins Leukot Essent Fatty Acids, 2011; 85(6) p 405-6.

[27] Cerf, ME and J Louw, High fat programming induces glucose intolerance in weanling Wistar rats. Horm Metab Res, 2010; 42(5) p 307-10.

[28] Larue-Achagiotis, C and J Le Magnen, Fast-induced changes in plasma glucose, insulin and free fatty acid concentration compared in rats during the night and day. Physiol Behav, 1983; 30(1) p 93-6.

[29] Dauchy, RT, et al., Dark-phase light contamination disrupts circadian rhythms in plasma measures of endocrine physiology and metabolism in rats. Comp Med, 2010; 60(5) p 348-56.

[30] Kemppainen, J, et al., Myocardial and skeletal muscle glucose uptake during exercise in humans. J Physiol, 2002; 542(Pt 2) p 403-12.

[31] Harizi, H, JB Corcuff, and N Gualde, Arachidonic-acid-derived eicosanoids: roles in biology and immunopathology. Trends Mol Med, 2008; 14(10) p 461-9.

[32] Horrillo, R, et al., 5-lipoxygenase activating protein signals adipose tissue inflammation and lipid dysfunction in experimental obesity. J Immunol, 2010; 184(7) p 3978-87.

[33] Oliver, E, et al., The role of inflammation and macrophage accumulation in the development of obesity-induced type 2 diabetes mellitus and the possible therapeutic effects of long-chain n-3 PUFA. Proc Nutr Soc, 2010; 69(2) p 232-43.

[34] Blok, WL, et al., Dietary fish-oil supplementation in experimental gram-negative infection and in cerebral malaria in mice. J Infect Dis, 1992; 165(5) p 898-903.

[35] Tai, ES, et al., Insulin resistance is associated with a metabolic profile of altered protein metabolism in Chinese and Asian-Indian men. Diabetologia, 2010; 53(4) p 757-67.

[36] Keys, A, Mediterranean diet and public health: personal reflections. Am J Clin Nutr, 1995; 61(6 Suppl) p 1321S-1323S.

[37] Dolecek, TA and G Granditis, Dietary polyunsaturated fatty acids and mortality in the Multiple Risk Factor Intervention Trial (MRFIT). World Rev Nutr Diet, 1991; 66 p 205-16.

[38] Ravnskov, U, Cholesterol was healthy in the end. World Rev Nutr Diet, 2009; 100 p 90-109.

[39] Garemo, M, RA Lenner, and B Strandvik, Swedish pre-school children eat too much junk food and sucrose. Acta Paediatr, 2007; 96(2) p 266-72.

[40] Tierney, AC, et al., Effects of dietary fat modification on insulin sensitivity and on other risk factors of the metabolic syndrome--LIPGENE: a European randomized dietary intervention study. Int J Obes (Lond), 2011; 35(6) p 800-9.

[41] Jebb, SA, et al., Effect of changing the amount and type of fat and carbohydrate on insulin sensitivity and cardiovascular risk: the RISCK (Reading, Imperial, Surrey, Cambridge, and Kings) trial. Am J Clin Nutr, 2010; 92(4) p 748-58.

[42] Lands, B, Measuring blood fatty acids as a surrogate indicator for coronary heart disease risk in population studies. World Rev Nutr Diet, 2009; 100 p 22-34.

[43] Gibson, RA, et al., Docosahexaenoic acid synthesis from alpha-linolenic acid is inhibited by diets high in polyunsaturated fatty acids. Prostaglandins Leukot Essent Fatty Acids, 2012.

[44] Verschuren, WM, et al., Serum total cholesterol and long-term coronary heart disease mortality in different cultures. Twenty-five-year follow-up of the seven countries study. JAMA, 1995; 274(2) p 131-6.

[45] Robinson, JG and NJ Stone, Antiatherosclerotic and antithrombotic effects of omega-3 fatty acids. Am J Cardiol, 2006; 98(4A) p 39i-49i.

[46] Savva, SC, et al., Association of adipose tissue arachidonic acid content with BMI and overweight status in children from Cyprus and Crete. Br J Nutr, 2004; 91(4) p 643-9.

[47] Kalupahana, NS, et al., Eicosapentaenoic acid prevents and reverses insulin resistance in high-fat diet-induced obese mice via modulation of adipose tissue inflammation. J Nutr, 2010; 140(11) p 1915-22.

[48] Fedor, D and DS Kelley, Prevention of insulin resistance by n-3 polyunsaturated fatty acids. Curr Opin Clin Nutr Metab Care, 2009; 12(2) p 138-46.

[49] Kashiyama, T, et al., Relationship between coronary plaque vulnerability and serum n-3/n-6 polyunsaturated fatty acid ratio. Circ J, 2011; 75(10) p 2432-8.

[50] Yoshimoto, M, et al., In vivo SPECT imaging with 111In-DOTA-c(RGDfK) to detect early pancreatic cancer in a hamster pancreatic carcinogenesis model. J Nucl Med, 2012; 53(5) p 765-71.

[51] Harlan Laboratories. Teklad Research Diets. http://www.harlan.com/products_and_services/research_models_and_services/laboratory_animal_diets[accessed 12 July 2012]

[52] Khan, S, et al., Dietary long-chain n-3 PUFAs increase LPL gene expression in adipose tissue of subjects with an atherogenic lipoprotein phenotype. J Lipid Res, 2002; 43(6) p 979-85.

[53] Lindeberg, S, [Paleolithic diet and evolution medicine: the key to diseases of the western world]. Lakartidningen, 2005; 102(26-27) p 1976-8.

[54] Cordain, L, et al., Origins and evolution of the Western diet: health implications for the 21st century. Am J Clin Nutr, 2005; 81(2) p 341-54.

[55] Qureshi, AI, et al., Regular egg consumption does not increase the risk of stroke and cardiovascular diseases. Med Sci Monit, 2007; 13(1) p CR1-8.

[56] Eaton, SB, The ancestral human diet: what was it and should it be a paradigm for contemporary nutrition? Proc Nutr Soc, 2006; 65(1) p 1-6.

[57] Milton, K, Diet and primate evolution. Sci Am, 1993; 269(2) p 86-93.

[58] Eaton, SB, M Konner, and M Shostak, Stone agers in the fast lane: chronic degenerative diseases in evolutionary perspective. Am J Med, 1988; 84(4) p 739-49.

[59] Johnson, J. Health benefits of grass farming. www.americangrassfedbeef.com/grass-fed-natural-beef.asp[accessed 12 July 2012]

[60] Kirschenbauer, HG, Fats and oils : an outline of their chemistry and technology. 2nd ed. ed. 1960, New York: Reinhold ; London : Chapman and Hall.

[61] Blasbalg, TL, et al., Changes in consumption of omega-3 and omega-6 fatty acids in the United States during the 20th century. Am J Clin Nutr, 2011; 93(5) p 950-62.

[62] Korotkova, M, et al., Maternal dietary intake of essential fatty acids affects adipose tissue growth and leptin mRNA expression in suckling rat pups. Pediatr Res, 2002; 52(1) p 78-84.

[63] Korotkova, M, et al., Gender-related long-term effects in adult rats by perinatal dietary ratio of n-6/n-3 fatty acids. Am J Physiol Regul Integr Comp Physiol, 2005; 288(3) p R575-9.

[64] Palsdottir, V, et al., Postnatal deficiency of essential fatty acids in mice results in resistance to diet-induced obesity and low plasma insulin during adulthood. Prostaglandins Leukot Essent Fatty Acids, 2011; 84(3-4) p 85-92.

[65] Cunha, TM, et al., A cascade of cytokines mediates mechanical inflammatory hypernociception in mice. Proc Natl Acad Sci U S A, 2005; 102(5) p 1755-60.

[66] Daley, CA, et al., A review of fatty acid profiles and antioxidant content in grass-fed and grain-fed beef. Nutr J, 2010; 9 p 10.

[67] Dietary Reference Intakes: The Essential Guide to Nutrient Requirements, ed. JJ Otten, JP Hellwig, and LD Meyers. 2006: The National Academies Press.

[68] van der Schalie, WH, et al., Animals as sentinels of human health hazards of environmental chemicals. Environ Health Perspect, 1999; 107(4) p 309-15.

[69] Hannah, JS, et al., Changes in lipoprotein concentrations during the development of noninsulin-dependent diabetes mellitus in obese rhesus monkeys (Macaca mulatta). J Clin Endocrinol Metab, 1991; 72(5) p 1067-72.

[70] Russell, JC and SD Proctor, Small animal models of cardiovascular disease: tools for the study of the roles of metabolic syndrome, dyslipidemia, and atherosclerosis. Cardiovasc Pathol, 2006; 15(6) p 318-30.

[71] Orr, MW and WH McNeill, Plagues and peoples, William H. McNeill. Touchstone (Nashv), 1988;(12) p 3-5.

[72] Panchal, SK and L Brown, Rodent models for metabolic syndrome research. J Biomed Biotechnol, 2011; 2011 p 351982.

[73] Buettner, R, et al., Defining high-fat-diet rat models: metabolic and molecular effects of different fat types. J Mol Endocrinol, 2006; 36(3) p 485-501.

[74] Lomba, A, et al., Obesity induced by a pair-fed high fat sucrose diet: methylation and expression pattern of genes related to energy homeostasis. Lipids Health Dis, 2010; 9 p 60.

[75] Chun, MR, et al., Differential effects of high-carbohydrate and high-fat diet composition on muscle insulin resistance in rats. J Korean Med Sci, 2010; 25(7) p 1053-9.

[76] Sweazea, KL, M Lekic, and BR Walker, Comparison of mechanisms involved in impaired vascular reactivity between high sucrose and high fat diets in rats. Nutr Metab (Lond), 2010; 7 p 48.

[77] Krygsman, A, et al., Development of glucose intolerance in Wistar rats fed low and moderate fat diets differing in fatty acid profile. Exp Clin Endocrinol Diabetes, 2010; 118(7) p 434-41.

[78] Ouwens, DM, et al., The role of epicardial and perivascular adipose tissue in the pathophysiology of cardiovascular disease. J Cell Mol Med, 2010; 14(9) p 2223-34.

[79] Rennison, JH, et al., Enhanced acyl-CoA dehydrogenase activity is associated with improved mitochondrial and contractile function in heart failure. Cardiovasc Res, 2008; 79(2) p 331-40.

[80] Rennison, JH, et al., Prolonged exposure to high dietary lipids is not associated with lipotoxicity in heart failure. J Mol Cell Cardiol, 2009; 46(6) p 883-90.

[81] Nishina, PM, et al., Atherosclerosis and plasma and liver lipids in nine inbred strains of mice. Lipids, 1993; 28(7) p 599-605.

[82] Guyenet, SJ. The Diet-Heart Hypothesis: Oxidized LDL, Part I http://wholehealthsource.blogspot.com/2009/07/diet-heart-hypothesis-oxidized-ldl-part.html[accessed 12 July 2012]

[83] Reiter, RJ, Potential biological consequences of excessive light exposure: melatonin suppression, DNA damage, cancer and neurodegenerative diseases. Neuro Endocrinol Lett, 2002; 23 Suppl 2 p 9-13.

[84] Vorona, RD, et al., Overweight and obese patients in a primary care population report less sleep than patients with a normal body mass index. Arch Intern Med, 2005; 165(1) p 25-30.

[85] Damiola, F, et al., Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. Genes Dev, 2000; 14(23) p 2950-61.

[86] Zvonic, S, et al., Characterization of peripheral circadian clocks in adipose tissues. Diabetes, 2006; 55(4) p 962-70.

[87] Loboda, A, et al., Diurnal variation of the human adipose transcriptome and the link to metabolic disease. BMC Med Genomics, 2009; 2 p 7.

[88] Bolli, GB and JE Gerich, The "dawn phenomenon"--a common occurrence in both non-insulin-dependent and insulin-dependent diabetes mellitus. N Engl J Med, 1984; 310(12) p 746-50.

[89] Shih, KC, et al., Effect of reversing dark-light cycles on normal diurnal variation and related metabolic disturbance in rats. Chin J Physiol, 2007; 50(2) p 69-76.

[90] Stavinoha, MA, et al., Diurnal variations in the responsiveness of cardiac and skeletal muscle to fatty acids. Am J Physiol Endocrinol Metab, 2004; 287(5) p E878-87.

[91] Miles, JM, et al., Nocturnal and postprandial free fatty acid kinetics in normal and type 2 diabetic subjects: effects of insulin sensitization therapy. Diabetes, 2003; 52(3) p 675-81.

[92] Hoffmann, P, et al., Circadian rhythm of eicosanoid formation as affected by dietary linoleate. Prostaglandins Leukot Med, 1986; 25(2-3) p 91-103.

[93] Durgan, DJ, et al., O-GlcNAcylation, novel post-translational modification linking myocardial metabolism and cardiomyocyte circadian clock. J Biol Chem, 2011; 286(52) p 44606-19.

[94] Cornelissen, G, Galli, C., Halberg, F., De Meester, F., Rise, P., Circadian time structure of fatty acids and vascular monitoring. J Appl Biomed, 2010; 8 p 93-109.

[95] Randle, PJ, et al., The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet, 1963; 1(7285) p 785-9.

[96] Randle, PJ, et al., The glucose fatty acid cycle in obesity and maturity onset diabetes mellitus. Ann N Y Acad Sci, 1965; 131(1) p 324-33.

[97] Cooksey, RC, et al., Mechanism of hexosamine-induced insulin resistance in transgenic mice overexpressing glutamine:fructose-6-phosphate amidotransferase: decreased glucose transporter GLUT4 translocation and reversal by treatment with thiazolidinedione. Endocrinology, 1999; 140(3) p 1151-7.

[98] Hazel, M, et al., Activation of the hexosamine signaling pathway in adipose tissue results in decreased serum adiponectin and skeletal muscle insulin resistance. Endocrinology, 2004; 145(5) p 2118-28.

[99] Buse, MG, Hexosamines, insulin resistance, and the complications of diabetes: current status. Am J Physiol Endocrinol Metab, 2006; 290(1) p E1-E8.

[100] Choi, CS, FN Lee, and JH Youn, Free fatty acids induce peripheral insulin resistance without increasing muscle hexosamine pathway product levels in rats. Diabetes, 2001; 50(2) p 418-24.

[101] Innis, SM, Effect of total parenteral nutrition with linoleic acid-rich emulsions on tissue omega 6 and omega 3 fatty acids in the rat. Lipids, 1986; 21(2) p 132-8.

[102] Boden, G, Obesity and free fatty acids. Endocrinol Metab Clin North Am, 2008; 37(3) p 635-46, viii-ix.

[103] Cooksey, RC and DA McClain, Increased hexosamine pathway flux and high fat feeding are not additive in inducing insulin resistance: evidence for a shared pathway. Amino Acids, 2011; 40(3) p 841-6.

[104] Rajamani, U, et al., The hexosamine biosynthetic pathway can mediate myocardial apoptosis in a rat model of diet-induced insulin resistance. Acta Physiol (Oxf), 2011; 202(2) p 151-7.

[105] Li, MD, et al., O-GlcNAc transferase is involved in glucocorticoid receptor-mediated transrepression. J Biol Chem, 2012; 287(16) p 12904-12.