EFFECTS OF DIETARY CALCIUM AND FIBER ON DIGESTIBILITY OF FAT AND ENERGY AND ON HEALTH INDICES OF CARDIOVASCULAR DISEASE

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DEDICATION

For my children

So they may grow to value education

and yearn to seek knowledge

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RESEARCH PROBLEM

Introduction

Dietary fat has been implicated in the etiology of chronic diseases, such as obesity (1-3), diabetes mellitus (4,5), cardiovascular disease (6-8) and several forms of cancer (9-11). Excess dietary fat is often associated with accumulation of excess weight, leading to undesirable fat deposition in the body and predisposing a risk for chronic disease development (12-14). Weight gain is the consequence of an imbalance between energy intake and energy expenditures. Fat is often blamed to tip this equation in favor of excess caloric intake partly because it is presumed to contribute a metabolizable energy value of more than twice that of either carbohydrate or protein (15). However, fat has to be absorbed before it can deposit its caloric value and exert its undesirable effects in the body. Fat absorption is influenced by several factors including the saturation level of fatty acids (16), dietary fiber (17, 18), and calcium (19, 20). Long chain saturated fatty acids usually remain in the intestines for a longer period of time than unsaturated fatty acids before they are absorbed (16). This provides an opportunity for the long chain fatty acids to interact with other molecules present in the intestinal milieu. Dietary fiber in the intestines acts as a resin by binding to fatty acids, thereby preventing their uptake by intestinal cells. Calcium, in the alkaline environment of the intestine, binds to fatty acids to form calcium soaps. These calcium-long chain fatty acid soaps are insoluble; thus the fatty acids are unavailable for absorption causing them to be removed from the body via feces. Alternatively, high amounts of calcium may result in calcium phosphate formation in the gut. Calcium phosphate binds bile acids, and through increasing excretion of bile acids in feces, may decrease emulsification of lipid in and absorption from the intestines (21). Therefore, even when fat is consumed, if it is fed with sufficient amounts of either fiber or calcium, its impact on energy retention, fat deposition, and ultimately disease etiology should be decreased. Yet, most diet and feed formulations assume that fat has an energy value on either a digestible, metabolizable, or net energy basis that is 2.25 times that of protein or carbohydrate or the metabolic energy equivalent of 9 kcal per gram of fat. This value has been used ever since its determination by Atwater a century ago (15). Atwater determined that fat was 95% digested based on the amount of fat that could be extracted from feces by anhydrous ether. Ether extraction, however, incompletely extracts total fecal fat because it fails to extract the fat trapped in ether insoluble compounds, e.g., calcium soaps; thus it results in an overestimate of fat digestibility. Therefore, in diets, the presence of fat with factors that may alter its digestibility or form, e.g., soap formation, will give fat a caloric value different from that proposed by Atwater. If the metabolizable energy value of fat is dependent on the composition of the diet or feed, diets or feeds presumed to be isocaloric may indeed not be isocaloric.

In contrast with the overestimate of metabolizable energy (ME) discussed above, net energy (NE) value for absorbed fat may be more than 2.25 times that for carbohydrate or protein. This is because dietary fatty acids can be stored directly by animals. In contrast, up to 40% of the ME of carbohydrate and protein is lost during the conversion of carbohydrate or protein into fatty acids for storage. Because this energy loss, i.e., heat increment, is lower for fat (approximately 2%) than for carbohydrate or protein, the net energy of absorbed fat will be more than 2.25 times the NE value of carbohydrate and protein. Retention of energy by animals will parallel NE more than ME; consequently, relative ME values for fat, carbohydrate and protein will underestimate the relative caloric retention by animals fed diets high in fat.

Extensive research has established the role of fiber in chronic disease prevention. Fiber binds to lipid components in the gut, particularly cholesterol, preventing their uptake and reducing their circulating levels; this is important particularly in reducing serum cholesterol levels and the risk for cardiovascular disease; fiber also dilutes carcinogens in the intestines, reducing their potentially harmful effects (22). Fiber is, therefore, currently considered an important constituent in diets for chronic disease prevention and reduction (14, 23). In contrast, less research has focused on effects of calcium preventing or reducing chronic diseases. Studies have demonstrated beneficial effects of calcium rich diets on reducing the risk of colorectal cancer, serum total and LDL cholesterol levels, and hypertension. But, few studies have examined the effects of calcium on body weight, body composition and fat deposition in humans.

We have previously demonstrated that fat digestibility, and consequently its metabolizable energy value, varies with type of fat and with calcium level in male Sprague-Dawley rats (24). Parallel studies in veal calves demonstrated that increasing dietary calcium from 0.52% to 1.24% of the diet decreased diet digestibility from 95 to 90% and increased fecal excretion of bile salts by 90% (25). In this study, we examined if similar effects could be obtained in other mammals. We chose two animal models for the digestibility study. The first animal model was a second rodent species, CD1 mice; this time we used all female animals specifically because previous work employed male animals and gender might alter the metabolic response to diet. The second animal model was Yorkshire pigs (males and females), chosen because of the close similarity in gastrointestinal anatomy and physiology between pigs and humans (26). In addition to studying the effect of type of fat (corn oil or beef tallow), calcium level, or type of fiber on fat, diet, and energy digestibility in animals, we also examined the dietary intake of young middle aged men and women to examine the relationship between dietary fat, calcium or fiber and body weight (assessed as body mass index), % body fat, weight distribution (determined by waist to hip ratio), blood pressure, serum triglycerides, serum total cholesterol, serum HDL cholesterol, serum LDL cholesterol, serum apolipoprotein A1, and serum apolipoprotein B levels. The animal studies were approved by the Laboratory Animal Use Committee and the human study was approved by the Institutional Review Board at Oklahoma State University. The approved Institutional Review Board form for the human study is presented as an Appendix to this manuscript.

Research Objectives

Our research objectives were:

1. To compare the caloric contribution of two sources of fat, animal (beef tallow) and plant (corn oil), with that of carbohydrate (sucrose) in mouse diets.

2. To compare the effects of dietary fiber supplementation from rice bran, cellulose, wheat bran, or beet pulp on the energy availability of beef tallow in mouse diets.

3. To measure the effect of calcium supplementation on fat digestibility and on energy retention by mice fed diets supplemented with either beef tallow or corn oil.

4. To examine whether the effects of the two fat sources and of calcium supplementation on fat and energy digestibility are similar in another animal model, the pig, that has a digestive tract similar to that of humans.

5. To compare the effects of beef tallow, corn oil, or sucrose supplementation on the caloric value and digestibility of diets fed to young pigs.

6. To study the effects of two levels of calcium on fat and energy digestibility by young pigs fed diets supplemented with added beef tallow or corn oil.

7. To compare the effects of supplementing diets with either carbohydrate (sucrose), fat (beef tallow or corn oil), or fat and calcium on plasma cholesterol levels of young pigs.

8. To examine the dietary intake of fat, fiber, and calcium in a sample population of young middle aged adults.

9. To measure body mass index, % body fat, waist to hip ratio, systolic and diastolic blood pressure, total serum cholesterol, HDL cholesterol, triglycerides, apolipoprotein A1 and apolipoprotein B levels in that sample population of men and women for associations with the subjects' dietary intake of fat, fiber, and calcium.

Hypotheses

The following were the null hypotheses developed for this study:

H1. Supplemental isocaloric amounts from the different energy sources sucrose, corn oil, or beef tallow produce equal effects on fat and energy digestibility in mice or pigs and similar cholesterol values in pigs.

H2. Supplementing high beef tallow or high corn oil diets with calcium does not affect the digestibility of either the fat or the diet in mice or pigs.

H3. Addition of fiber to a high beef tallow diet does not affect the digestibility of the tallow or the diet of mice.

H4. The nutrient adequacy of the diets of human subjects is the same regardless of gender or the level of fat, fiber, or calcium in the diet.

H5. Fat, fiber, or calcium intake by human subjects does not affect anthropometric measures or indices of cardiovascular disease risk factors.

Assumptions

The following conditions were assumed in the methodology of the study:

1. Animals sacrificed at the beginning of each experiment had body compositions representative of the initial body composition of the remaining experimental animals.

2. The two pigs per treatment that were sacrificed at the end of the experimental period had body compositions representative of the body composition of other pigs in the same treatment group.

3. All the nutrient needs of mice and pigs were met by the diets provided.

4. Mouse and pig fecal excretion values obtained during the collection period per unit of feed consumed were representative of values for the total feeding and energy retention study.

5. Carcass, feed, feed refusal, and fecal samples obtained for analysis were representative of the carcass, feed, feed refusals, and feces, respectively.

6. Dietary data obtained from the human subjects were representative of the dietary intake of the subjects.

7. Anthropometric and clinical measurements performed on the human subjects were accurate and precise.

8. All chemical analyses in each trial were accurate and precise.

Definitions

Gross energy: The heat of combustion (measured in kcal) liberated when a substance is completely oxidized in a bomb calorimeter.

Fecal energy: The gross energy of feces consisting of undigested food and other metabolic products.

Digestible energy: The gross energy of total food ingested minus the gross energy of total feces excreted.

True metabolizable energy: The gross energy of ingested food minus fecal energy of food origin, minus energy in gaseous products of digestion, minus energy in heat of fermentation (heat produced in the digestive tract as a result of microbial fermentation),

minus urinary energy of food origin.

Fat digestibility: Fat content of ingested food minus the fat content in excreted feces divided by the fat content of the ingested food and multiplied by one hundred.

Energy retention: The percentage of the gross energy of the food ingested which led to an increase in the body energy content, determined by measuring the caloric content of the carcass. It is equal to gain in body energy divided by energy intake and multiplied by one hundred.

Body mass index (BMI): An indicator of a person's weight in relation to height. The index is determined by dividing weight (kg) by the square of the height (m).

Waist to hip ratio (WHR): An indicator of a person's fat distribution. The ratio is determined by dividing the waist circumference by the hip circumference.

LDL cholesterol: Low density lipoprotein contains 25% protein. LDL cholesterol comprises 50-70% of total plasma cholesterol. It functions in delivering cholesterol to peripheral tissues in the body.

HDL cholesterol: High density lipoprotein contains about 50% protein. HDL cholesterol comprises 20-30% of the total plasma cholesterol. It functions in the uptake and removal of cholesterol from peripheral tissues in the body.

Apolipoprotein A1: The major protein in HDL, representing about 60% of the protein component of that particle.

Apolipoprotein B: The major protein in LDL, representing about 90% of the protein component of that particle.

Format of Dissertation

Each of the experiments was organized as an individual manuscript for publication. Chapters III, IV, V, and VI were written in journal article format using the guidelines for Nutrition Research. The other chapters follow traditional format.

CHAPTER II

REVIEW OF THE LITERATURE

Excessive consumption of dietary fat has been implicated in the etiology of obesity and cardiovascular disease. In addition, obesity is an independent risk factor in the development of cardiovascular disease (27-29) and hypertension (30). A review of the research investigating the role of fat in obesity and cardiovascular disease as well as the impact of the level of dietary fat and its properties, dietary fiber, and calcium on fat absorption is summarized in this chapter.

Dietary Fat and Weight Gain

The evidence linking high fat intake and obesity is supported by both epidemiological and experimental studies. Fat is implicated as causing an imbalance between energy intake and energy expenditure (31).

Epidemiological Studies

Obesity is more prevalent in countries, such as in the United States and the United Kingdom, that have diets with a high fat to energy ratio, and less prevalent in countries, such as China and Japan, that have diets containing low fat to energy ratio (32). Furthermore, immigrants from countries with low fat diets increase their body size when exposed to westernized high fat diets (32). Cross sectional studies within countries have also shown that obesity and overweight are more prevalent in people with high fat intake. In a study examining the incidence of obesity and overweight in Scottish men and women, Bolton-Smith and Woodward (33) found that excessive body weights were 150% more prevalent in individuals with the highest intake of fat to sugar ratio than in individuals with the lowest fat to sugar ratio. A positive association between fat intake and body mass index was also observed in women participants of the Nurses' Health Study (34). Romieu *et al.* (2) found that in adult women, after adjusting for age and total caloric intake, body mass index was associated with intake of total fat and saturated fatty acids. Dreon *et al.* (35) detected a positive correlation between % body fat and intakes of total fat, saturated fatty acids, and monounsaturated fatty acids in middle-aged men. Within- population studies demonstrated that exposure to high fat diets and a sedentary lifestyle increased the prevalence of obesity in a population, like the Pima Indians of Arizona, who previously consumed a traditional low fat diet and a high physical activity lifestyle (36). Klesges *et al.* (37) found that in a group of men and women, percent energy from fat was positively correlated with body mass index and that weight gain over a two-year period was positively associated with increases in dietary fat consumption. Slattery *et al.* (38) observed that waist to hip ratio was positively correlated with % of calories from fat in the diet of white men and women and black men participants of the Coronary Artery Risk Development in Young Adults study. A small cross-sectional study by Astrup *et al.* (39) provided further evidence relating dietary fat to obesity, in which, a 10 kg change in body fat was associated with an increase in dietary fat intake of 1.6% or more.

Experimental Studies

Controlled experimental research in humans has provided further evidence linking fat intake to weight gain. In the Women's Health Trial Feasibility Study (40), women with *ad libitum* access to a diet with 20% of energy from fat lost weight while women with similar access to a diet with unrestricted fat level did not. Horton *et al.* (41) fed an extra 50% of energy requirements from either fat or carbohydrates to male subjects and observed that fat overfeeding led to a 90-95% storage of the excess calories while carbohydrate overfeeding led to a lower, 75-80%, storage of the excess energy. Sims *et al.* (42) supplemented the diet of normal weight men with fat added to frozen meals for eight months and observed that men receiving the high fat diet gained a higher proportion of body weight relative to ingested energy. Marckmann (43) observed a 0.5 kg weight gain in individuals who supplemented their daily intake with 15 g of fish oil for 6 weeks and a 2.5 kg weight gain in individuals supplementing their diet with 6 g of fish oil for 10 weeks. Miller *et al* (44) found a significant positive association between percent of calories from fat in the diet and percent body fat of subjects; obese subjects consumed a diet with 35% of calories from fat, while lean subjects consumed diets with only 29% of calories from fat. Therefore, dietary fat is related to weight gain and is thought to do so, mainly, because it increases energy intake through its high energy density, the high palatability of high fat diets, or the minimal effect it has on appetite suppression (31). These studies relied on feeding equivalent metabolizable energy amounts of fat and carbohydrate in order to compare the net energy contributions of these two energy sources. Such comparisons may be erroneous because more energy is lost converting injested carbohydrate to body fat than is lost converting injested fat to body fat.

The Caloric Value of Fat.

Estimates of the metabolizable energy of fat currently in use are based on values derived by Atwater and Bryant (15) at the turn of the century. These values attribute a caloric value to fat 2.25 times that of carbohydrates. Therefore, a certain amount of fat will provide 225% as much energy as an equal weight of carbohydrates. However, studies in animals using different sources of fat have demonstrated that fat may provide an energy value higher than 9 kcal/g. Jensen *et al.* (45) found that a fat with a gross energy value of 7.71 kcal/g produced a 10.16 kcal/g value for net energy in turkeys. Later, Donato and Hegsted (46) obtained a net energy value of 11.1 kcal/g, 124% the expected 9 kcal/g, in rats when the metabolizable energy value of carbohydrates was set at 4 kcal/g. This extra-caloric content of fat may be due to the following: 1) the ability of fat to slow the rate of passage of nutrients in the digestive tract and thus, enhance the total digestibility and caloric value of the other nutrients present in the diet; 2) endogenous excretions of energy may be reduced by added lipid, and 3) fat is more efficiently stored in adipose tissue, at a metabolic cost of 2%, than carbohydrate, with an estimated metabolic loss of 24% (47-49). However, studies have indicated that this extra-caloric value of fat is only observed when the level of fat in diets is low. Sibbald and Kramer (50) reported that as the level of beef tallow in the diet of adult roosters increased from 5, 10, to 15%, the metabolizable energy value of beef tallow decreased from 9.04, 8.28, to 7.82 kcal, respectively. They concluded that an interaction between fat and other non-fat components in the diet peaked at the low level of fat inclusion beyond which, added fat diluted the interaction making it less apparent. Mateos and Sell (51) reported that the apparent metabolizable energy of yellow grease was highest, at 9.37 kcal/g, when fed at 3% of the diet and lowest, at 8.65 kcal/g, when fed at a 15% level in the diet of hens. Eusebio *et al.* (52) studied the performance of 10-week old pigs fed a diet with up to 38% fat from either tallow, lard, soybean oil, or coconut oil. They observed no significant differences in feed conversion among the treatments but regardless of the fat source, as the level of fat in the diet increased, rate of gain decreased. Frobish *et al.* (53) fed 3-week old pigs diets containing either 0, 5, or 10% added lard. Weight gain of the pigs and feed efficiency increased when lard was increased from 0 to 5%, but decreased when fat was increased from 5 to 10% of the diet. Therefore, even though fat has a bomb calorimeter value 2.25 times that of carbohydrates, in vivo, fat could have an net energy value higher or lower than expected depending on the conditions under which it is presented to the body. Fat could contribute a higher than expected caloric value if it is present at a level appropriate for enhancing the digestibility and caloric contribution of other constituents in the diet. Fat could, also, impart a lower than expected caloric value depending on the level at which it is fed and on other factors such as properties of the fatty acids and the type of dietary fiber and level of dietary calcium, as is discussed later in this chapter.

Fat and Energy Intake.

Studies have confirmed that high fat diets are palatable to humans and are therefore consumed at a greater quantity than less palatable low fat diets. Lissner *et al.* (54) fed females, of varying body weight, a low fat diet providing 15-20% of energy from fat, a moderate fat diet, with 30-35% of energy from fat, and a high fat diet, with 45-50% of energy from fat, for two weeks each. Based on determined energy requirements, subjects on the low fat diet had an 11.3% energy deficit, but those on the high fat diet consumed a 15.4% energy surfeit ; therefore, the energy intake of subjects increased as the % of fat in the diet increased. Thomas *et al.*(55) reported that participants ate more when fed a high fat diet than when fed a high carbohydrate low fat diet. Even though the sensory qualities of fat may promote over-consumption of high fat foods, an increased preference for fat and high fat food may be apparent in overweight individuals (12). Drewnowski *et al.* (56) observed that obese and previously obese subjects preferred higher levels of fat in mixtures of dairy products and sugar than their lean counterparts. Mela and Sacchetti (57) saw a positive relationship between the preference for fat in food and % body fat in normal-weight subjects. However, although fat enhances the palatability of food, other factors may also influence the amount and type of food individuals consume (58).

Fat Intake and Satiety.

Fat consumption stimulates the release of cholecystokinin, a hormone that mediates satiation and possibly early-phase satiety (59). High fat ingestion also increases enterostatin, a peptide that decreases food intake in rats (60). Satiation is different from satiety in that the former describes the process in operation while foods are being eaten and controls meal size, while the latter describes the state engendered as a consequence of consumption and measures the capacity of food to control subsequent hunger and eating (61). Golay and Bobbioni (62) proposed that fat had a more marked effect on satiation than on postingestive satiety. Green *et al.* (63) found that subjects consumed more energy when exposed to high fat foods than when exposed to lower fat foods, leading them to conclude that fat may have a weak effect on satiety. Thus, although fat produces physiological signals to reduce food intake, the emulsified fat in the intestine, required to stimulate the release of satiety signals, may be diluted by the presence of other nutrients. Additionally, satiety signals require some time before they are operational, lagging in time behind the potent oral stimulation of high fat foods, which leads to a quick ingestion of high energy-high fat foods and therefore over-consumption before the satiety signals have an opportunity to elicit their effects (61). Satiety signals produced from high fat foods may also be weak because high fat foods usually are smaller in volume for a given energy content; a small volume produces a relatively small stomach distention per unit of energy ingested, thus not eliciting a feeling of fullness (13).

Obesity and Cardiovascular Disease

Obesity, or excess body fat, is an important risk factor in the development of cardiovascular disease (64). The anatomical distribution of body fat is a determinant of disease development (65). Vague (66), in 1956, observed that patients with greater upper (android) vs. lower (gynoid) body fat were more prone to certain disorders, that men were more android than women, and that diabetic patients were more android than non-diabetics. Terry *et al.* (67), in a twenty- three year longitudinal study, examined measurements of waist to hip ratio and body mass index in relation to the incidence of ischemic heart disease and stroke mortality in 84,910 white male US Army veterans discharged between the years 1946 and 1947. They divided their subjects into four groups based on age; 16-20, 21-25, 26-30, and 31-35 years of age. They found that both waist to hip ratio and body mass index contributed to risk of premature ischaemic heart disease across all age groups, and that waist to hip ratio was a significant predictor of cerebrovascular disease mortality in men under 26 years of age. In an epidemiological study of associations between waist to height ratio, body mass index, and waist to hip ratio and risk factors for coronary heart disease, Hsieh and Yoshinaga (68) found that for 3,131 men between the ages of 22 and 82 years, all three indicators of obesity were positively correlated with systolic blood pressure, diastolic blood pressure, fasting blood triglyceride, and fasting blood cholesterol; but, they were inversely correlated with HDL cholesterol levels. Tian *et al.* (69) examined the relationship between serum lipids and dietary and non-dietary factors in 314 Chinese men and 317 Chinese women. They observed that when the percent caloric contributions of fat and saturated fat to the diet were low (29% and 6.9%, respectively), dietary intake had no association with serum lipids. Serum lipid levels in that population were strongly related to body mass index; body mass index was positively related to serum total cholesterol, triglycerides, and LDL cholesterol and inversely related to HDL cholesterol. Chumlea *et al.* (70) examined the relationship between body fat distribution and blood lipids in 41 white men and 63 white women, 67-92 years of age. They observed that a large abdominal circumference produced negative health alterations in blood lipid levels. Overall fatness and a central fat pattern was associated with lower HDL cholesterol and higher blood triglycerides. Galanis *et al.* (71) observed similar relationships between obesity and risk factors for coronary heart disease in 178 Samoan men and 147 Samoan women. They found that in men, body mass index and abdomen to hip circumference ratio were positively associated with fasting serum levels of total cholesterol, total cholesterol to HDL cholesterol ratio, apolipoprotein B, and the log of triglycerides and insulin concentrations, but negatively associated with HDL cholesterol. Results in women showed that body mass index was negatively correlated with HDL cholesterol and positively correlated with the log of triglyceride and insulin concentrations, only. Raitakari *et al.* (72) reported that in Finnish adolescents and young adults, body mass index was positively related to serum LDL cholesterol, triglycerides, systolic blood pressure, and diastolic blood pressure but negatively related to serum HDL cholesterol. Ward *et al.* (73) found that abdomen to hip ratio, independent of body mass index, was inversely related to serum HDL cholesterol and positively related to serum triglycerides in 878, 43-85 years of age, male participants of the Normative Aging Study. DiPietro *et al.* (74) observed that overall adiposity and chest skinfold measurements were higher in 65-74 year old participants of the Chicago Heart Study who had higher levels of systolic blood pressure, diastolic blood pressure, serum cholesterol, and serum triglycerides; however, these associations were secondary to other more powerful risk factors, such as race, gender, old age, and the existence of a disease state of hypertension or diabetes. Ernst *et al.* (75) examined data from the three National Health and Nutrition Examination Surveys (NHANES) I, II, and III of adults 20 years and older to study trends in energy and fat consumption, prevalence of overweight in the population, the association between overweight and blood pressure or blood cholesterol, and the prevalence of high blood pressure and high blood cholesterol among the overweight and the underweight. Their results suggested that, for the years covered by the surveys, total energy intake increased, total and saturated fat intake decreased, but still remained above recommendations, and overweight increased. High diastolic and systolic blood pressures increased with increased body mass index in both black and white men and women. Overweight white men and women had the highest prevalence of hypertension compared to normal weight counterparts; the difference in the prevalence of high blood pressure between the two different weight groups was higher in white than in black subjects. Serum cholesterol levels were similarly elevated by higher body mass index values for all subjects. However, the difference in the prevalence of high blood cholesterol among overweight versus normal weight subjects was highest in black men and lowest in black women. Loos and Halais (76) observed that waist to hip ratio, an indicator of abdominal obesity, was more highly correlated with cardiovascular disease factors than body mass index, an indicator of overall obesity, in 255 women and 184 men between the ages of 18 and 71 years. They observed that men under 50 years of age had exhibited the most marked increase in waist to hip ratio with age, while women had the most marked elevations of this ratio after age 50. In these two groups, where abdominal obesity predominates, waist to hip ratio and body mass index were interrelated; however, in women under fifty, with a characteristic gluteo-femoral obesity, body mass index was not associated with cardiovascular disease risk. For those women, elevations of waist to hip ratio were highly associated with elevations of blood cholesterol. Body mass index did not distinguish between abdominal and gluteo-femoral obesity, whereas, waist to hip ratio did. The investigators recommended that both indices could be used together in assessing cardiovascular disease risk. Matsuzawa *et al.* (77) proposed the use of visceral fat obesity, denoting the predominance of fat accumulation in the intra-abdominal cavity as measured by computed tomography, as an additional indicator of atherosclerosis. They found that visceral fat obesity was present in 90% of obese patients with ischaemic heart disease but only 40% of non-obese patients with coronary artery disease. They also reported that among non-obese individuals, those with coronary artery disease had twice as much visceral fat as did their healthy counterparts; differences in body mass index or subcutaneous fat measurements were not detected between the two groups. Mauriege *et al.* (78) found that subcutaneous abdominal fat accumulation and larger abdominal adipocytes were associated with higher risk of cardiovascular disease in 54 healthy young males. Abdominal fat cell weight also was positively correlated with plasma triglycerides, cholesterol, VLDL cholesterol, LDL cholesterol, apolipoprotein B and inversely correlated with the HDL cholesterol to LDL cholesterol ratio. These studies demonstrate the strong relationship between excess body fat and its abdominal distribution and an increased risk of cardiovascular disease.

Dietary Fat and Cardiovascular Disease

In addition to potentially indirectly influencing the risk of cardiovascular disease by way of increased body fat deposition, dietary fat has been directly implicated in the risk of atherosclerosis by altering blood lipid levels. The epidemiological evidence linking dietary lipids to cardiovascular disease is overwhelming. Kritchevsky *et al.* (79), in 1954, demonstrated that saturated fatty acids were more atherogenic for rabbits than unsaturated fatty acids. Later, Keys *et al.* (80) examined metabolic studies in healthy humans and were able to derive a formula quantitating the relationship between serum cholesterol and the level of dietary cholesterol, saturated and polyunsaturated fat. There results were further confirmed by Hegsted *et al.* (81), who also formulated an equation predicting changes in serum cholesterol based on changes in the cholesterol intake and the percent of calories from saturated and unsaturated fat in the diet. Both Keys and Hegsted and their coworkers (81, 82) found that intakes of saturated fatty acids (with the exception of short chain fatty acids and stearic acid) were correlated with total serum cholesterol levels (with myristic acid being twice as potent as palmitic acid in that effect). They observed that polyunsaturated fatty acids decreased blood cholesterol. They determined that monounsaturated fatty acids as well as stearic acid had neutral effects on circulating cholesterol levels, and that dietary cholesterol between 100-600 mg/day linearly increased serum cholesterol, however, this effect was influenced by the level of saturated and polyunsaturated fatty acids in the diet. Mensink and Katan (83) also derived equations across populations linking dietary fat to blood lipids. They found that alterations in total serum cholesterol were mostly the consequence of alterations in serum LDL cholesterol, which increased when the level of saturated fatty acids in the diet increased. Their meta-analysis of 27 controlled trials lead to findings that saturated fatty acids increased, not only LDL cholesterol, but also HDL cholesterol levels. Grundy *et al.* (84) observed that substituting monounsaturated fatty acids for saturated fatty acids in the diets of hypercholesterolimic subjects reduced plasma total and LDL cholesterol while maintaining HDL cholesterol levels. Hegsted *et al.* (6) evaluated experimental data (up to the year 1991) in humans and concluded that saturated fatty acids were the main determinants of increased serum cholesterol, polyunsaturated fatty acids lowered serum cholesterol; monounsaturated fatty acids seemed to have no independent effect on serum total or LDL cholesterol but rather their consumption may alter the dietary content of saturated and polyunsaturated fatty acids resulting in favorable blood lipid levels. Cox *et al.* (85) fed two isocaloric diets with 38% of calories from fat, in a double crossover design, to 67 free living adults. The diet was either high in saturated fatty acids (providing

26% of calories) and low in polyunsaturated fatty acids (providing 2% of calories), or high in polyunsaturated fatty acids (providing 23% of calories) and low in saturated fatty acids (providing 9% of calories). They found that in 47 subjects, whose plasma lipid levels responded to dietary changes, increasing the saturated fatty acid intake correlated with elevations in plasma total cholesterol, triglycerides, and apolipoprotein B. Cobb *et al.* (86) observed changes in plasma lipoprotein levels in response to changes in dietary composition in 63 males and females. Both sexes experienced elevations in circulating levels of total and LDL cholesterol and decreases in HDL cholesterol in response to a high saturated and low polyunsaturated fatty acid containing diet. However, for normolipidemic females the drop in the potentially protective plasma HDL cholesterol was larger than that observed in normolipidemic males. In females, the change in HDL cholesterol was dependent on baseline HDL levels, changes in plasma triglycerides, and changes in the ratio of dietary saturated to polyunsaturated fatty acids. In males, diet was the only predictor of changes in plasma HDL levels. However, Denke (87) suggested that low total and saturated fat diets are beneficial to women since these diets, despite lowering HDL cholesterol, lower total and LDL cholesterol thus, lowering overall risk of coronary heart disease, similar to the benefits observed in men. Additionally, diets low in total and saturated fat are often abundant in fruits, vegetables, and grain products; these foods provide fiber and other beneficial nutrients that reduce the risk of coronary heart disease.

Population studies provided information correlating dietary fat to atherosclerosis and coronary heart disease incidence. Posner *et al.* (7) examined the relationship between dietary lipids and the 16-year incidence of coronary heart disease morbidity and mortality in middle aged (45-55 years old) male participants of the Framingham Study. They observed that the percent of energy from total fat and monounsaturated fatty acids was positively related to increased incidence of coronary heart disease and that the monounsaturated fatty acids in that population came from animal food sources also high in saturated fatty acids. Saturated fatty acids in that study seemed independently related to increased incidence of the disease even after adjusting for serum cholesterol levels. Artaud-Wild *et al.* (88) analyzed coronary heart disease mortality rates in 40 countries. They found that the incidence increased significantly with increases in dietary cholesterol and saturated fat intakes in all the studied countries. However, high dietary cholesterol and saturated fatty acid intakes were also associated with low intakes of fiber, vitamin E, and folic acid; suggesting that sub-optimal intakes of those nutrients as well as fat may be associated with the incidence of coronary heart disease. Raitakari *et al.* (72) examined associations between lifestyle and cardiovascular disease risk factors in Finns between the ages of 15 and 24 years. They observed that the preference for butter over soft vegetable margarine was associated with higher levels of serum LDL cholesterol in both males and females. Ascherio *et al.* (89) examined the association between fat intake and incidence of coronary heart disease in 43,757 health professional men 40 to 75 years of age in the United States. They observed significant positive associations between saturated fat intake as well as cholesterol intake and risk of coronary disease, however the association was weakened after adjustment for dietary fiber intake. They also reported an inverse association between intake of linolenic acid and risk of myocardial infarction. Kromhout *et al.* (90) observed an inverse relationship between fish consumption (rich in linolenic acid) and mortality from coronary heart disease. Bang and Dyerberg (91) observed a low rate of coronary heart disease in Greenland Eskimos, a population with a high intake of linolenic acid intake from fish. Shaefer *et al.* (92) summarized the possible reasons for the protective effects of n-3 polyunsaturated fatty acids. They suggested that the decrease in LDL levels observed when substituting n-3 fatty acids for saturated fatty acids was mostly attributable to the removal of the saturated fat and not the addition of the n-3 fatty acids; however, n-3 fatty acids may exert protective effects by decreasing serum triglycerides levels, platelet aggregation, and blood pressure as well as altering lipoprotein metabolism, increasing bleeding time, and decreasing the body's immune response. In contrast, Nordoy *et al.* (93) fed a very small group of healthy men diets either high or low in total and saturated fat content with or without added n-3 fatty acids. They found that regardless of the level of saturated fatty acids in the diet, n-3 fatty acids significantly lowered plasma total cholesterol, very low density lipoprotein (VLDL) cholesterol, HDL cholesterol, and total triglycerides. Moreover, the low saturated fatty acid diets produced lower total cholesterol, LDL cholesterol, and HDL cholesterol concentrations than the high saturated fatty acid diets. Optimal plasma lipid profiles in that study were produced by feeding a diet low in saturated fatty acids and high in n-3 fatty acids. Fraser (94) suggested that because serum concentrations of triglycerides are inversely related to concentrations of HDL cholesterol, then diets which reduce serum triglycerides may increase serum HDL cholesterol levels. Norum (95), in a review of findings linking dietary fat to blood lipids, stated that the concentration of plasma triglycerides was higher when the intake of saturated fatty acid was high than when the intake of n-3 or n-6 polyunsaturated fatty acids was high.

Long term clinical intervention studies demonstrated the benefits of reducing total fat, saturated fatty acid, and cholesterol intakes. Dayton *et al.* (96) divided 846 older men into a control group with a diet providing 40% of calories from mostly animal fat, and an experimental group with a diet supplying 40% of calories from fat but, two thirds of which was from vegetable oils. After eight years of follow-up, those in the experimental group had 13% lower serum cholesterol concentrations and 23% lower incidence of coronary deaths and myocardial infarction than those in the control group. It should be noted, however, that the experimental group consumed 365 mg of cholesterol/day, an amount almost half the 653 mg /day consumed by the control group; therefore, the favorable results obtained by substituting vegetable fat for animal fat may have been confounded by differences in dietary cholesterol. Hjermann *et al.* (97) observed that in 1,232 middle aged men, those who consumed diets with 28% of calories from fat, less than 300 mg of cholesterol per day, and equal amounts of saturated and polyunsaturated fatty acids had 13% lower serum total cholesterol, 20% higher HDL cholesterol levels, and 20% lower serum triglycerides than those who consumed diets with 44% of calories from fat, 500-600 mg daily dietary cholesterol, and 2.5 times more saturated fatty acids than polyunsaturated fatty acids. Puska *et al.* (98) observed reductions in serum cholesterol and in systolic and diastolic blood pressure, in men and decreases in blood pressure in women members of a Finnish community who received a community-based health intervention program for ten years. The incidence of mortality from coronary artery disease in that community decreased by 24% in men and 51% in women, significantly lower than other communities or counties in Finland during the same time period. Data from the Multiple Risk Factor Intervention Trial (99), involving 12,866 men between the ages of 35 to 57 years, revealed that men who received instruction on a low saturated fat and low cholesterol diet had lower serum cholesterol levels after 72 months of follow up than men who did not receive the intervention. Leren (100) reported than in the Oslo Heart Study, survivors of myocardial infarction who were treated with a diet low in cholesterol and saturated fatty acids had lower serum cholesterol levels and incidence of coronary artery disease after 5 years than counterparts not receiving the dietary treatment. Arntzenius *et al.* (101) examined the effects of treating 39 coronary atherosclerosis patients with a vegetarian diet with less than 100 mg of cholesterol per day and a high polyunsaturated to saturated fatty acid ratio of 2 to 1. Subjects had a 10% decrease in serum total cholesterol, an 8.5% reduction in the ratio of total cholesterol to HDL cholesterol, and lower systolic blood pressure after two years of being on the dietary treatment compared to levels prior to the intervention. Recently, findings by Drs. Gould and Ornish and their coworkers received news media exposure. Ornish *et al.* (102) fed patients with documented coronary atherosclerosis a strict vegetarian diet containing less than 10% of calories from fat and less than 50 mg of daily cholesterol; this resulted in a 24% reduction in serum total cholesterol, 37% lower LDL cholesterol, and 69% decrease in the ratio of LDL to HDL cholesterol after one year on the dietary intervention. Gould *et al.* (103) reevaluated those patients after five years and found improvements in size and severity of myocardial perfusion abnormalities in individuals who received the experimental treatment (102) and worsened outcomes in those who did not.

Because of the findings linking fat intake to cardiovascular disease risk, dietary goals have been established to support health in the general American population (23). These goals recommend a total fat intake of 30% or less of calories, with saturated fatty acids supplying no more than 10% of calories, relatively equal amounts of saturated, polyunsaturated and monounsaturated fatty acids, and a daily dietary cholesterol intake of 300 mg or less. Furthermore, the United States Department of Agriculture and the United States Department of Health and Human Services have developed the Dietary Guidelines for Americans (14) for the same purpose. Current medical nutrition therapy for the reduction of heart disease follows the recommendations of the Adult Treatment Panel II of the National Cholesterol Education Program (104). These recommendations include a two step dietary approach. Individuals with elevated LDL cholesterol and fewer than two additional risk factors follow the Step I diet, which is similar to the dietary recommendations made for the general public. Individuals who do not respond to the Step I diet or need further reductions in LDL cholesterol are placed on the Step II diet, which limits saturated fatty acid intake to no more than 7% and cholesterol intake to no more than 200 mg/day.

Diet and Fat Digestibility

The negative physiological correlates of fat intake can only be produced if fat is digested and absorbed. Fat digestibility is influenced by the type of dietary fat, dietary fiber, and the level of dietary calcium.

Properties of Dietary Fat and Fat Digestibility

Physical properties of dietary fat, such as its melting point, fatty acid chain length and fatty acid saturation, as well as the amount of fat in the diet all can affect the digestibility of fat.

Early observations reported that the digestibility of fat was related to its melting point. Langworthy and Holmes (105) reported a digestibility of 88% for mutton fat (melting point = 50°C), 80.1% for oleostearin (melting point = 50°C), and 81.9% for deer fat (melting point = 51.4°C) in humans. Cheng *et al.* (106) observed a coefficient of digestibility of 92.4 for bland lard (melting point = 47.8°C), 66.2 for blended lard (melting point = 55.2°C), 58.0 for hydrogenated lard (melting point = 55.4°C), and 17.3 for a more hydrogenated lard (melting point = 61.0°C).

Fatty Acid Chain Length. Callaway *et al.* (107) suggested, in 1956, that the chain length of fatty acids was an important factor associated with fat digestibility. Lloyd and Crampton (108) fed diets containing 20% of calories from fat with fatty acids of varying chain lengths to pigs and guinea pigs. They observed that the apparent digestibility of the fatty acids was inversely correlated with the chain length of the fatty acid and that up to 30% of the variation in fat digestibility was explained by the chain length of the fatty acids in the tested fats and oils. Bach and Babayan (109) reported that triglycerides with fatty acids of chain lengths containing between eight and fourteen carbons had higher digestibilities than triglycerides with fatty acids of sixteen or more carbons. Braud and Newport (110) fed young pigs whole milk or substituted the butter fat in that milk with either beef tallow, coconut oil, or soybean oil. Pigs fed the beef tallow or coconut oil diets gained less weight than those fed the butterfat or the soybean oil diets. Pigs fed the beef tallow (high in long chain stearic acid) diet had the highest amount of fatty acids present in the small intestinal content and the lowest apparent fat digestibility. Leeson and Atteh (111) fed Large White male turkey poults supplemental isoenergetic amounts from either tallow, corn oil, soybean oil, animal-vegetable blend fat, or canola oil. Diets with added fat from vegetable oil sources produced greater fat retention. Diets containing tallow or a vegetable-animal blend fat resulted in the highest fecal fat excretion. Thus, saturated fatty acids were not well digested by birds in that study. Cera *et al.* (112) fed pigs diets containing equal amounts of either coconut oil (composed of 60% medium chain fatty acids with 14 or less carbons), corn oil (composed of 95% long chain fatty acids of 16 or more carbons), or beef tallow (composed of 95% long chain fatty acids, but more of which were saturated when compared to the fatty acids in corn oil). They found that the apparent digestibility of fat ether extract was lowest for beef tallow, followed by corn oil, and highest for coconut oil. Previous results in our laboratories (24) mirrored these findings. We fed isocalorically calculated amounts of either coconut oil, corn oil, or tallow supplemented diets to growing Sprague-Dawley rats and observed that the added tallow was less well digested than coconut oil, which in turn was less well digested than corn oil. Rats fed either the tallow or the coconut containing diet had less body fat than rats fed the high corn oil diet. These studies made apparent the fact that fat digestibility is significantly altered by the chain length of the fatty acids and that the degree of saturation of fatty acids is an additional contributor to the efficiency with which fat is digested.

Fatty Acid Saturation. Fatty acids of the same chain length differ in digestibility when they have different degrees of saturation. Carroll (113), fed diets containing either stearic or oleic acid (both 18 carbons in length) to Sprague-Dawley rats and obtained apparent digestibilities of 12 and 48% for stearic acid and oleic acid, respectively. Similar findings were observed by Bayley and Lewis (114), whereby saturated fatty acids were less well absorbed than unsaturated fatty acids when fed to pigs. Hamilton and McDonald (115) observed that the two saturated fatty acids palmitic and stearic in feeds containing beef tallow or lard were poorly digested compared to unsaturated fatty acids of the same chain length. However, they noted that more of these two fatty acids were absorbed when the fat source was lard than when the fat source was tallow. Palmitic acid in lard is esterified at the sn-2 position, which permits its absorption as 2-monopalmitin. Mattson *et al.* (116) showed that the position occupied by the fatty acids stearate and oleate on the glycerol molecule determined the digestibility of the triglyceride by rats. Small (117) stated that the location of saturated fatty acids in the sn-1 and 3 positions, as is the case in naturally occurring vegetable fats, renders the fats less well absorbed than randomized fats with saturated fatty acids placed in the sn-2 position. Lien *et al.* (118) found that mixtures of coconut oil and palm olein were better absorbed when the fats were randomized to locate more of the saturated fatty acids on the sn-2 position than when natural, non-randomized, fats were fed to rats. Brink *et al.* (119) similarly obtained lower fat and energy absorption when rats were fed 2-oleoyl-distearate (stearic acid on the sn-1,3 position) than when fed 1-oleoyl-distearate (stearic acid on the sn-2 position). De Schrijver *et al.* (120) fed male Wistar rats diets with 9.1% added fat from either beef tallow, native or randomized fish oil, or native or randomized peanut oil. In that study, randomization did not affect apparent digestibility of total dietary fat, but the beef tallow diet had the lowest apparent digestibility. Awad *et al.* (121) fed rats purified diets containing 14% of added fat from either the saturated fatty acid sources beef fat or butterfat, or from the highly polyunsaturated safflower oil. Fecal fatty acid excretion was higher from the high saturated fat diets than from the high polyunsaturated fat diet; compared to the high safflower oil diet, the high butterfat diet and the high beef fat diet resulted in twice and four times as much fecal free fatty acid excretion, respectively. Wiseman and Salvador

(122) reported that the apparent metabolizable energy of fat decreased as the saturated fatty acid content in the fat increased. Toyomizu *et al.* (123) observed that mice fed diets containing beef tallow or hydrogenated beef tallow had lower mature body weights and lower % body fat than mice fed soybean oil or linseed oil containing diets. Therefore, the more saturated the fatty acid, the lower its absorption and the more the saturated fatty acid is positioned in the sn-1,3 position on the glycerol backbone, the lower the absorption of the fat containing the saturated fatty acids.

Dietary Fiber and Fat Digestibility

Dietary fiber has the ability to interfere with digestion and absorption of fat, thus increasing its excretion in feces (124). Noblet and Perez (125) provided equations for predicting the digestible and metabolizable energy values of energy and nutrients in diets for growing pigs and found that the digestibility coefficient of energy was negatively affected by the dietary fiber content of the diet. Digestive interactions between fat and fiber and fat and ash were observed that might influence the caloric contribution of diets. Lairon (126) observed that fiber supplementation resulted in a 610% increase in the ileal excretion of triglycerides plus diglycerides and a 400% increase in ileal excretion of monoglycerides plus free fatty acids compared to low fiber diets in 6 ileostomized subjects. Hingham and Read (127) reported an increase in stomal fat losses in ileostomized individuals as a result of interactions between fat and fiber in the small intestine. Patients fed guar gum had a 50% increase in the amount of lipid in the ileostomy fluid. However, the type of fiber influences the effects on digestibility. Ikeda *et al.* (17) reported that dietary fiber significantly lowered triglyceride absorption in lymph cannulated rats. Triglyceride absorption was low with chitosan, intermediate with guar gum, and high with cellulose as fiber sources in intragastrically administered emulsions. Guar gum also affected the absorption of cholesterol and triglycerides. Ebihara and Schneeman (128) found that isotopically labeled triolein and cholesterol were higher in the intestinal contents of rats fed glucomannan and guar gum than of rats fed cellulose. Morgan *et al.* (129) observed a decrease in energy digestibility as the fiber content of the diet increased. Energy digestibility was most suppressed by the addition of straw and oatfeed, compared with the addition of sugar beet pulp to the diets of growing pigs. Myer and Combs (130) reported reduced efficiency of feed conversion, dry matter digestibility, and energy digestibility upon the addition of 40% ground oats to the feed of growing-finishing swine. Cummings *et al.* (131) increased the fiber intake of human subjects from 17 to 45 g/day by the addition of wheat fiber to their metabolically controlled diets. Increasing dietary fiber increased fecal weight from 79 to 228 g/day, fecal fat from 1.75 to 2.75 g/day, and fecal bile acids output from 199 to 279 mg/day; however, fecal bile acids were diluted on the high fiber diet decreasing to 6.2 from 9.5 mg/g of fecal solids on the low fiber diet. Galloway *et al.* (132) reported that Albino Swiss rats fed a high (26% of diet) fiber (cellulose) had lower weight gain and more fecal weight than animals fed a low (2% of diet) fiber diet. Fiber in that study was the main determinant of fecal bile acid concentration, with fiber diluting the concentration of bile acids in the feces. Similar findings in humans (133) demonstrated low fecal bile acid concentrations (per g of dry stool) when subjects were fed high fiber diets, compared to low fiber diets. Bile acids are synthesized by the liver from cholesterol and enter into the intestinal tract with bile to aid in fat digestion. They are then reabsorbed via the enterohepatic circulation; however, unabsorbed bile acids are excreted in the feces and are important in the regulation of the body's bile acid and therefore, circulating cholesterol pool (134).

Dietary fiber has been implicated in the reduction of circulating lipid levels. Rimm *et al.* (135) examined the relationship between dietary fiber and coronary heart disease in 43,757 US male health professionals. They reported a strong inverse association between fiber intake and risk for coronary disease. They suggested that fiber, independent of fat intake, was important in the prevention of coronary disease. Landin *et al.* (136) observed that a preprandial dose of guar gum reduced fasting levels of circulating cholesterol and triacylglycerol in healthy male subjects. Frape and Jones (137) supplemented the daily diet of healthy middle-aged volunteers with 6 g of fiber from sugar-beet root for three weeks and obtained an 8.5% and 9.6% reduction in the levels of fasting total cholesterol and LDL cholesterol, respectively. Cara *et al.* (138) added fiber from either oat bran, rice bran, wheat fiber, or wheat germ to test meals of six normolipidemic male subjects. The addition of fiber reduced the postprandial rise in triglycerides and the overall chylomicron cholesterol response. Postprandial cholesterol levels decreased with fiber and were lowest with oat bran. Marckmann *et al.* (139) fed a high fiber (1.38 g/100 kcal) low fat (28% of energy) diet to 21 healthy middle-aged Danes. The diet resulted in lower serum concentrations of LDL and HDL cholesterol when compared with feeding a low fiber (1.09 g/100 kcal) high fat (39% of energy) diet. Arjmandi *et al.* (140) fed 38 hypercholesterolemic women added fiber from either sunflower seed or flaxseed. They observed that for every one gram increase in soluble fiber, total serum cholesterol decreased by 2.2 mg/dl and LDL cholesterol decreased by 2.7 mg/dl, and for every one gram increase in insoluble fiber, total serum cholesterol decreased by 1.6 mg/dl and LDL cholesterol decreased by 1.9 mg/dl.

Schneeman (22) suggested that the fiber in the small intestine can increase the viscosity of the intestinal contents thus slowing diffusion, reducing mixing, and lowering the rate of nutrient absorption. Fiber can also interact with micelle components such as bile acids thereby reducing plasma cholesterol concentrations. Ikeda *et al.* (17) theorized possible mechanisms involved in the reduction of cholesterol absorption observed with dietary fiber. First, fiber reduces gastric emptying and transit times; second, fiber adsorbs bile salts, thus, inhibiting the micellar solubility of cholesterol and lipid digestion products; and third, fiber, particularly the viscous type, reduces the accessibility of micelles to the surface of intestinal absorptive cells. Suzuki *et al.* (141) suggested that dietary fiber may decrease the intestinal absorption of lipid by decreasing transit time or by inhibiting contact with intestinal absorptive cells.

Calcium and Fat Digestibility

Calcium has the ability to alter fat absorption and thus its conversion to energy and blood lipids. Yacowitz *et al.* (142) fed high calcium diets to rats and noted that fat and calcium combined in the gut to form non-digestible calcium soaps that were excreted in the feces. French (143) found that calcium utilization in the Albino rat decreased as fat in the diet increased. Calcium was best absorbed when the ratio of calcium to fat in the diet was 0.063 to 1. Holt *et al.* (144) observed that feeding high fat diets resulted in the formation of insoluble calcium fatty acid soaps, rendering calcium unavailable for absorption by infants and children. Bassett *et al.* (145) noted that increasing calcium intake increased the total amount of fecal fat whereas reducing the amount of dietary calcium improved the absorption of fatty acids in patients with ideopathic steatorrhea. Cheng *et al.* (106) observed that supplemental calcium and magnesium in the diet reduced the apparent absorption of trilaurin from 97.3% to 70.5%, trimyristin from 76.6% to 37.7%, tripalmitin from 27.9% to 12.8%, and tristearin from 18.9% to 10.6% in rats. Calcium and magnesium caused an increase in fecal soap excretion and depressed the digestibility more for high melting point triglycerides and hydrogenated fats than for low melting point fats. Calcium was, similarly, shown to depress the digestibility and metabolizable energy of tallow in rats fed 230 mg (vs. 62 mg) of calcium from calcium carbonate/day (24). Lapre *et al.* (146) reported that supplementing a high milk fat or high palm oil rat diet with calcium phosphate (9 g/kg diet) increased fecal output and increased total fecal excretion of fatty acids but reduced concentrations of fatty acids in fecal water. This suggests that calcium formed fatty acid soap complexes, which reduced fatty acid absorption and solubility. Lupton *et al.* (147) reported similar increases in fecal weight and total fecal lipid excretion with increases in the level of calcium in the diet of rats. Brink *et al.* (119) showed that calcium (1 g /100 g diet), from calcium carbonate, increased fecal excretion and reduced the absorption of the saturated fatty acids palmitic and stearic acid in rats. The effect of calcium on fatty acid excretion and absorption was further enhanced when the fatty acids were positioned at the sn-1,3 position vs. the sn-2,3 position. Aoyama *et al.* (148) similarly found that calcium fortification of the diet of rats reduced the apparent absorption efficiency of fat with long chain saturated fatty acids, especially when these fatty acids were positioned at the sn-1,3 sites on the glycerol backbone, and the apparent absorption efficiency of saturated fatty acids in foodstuffs such as chocolate (cocoa butter had palmitic and stearic acid on the sn-1,3 positions). Rouvinen and Kiiskinen (149) fed beef tallow or rapeseed oil containing diets with various levels of ash (4,8,and 14% dry matter) from either limestone grist or bone meal to adult male minks. As the ash level in the diet increased from 4 to 14%, it decreased the digestibility of beef tallow from 76 to 67%, and from 87 to 66% for the limestone grist and the bone meal containing diets, respectively. The digestibility of rapeseed oil also decreased with increases in ash content, but the effect was not as large as it was with beef tallow; the digestibility of rapeseed oil changed from 94 to 85% and 96 to 94% with limestone grit and bone meal, respectively. Consequently, the metabolizable energy of the diets decreased with increasing ash level; the metabolizable energy of the beef tallow containing diet was reduced from 17.0 to 14.4 and from 18.6 to 14.5 MJ/kg of dry matter, and that of the rapeseed oil containing diet was diminished from 18.5 to 15.6 and 19.4 to 16.7 MJ/kg of dry matter with the limestone grit and bone meal inclusion, respectively. Awad *et al.* (150) fed Sprague-Dawley weanling male rats a diet with 14% beef fat with or without an added 1% of calcium from calcium carbonate. The excess dietary calcium reduced the free bile acid and free fatty acid content of fecal water by 54 and 44%, respectively by altering the solubility of these lipids. Lapre *et al.* (151) observed that supplemental calcium (from 9 mg calcium phosphate/g diet) greatly increased the fecal excretion of fatty acids by Wistar rats; the increase was more significant for diets containing fats with saturated fatty acids than for those with polyunsaturated fatty acids. Hambly *et al.* (152) reported higher weight gain in rats fed a high fat low calcium and fiber diet than in rats fed a low fat high calcium and fiber diet. The nutrient to energy ratio and the energy intake of both diets were the same, but the availability of energy from fat was greater when calcium and fiber levels were lower in the diet. Suzuki *et al.* (141) observed that fecal fatty acid-calcium soaps excretion increased from 3 μg/g of feces to 495 μg/g of feces when healthy male subjects switched from a low fat (14% of energy/day) to a high fat (53% of energy/day) diet. The investigators in that study suggested that in the presence of sufficient calcium, the long-chain fatty acids in the diet are rendered insoluble by conversion to calcium soaps. Calcium can, therefore, reduce the solubility, absorption, digestibility and metabolizable energy of fat.

Through its impact on fat digestibility and lipid solubility, calcium potentially can alter cholesterol metabolism. Yacowitz (21) added 0.89 g of calcium from either calcium carbonate or calcium gluconate to the diet of subjects with elevated serum lipid levels and observed an increase in fecal fat excretion and a decrease in serum triglycerides and cholesterol levels. As mentioned above, calcium reduced the solubility of bile acids (important in cholesterol metabolism) in the fecal water from rats (150). Van der Meer *et al.* (153) supplemented the diet of 12 healthy men with 35.5 mmol of calcium/day from calcium carbonate; this resulted in a 53% increase in the fecal excretion of bile acids. Denke *et al.* (154) fed 13 moderately hypercholesterolimic men a diet either low in calcium (410 mg/day) or high in calcium (2200 mg/day) and observed an increase in the daily fecal excretion of saturated fatty acids from 6 to 13% upon increasing dietary calcium. Circulating lipid levels were also affected by calcium fortification such that serum total cholesterol decreased by 6%, LDL cholesterol decreased by 11%, and apolipoprotein B decreased by 7%, without affecting HDL cholesterol levels. The researchers in that study stated that calcium supplementation was effective in favorably altering serum lipid levels and could be part of a cholesterol lowering strategy.

CHAPTER III

ENERGY VALUE OF CARBOHYDRATE AND LIPIDS WITH ADDED CALCIUM FOR GROWING MICE

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Abstract

Female CD1 weanling mice (16 g initially) were limit fed a purified diet alone or with added isocaloric amounts (5.4 kcal metabolizable energy per day) from either sucrose, corn oil, or tallow. In addition, diets with supplemental fat contained either 0.60% or 1.5% calcium. Growth rates, digestibilities, and energy retentions were measured during the 28 day study. Fecal fat and fecal soap excretion were greater ($P<0.06$) for animals fed tallow than for those fed corn oil. Mean metabolizable energy (Atwater) values for sucrose, tallow, and corn oil averaged 4.01, 7.96, and 8.94 kcal, respectively. Retention of digested energy from sucrose, tallow and corn oil averaged 13%, 10% and 21%, respectively. This means that per gram of added nutrient, retained energy from tallow averaged 1.60 and that from corn oil averaged 4.11 times that of added sucrose. Retained energy from added corn oil was greater ($P<0.01$) than from added tallow. On a retained energy basis, the relative value for corn oil was greater and the relative value for tallow was less than the metabolizable energy ratio of fat to carbohydrate proposed by Atwater of 2.25. Mice fed corn oil had a higher ($P<0.01$) percentage of body fat and a lower ($P<0.01$) percentage of body protein. Added calcium depressed ($P<0.01$) digestibilities of both dry matter and energy, with a greater ($P<0.01$) effect on tallow than on corn oil. The metabolizable energy value of fat in diets for growing mice varied from 75 to 108% of Atwater values and the retention of added energy varied with fat source in this study, being 156% greater ($P<0.01$) for corn oil than for tallow.

Key words: Tallow, Corn oil, Mice, Energy, Calcium

Introduction

Much public concern has been raised over fat intake and its relationship to obesity, atherosclerosis, colon cancer, and endocrine related cancers (1-6). However, fat digestibility and absorption can vary with source of fat and other diet components (7). For example, fat digestibility is depressed by adding calcium to the diet (8-10) because calcium binds fatty acids in the digestive tract to form insoluble, indigestible fatty acid soaps (11, 12). Fat digestibility also can be affected by the saturation level of the fatty acids (13) with saturated fats being less completely absorbed than unsaturated fats (8,13). Previous studies in our laboratories (8) utilizing male rats have shown that digestibilities and metabolizable energy values were lower for tallow than corn oil supplemented diets,. especially in the presence of calcium. Donato and Hegsted (14) have found that tallow or corn oil diets fed to male rats had a higher caloric value than expected, an average of 11.5 kcal/g, compared to sucrose at 3.9 kcal/g. Wiseman and Salvador (15) found that apparent metabolizable energy was inversely proportional to fatty acid saturation for young chicks. Noblet and Perez (16) found that fat digestibility was lower (79%) than generally accepted (95%), possibly due to minerals included in the diet. If the caloric value of fat is a function of its digestibility (7), then diet composition will alter the caloric value of fat. For ease of handling and body composition measurement, mice were used in this experiment. Females were used because most previous work has used males and gender may alter diet effects on body composition. This study was designed to: 1) compare the caloric value of two fat sources, one animal (tallow) and one plant source (corn oil), with that of carbohydrate (sucrose); and 2) measure the effect of calcium supplementation on digestibility and energy retention from diets supplemented with these two sources of fat.

Materials and Methods

Forty-two female CD1 weanling mice (16 g initially) were stratified by weight and assigned (six animals per group) to 7 groups; one group was used to determine initial body composition and the other 6 groups were assigned to the 6 dietary treatments which were limit fed for 4 weeks. The compositions of the diets are shown in Table 1. Except for the basal diet, all diets were isocaloric on a metabolizable energy basis. The basal mixture, which served as a base for all diets, was composed of casein, corn oil, sucrose, vitamin mix (AIN-76A), mineral mix (AIN-76), and choline chloride. It was formulated to meet all nutrient needs (17). Each animal was caged individually in a wire bottom hanging stainless steel cage and had *ad libitum* access to water in a climate-controlled room with a 12-hour light:dark cycle. Animals were given their daily ration, which was kept refrigerated until it was offered, once at 1600 and were free to consume it *ad libitum* throughout the 24 hour span. The protocol of the study was approved by the Laboratory Animal Use Committee at Oklahoma State University.

Animals were weighed initially and weekly during the 28 day study. Feed refusals, collected daily from each animal and frozen until the end of the experimental period, were used to calculate dry matter, energy and nutrient intakes. Diets and feed refusals were analyzed for gross energy by oxygen bomb calorimetry (Parr, 1261 Calorimeter Parr Inst. Co. Moline, IL, 1988), nitrogen by the AOAC Kjeldahl method (18) utilizing Tecator Kjeltech instrumentation for digestion and distillation, and lipid by ether extraction (18). Diet samples also were analyzed for calcium content utilizing an atomic absorption spectrophotometer (Perkin Elmer Atomic Absorption Spectrophotometer, model 5100, Perkin Elmer, Norwalk, CT) (19).

Initial body composition was obtained by sacrificing six mice at the start of the trial. Final body composition was obtained by sacrificing all the remaining mice at the end of the 28-day experimental period. Body composition of all animals was determined as follows. The large intestine and cecum were removed to avoid undigested food; we chose not to fast the animals prior to sacrifice for fear that that may alter body composition analyses. The remaining empty body was autoclaved and ground with a mini-food processor to produce a homogeneous tissue sample (8). Dry weight was obtained by lyophilizing each carcass individually for eight days. Body fat, nitrogen, and energy content were determined as previously described for the diets. The difference between initial and final body composition was used to calculate retention of specific components and energy. During weeks 2, 3, and 4, each animal's feces were collected, weighed and analyzed separately. Feces were dried at 100^{o}C for 48 hours. Gross energy of feces was determined by oxygen bomb calorimetry. Fecal fat was obtained by first extracting the samples in petroleum ether to determine ether-soluble fat content and then further extracted in a 40:10:1 isopropanol:heptane:1N sulfuric acid solution to release the fatty acids bound as soap (8, 20). To calculate digestibilities of each supplement which was added to the basal diet, the contribution of the basal diet to feed intake and fecal output were subtracted from feed intake and fecal output of the supplemented diets for the last 21 day period of the trial. Energy retention from each supplement was calculated in a similar fashion based on initial and final body compositions of mice fed the basal and the supplemented diets for the duration of the trial.

Statistical analysis of the data used the General Linear Models procedure of SAS (21) and absolute probabilities will be presented and discussed. Treatment means were contrasted using the Duncan's Multiple Range Test. However, because specific treatment contrasts are interpreted more readily, orthogonal contrasts will be presented and discussed. Differences examined by orthogonal contrasts included 1) basal versus energy-supplemented diets; 2) sucrose versus fat-supplemented diets; 3) tallow versus corn oil; 4) low versus high calcium; and 5) interaction of fat source and calcium concentration. These factors were the primary foci for discussion.

Results

Orthogonal contrasts representing average effects of added energy, sucrose versus lipids, lipid source (corn oil versus tallow), calcium level, and the interaction between lipid source and calcium level are presented in Table 2. These will be discussed sequentially.

Energy Supplementation

Differences among diets in daily dry matter intakes were as expected, being greater with added energy and less for diets with fat added than with sucrose added. Supplementing the basal diet with 5.4 kcal of metabolizable energy daily, when averaged across energy sources, increased daily gain ($P<0.02$), fecal output of dry matter, fat and soap ($P<0.02$), digested energy content of the diet (kcal/g; $P<0.01$) and body energy retention ($P<0.01$) (Table 2). Added energy increased fat but decreased ($P<0.01$) protein content of the body.

Energy Source

Compared with mice fed sucrose, those fed added fat tended to have higher ($P<0.10$) gain:feed ratio, fecal dry matter and soap excretion ($P<0.01$) and digestibility of added source ($P<0.01$) but lower ($P<0.01$) digestibilities for added dry matter and energy. Digested energy content of the diet (kcal/g) and of supplemental dry matter was greater ($P<0.01$) with fat than with sucrose added to the diet. Energy retention of the added energy per gram fed also tended to be greater ($P<0.07$) from added fat than from added sucrose.

Fat Source

Digestibility of dry matter and energy was lower ($P<0.01$) for tallow than for corn oil added to the diet although an interaction with calcium level was detected ($P<0.01$ and $P<0.08$). Added calcium depressed digestibility more for tallow than for corn oil. The high calcium-tallow diet had lower ($P<0.01$) energy and dry matter digestibilities than any other diet. Retention of added energy per day or per g fed was greater ($P<0.01$) from added corn oil than from added tallow; efficiency of energy retention, calculated as retained divided by digested energy, for corn oil averaged more than twice that for tallow (21% versus 10%; $P<0.02$). In their carcasses, when averaged across calcium levels, mice fed corn oil had more dry matter and fat ($P<0.03$; $P<0.01$) but less protein ($P<0.01$) than mice fed tallow.

Added Calcium

Because calcium was added only to the fat-supplemented diets, discussion and interpretation must be restricted to such diets. Added calcium increased fecal dry matter output ($P<0.01$) and depressed digestibility of diet dry matter and energy ($P<0.01$), especially with the tallow diet (interaction $P<0.08$). However, added calcium did not alter retention of either fed or digested energy.

Interactions Between Calcium Addition and Fat Source

Fecal dry matter excretion was increased more (interaction $P<0.04$) by adding calcium to the tallow than to the corn oil diet. Added calcium reduced digestibility of dry matter and energy, both of the total diet and of the added materials, (interactions $P<0.01$; $P<0.02$ and $P<0.01$; $P<0.08$, respectively) more with tallow than with corn oil in the diet.

Discussion

Compared with added sucrose, supplementation of a basal diet with equal kilocalories from fat (tallow or corn oil) reduced digestibilities of dry matter and energy but increased digestibility of fat. Compared with supplemental corn oil, supplemental tallow resulted in lower digestibilities for both dry matter and energy. Fat content of the body was greater with the corn oil diet than with the tallow diet. These results agree with previous results obtained feeding high fat diets to male weanling rats (8) and other studies that have shown that saturated fats are less completely absorbed than unsaturated fats (22-24).

Standard physiological fuel (Atwater) values are compared with the values we measured for digested (metabolized) energy, retained energy, and heat increment (determined by subtraction as the difference between metabolized and retained energy) for the added nutrients shown in Figure 1.

Metabolizable energy is equal to digested energy minus urinary energy loss. Unlike effects with added protein, added sucrose and lipid calories are not partially lost in urine. Thus, the digestible energy values measured should equate directly with the metabolizable energy values proposed by Atwater. Like Atwater's estimates, metabolizable energy values for the added fats were much higher than for sucrose (averaging 7.96 and 8.94 kcal/g for tallow and corn oil versus 4.01 for sucrose as shown in Figure 1). Digested energy averaged 84% and 94% of the gross energy value (bomb calorimetry value of 9.47 kcal/g) for tallow and corn oil, respectively. Although these values are reasonably close to the Atwater values for carbohydrate (4 kcal/g) and lipid (9 kcal/g), our metabolizable energy value was consistently lower for tallow than for corn oil suggesting that metabolizable energy values can differ among sources of lipid. This is consistent with observations in which high saturated fatty acid content of lipids, as in tallow, resulted in lower fat digestibility when compared to lipids with less saturated and more unsaturated fatty acid content (13, 23, 25, 26).

Another method of contrasting caloric value is to compare the amounts of energy retained by the body (net energy), not simply the amounts digested. In this study, retained energy from supplemented sucrose, tallow and corn oil averaged 0.53, 0.85 and 2.18 kcal/g fed. Such a comparison yields a markedly different ratio for fat to carbohydrate than either metabolizable energy or Atwater values do, as has been noted previously (14). Thus, if we set the caloric value for retained or net energy from sucrose at 4.00 kcal/g (to parallel the Atwater value for carbohydrate), then the amounts of energy retained (net energy) calculate to be 6.4 kcal/g of tallow and 16.4 kcal/g of corn oil in this study. That the proportion of dietary energy retained is greater for some lipids than for others has been observed previously (8, 14, 27-29) and may be due to several factors. Explanations as compiled by Reid (30) and Dale and Fuller (31) include: 1) fat in a diet enhances total digestibility and caloric value of other diet components including proteins and carbohydrates; 2) endogenous excretions of energy may be reduced by added lipid, and 3) heat loss is lower for synthesis of stored lipid from dietary fat than from dietary carbohydrate. In this study, efficiency of retention of digested energy averaged more than twice as high for corn oil than tallow (21 versus 10%).

The difference between metabolizable energy (Atwater values) and retained (net) energy equals the amount of energy lost as heat, often called the heat increment. The heat increment expressed as a percentage of metabolizable energy was similar for supplemental tallow and sucrose (83% and 87%) but slightly lower with supplemental corn oil (72%) calculated from values in Figure 1 (see footnote).

Supplementing high fat diets with calcium reduced energy digestibility and increased fat excretion; this agrees with results by others (8, 10, 12, 32). Fecal soap levels tended to be increased by calcium addition. Presumably, this is due to the formation of insoluble soaps that render the fatty acids unavailable for absorption (11, 12, 33, 34). Added calcium depressed the digestibility more for tallow than for corn oil supplemented diets. Calcium salts of more saturated fatty acids, as from tallow, should be less easily ionized and solubilized than salts of less saturated fatty acids (26). At 1.5 versus 0.6% of dry weight of a diet containing tallow, calcium reduced the digestibility of energy of tallow by 26% (6.79 versus 9.12 kcal/g as shown in Figure 1), and retention of energy by 17% (0.77 versus 0.93 kcal/g tallow). In contrast, added calcium reduced energy digestibility of corn oil by only 16% (8.14 vs. 9.74 kcal/day) and added calcium tended to increase (11%) retention of dietary energy from corn oil (2.29 vs. 2.07 kcal/g corn oil tallow).

In conclusion, calculating the caloric content of mixed diets from analysis of nutrients (protein, fat and carbohydrate) may not accurately predict either digested or retained energy. The source of fat can alter both digestibility and retention of digested energy. Supplemental calcium can depress digestibility, especially with tallow. Consequently, source of fat and the calcium content of a diet must be evaluated because these components can influence availability of energy from the diet.

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Table 1. Composition of Experimental Diets, Percentage By Weight.

Diet	Basal	Sucrose	Tallow	Corn Oil	Tallow	Corn oil
Calcium	Low	Low	Low	Low	1.5%	1.5%
Tallow	0	0	14.27	0	13.94	0
Corn Oil	6.87	5.00	5.89	20.16	5.76	19.66
$CaCO_3$	0	0	0	0	2.31	2.31
Sucrose	51.89	65.00	44.48	44.48	43.46	42.48
Casein	27.49	20.00	23.57	23.57	23.02	23.02
Cellufil	6.87	5.00	5.89	5.89	5.76	5.76
Minerals.	4.81	3.50	4.12	4.12	4.03	4.03
Vitamins.	1.37	1.00	1.18	1.18	1.15	1.15
Methionine	0.41	0.30	0.35	0.35	0.35	0.35
Choline Cl	0.27	0.20	0.24	0.24	0.23	0.23
Amount fed, g/day	3.64	5.00	4.24	4.24	4.34	4.34
Kcal/ration/day		19.31	19.29	19.29	19.29	19.11
	13.87					
Ca %, by analysis	0.70	0.51	0.60	0.60	1.50	1.50

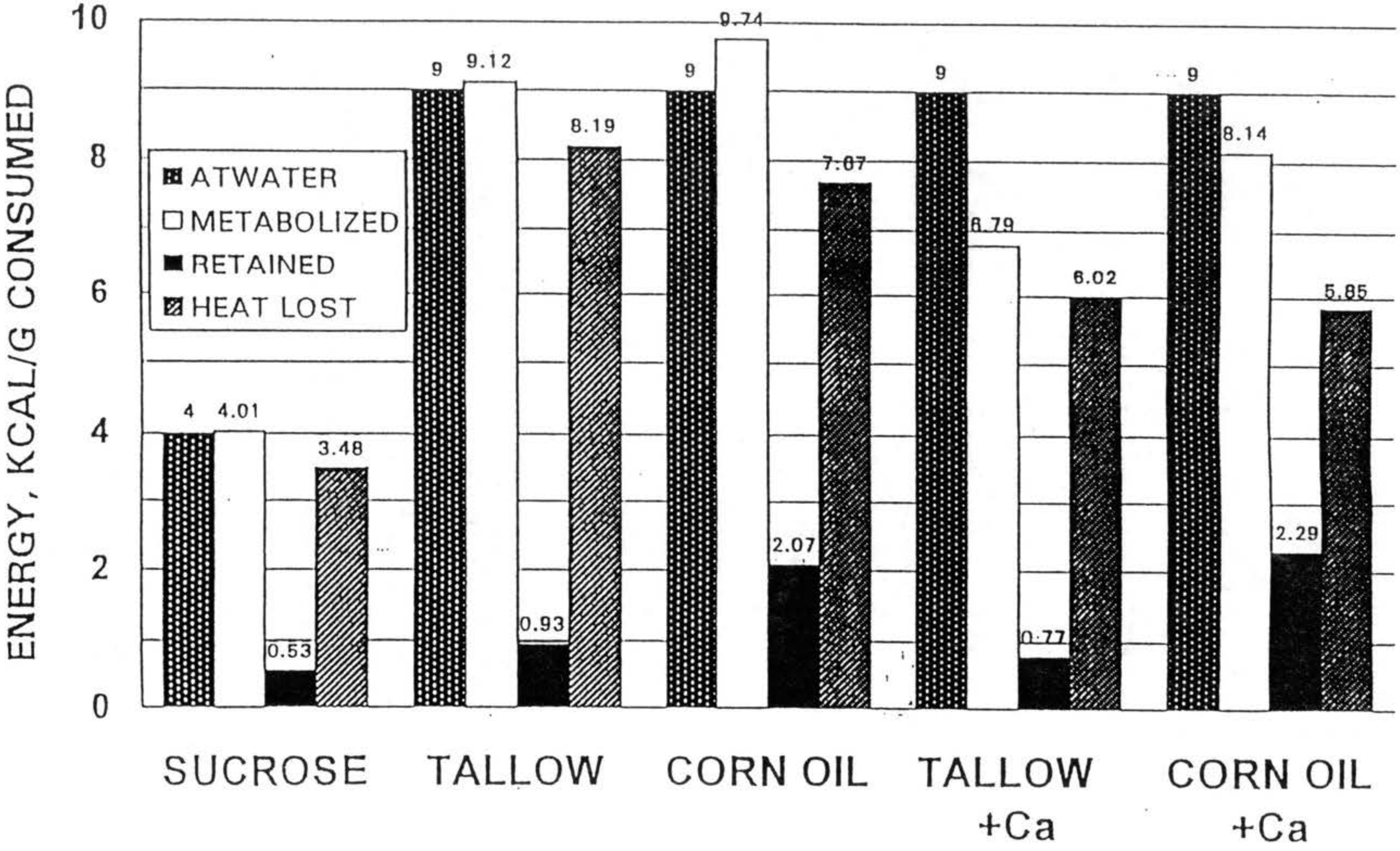
Table 2. Results of Energy Supplementation

	DIET							MAIN EFFECTS AND INTERACTIONS (P <)				
Diet	Basal	Sucrose	Tallow	Corn Oil	Tallow	Corn Oil	Standard error	Added Energy	Fat vs. CHO	Tallow vs. Corn Oil	Added Ca	Ca by fat source interaction
Calcium, %	0.70%	0.51%	0.60%	0.60%	1.50%	1.50%						
Feed intake, g/day	3.48	4.48	3.88	3.96	3.80	3.97	0.05	0.01	0.01	0.03	0.54	0.39
Gain, g/day	0.34	0.44	0.45	0.56	0.41	0.50	0.05	0.02	0.49	0.06	0.35	0.82
Gain/Feed	0.096	0.098	0.114	0.140	0.107	0.125	0.02	0.15	0.10	0.09	0.39	0.75
Fecal output, g/28 days												
Dry matter	10.9^{c}	9.9^{c}	10.8^{c}	10.7^{c}	15.3^{a}	13.4^{b}	0.41	0.02	0.01	0.02	0.01	0.04
Fat	0.46	0.63	0.93	0.65	0.86	0.73	0.10	0.01	0.18	0.06	0.94	0.47
Soap	0.09^{d}	0.11^{d}	0.37^{b}	0.19^{c}	0.45^{a}	0.21^{c}	0.02	0.01	0.01	0.01	0.06	0.26
Diet digestibility, %:												
Dry Matter	88.9^{c}	92.1^{a}	90.1^{b}	90.4^{b}	85.6^{d}	88.0^{c}	0.32	0.31	0.01	0.01	0.01	0.01
Energy	91.6	93.6	90.9	93.2	88.4	92.5	0.37	0.74	0.01	0.01	0.01	0.02
Fat	95.2	93.3	96.2	97.2	96.4	97.0	0.83	0.37	0.01	0.36	0.97	0.82
Digestibility of test material												
Dry Matter	-	101.0^{a}	97.4^{a}	99.5^{a}	68.9^{c}	83.4^{b}	1.95	-	0.01	0.01	0.01	0.01
Energy	-	99.5^{a}	79.3^{b}	95.5^{a}	66.4^{c}	91.9^{a}	2.52	-	0.01	0.01	0.01	0.08
Digested energy, kcal/g:												
Diet	4.13^{d}	4.10^{d}	4.84^{a}	4.93^{a}	4.56^{c}	4.78^{b}	0.02	0.01	0.01	0.01	0.01	0.01
Added Source	-	4.01^{e}	9.12^{b}	9.74^{a}	6.79^{d}	8.14^{c}	0.13	-	0.01	0.01	0.01	0.02
Final body composition												
Dry Matter, %	39.2	41.8	39.9	42.8	39.6	44.4	1.65	0.17	0.95	0.03	0.69	0.56
Protein, % of dry matter	47.0	41.9	44.2	37.7	44.0	36.8	1.70	0.01	0.53	0.01	0.73	0.84
Fat, % of dry matter	38.6	45.3	44.0	49.7	42.8	51.6	2.18	0.01	0.47	0.01	0.87	0.26
Retained dietary energy												
Kcal/day	1.15	1.69	1.61	2.15	1.47	2.20	0.18	0.01	0.42	0.01	0.80	0.61
Of kcal fed, %	7.31	8.58	7.73	10.21	7.44	10.73	0.86	0.10	0.64	0.01	0.90	0.64
Of kcal digested, %	7.98	9.17	8.52	10.96	8.43	11.60	0.94	0.10	0.51	0.01	0.78	0.70
Retention of added Energy												
kcal/day		0.53	0.46	1.00	0.32	1.05	0.20	-	0.45	0.01	0.82	0.63
kcal/added g fed		0.53	0.93	2.07	0.77	2.29	0.46	-	0.07	0.01	0.95	0.68
kcal/kcal digested	-	0.13	0.10	0.19	0.10	0.23	0.04	-	0.65	0.02	0.61	0.65

Means within a row with different superscripts differ ($P<0.05$)

Figure Legend:

Figure 1. Atwater (physiological fuel) values, measured metabolized (digested) energy, retained energy, and heat lost (difference between digested and retained energy) in kilo calories per gram of added sucrose, tallow, and corn oil, with or without added calcium. Heat loss (by calculation) as a percentage of digested energy for each of the five diets was 87%, 90%, 79%, 89%, and 72%, respectively.


ENERGY, KCAL/G CONSUMED
10
8
6
4
2
0
ATWATER
METABOLIZED
RETAINED
HEAT LOST
4
4.01
0.53
3.48
9
9.12
0.93
8.19
9
9.74
2.07
7.07
9
6.79
0.77
6.02
9
8.14
2.29
5.85
SUCROSE
TALLOW
CORN OIL
TALLOW +Ca
CORN OIL +Ca

CHAPTER IV

EFFECTS OF LEVEL OF CALCIUM OR FIBER TYPE ON DIGESTIBILITY OF A TALLOW-SUPPLEMENTED DIET BY MICE

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Abstract

Female CD1 weanling mice (16 g) were used to study effects of calcium and fiber level on growth and digestibility. Mice were limit fed a basal AIN purified diet with 14% tallow added (4.4 kcal/day) with calcium at either 0.6, 1.0, 1.5, 2.0, or 2.5% of diet dry matter. In addition, 10% (0.22 g/day) total dietary fiber was added to the 0.6% calcium diet from either cellufil, rice bran, wheat bran, or beet pulp. Growth rates, digestibilities, energy retention, and body composition were measured during the 28 day trial. Added calcium linearly ($P<0.01$) depressed digestibility of dry matter (83 versus 90%), digestibility of energy (87 versus 91%), and calculated Atwater values, while it increased fecal excretion of soap ($P<0.01$)and fat ($P<0.10$). For every 1% increase in dietary calcium, metabolizable energy decreased by 2%. Added fiber also increased ($P<0.06$) fecal fat and soap excretion and depressed dry matter and energy digestibility ($P<0.01$).

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Of the 4 tested fibers, mice fed the rice bran diet had the lowest ($P<.05$) digestibilities of dry matter, energy and fat. By depressing tallow digestibility and increasing fecal fat excretion, caloric value can be altered markedly by adding either calcium or fiber to a diet rich in fat. KEY WORDS: Calcium, Tallow, Fiber, Mice

Introduction

Dietary fat, particularly saturated fat, has been linked to increased body fat and obesity and the incidence of degenerative diseases (1-3). A fundamental concern of dietary fat is its high caloric value; however, other dietary components may have important effects on the availability of dietary fat. Digestibility and energy value of fat is not constant, but is reduced when fiber is included in the diet, possibly due to altered gastric emptying and intestinal transit time or adsorption to bile salts (4-6). Fat digestibility also is reduced when calcium content of the diet is increased (7-9). Reacting with fatty acids to form insoluble calcium-fatty acid soaps, calcium renders fatty acids unavailable for absorption (10, 11). If the caloric value of fat is a function of its digestibility (12), then the presence of interfering agents like fiber or calcium in the diet should alter the caloric value of fat. This study was designed to study the 1) effects of calcium supplementation on fat and energy digestibility; and 2) effects of dietary fiber supplementation (rice bran, cellulose, wheat bran, or beet pulp) on energy availability of beef tallow.

Materials and Methods

Sixty-six female CD1 weanling mice (16g) were assigned to treatments to produce equal weight groups (six animals each). One group of six mice was used for analysis of initial body composition. The remaining 60 mice were assigned to 10 dietary treatments described in Table l. Values given are based on measured intakes, not the amounts offered. Diet compositions are shown in Table 2. The basal mixture, which was included in each diet, was formulated from casein, corn oil, sucrose, vitamin mix (AIN-76A), mineral mix (AIN-76), and choline chloride, to meet all nutrient needs (13). Except for the basal diet, diets all had tallow added and were provided isocalorically (metabolizable energy basis) by feeding specifically weighed amounts daily. When fiber sources were added, the portion of fiber source that was not dietary fiber replaced sucrose in the diet. Each animal was caged individually in a wire-bottom hanging stainless steel cage and given *ad libitum* access to water in a climate controlled room with a 12 hour dark: light cycle. Animals were fed once daily at 1600. The protocol and methodology of the study was approved by the Laboratory Animal Use Committee at Oklahoma State University.

Animals were weighed initially and weekly thereafter during the 28 day trial. Feed refusals, collected daily from each animal, were frozen until the end of the experimental period and analyzed to determine intake of nutrients and energy. Diets, feed refused, and feces were analyzed for gross energy by oxygen bomb calorimetry (Parr, 1261 Calorimeter Parr Inst. Co. Moline, IL), nitrogen by the AOAC Kjeldahl method (14) utilizing Tecator Kjeltech instruments for digestion and distillation, and lipid by ether extraction (14). Diet samples also were analyzed for calcium content utilizing an atomic absorption spectrophotometer (Perkin Elmer Atomic Absorption Spectrophotometer, model 5100, Perkin Elmer, Norwalk, CT) (15).

Initial body composition was obtained by sacrificing six mice at the start of the trial. Final body composition was obtained by sacrificing all mice after the 28 day experiment.

Each whole carcass, minus the large intestine and cecum which were removed to exclude undigested food, was autoclaved to ease grinding and obtain homogeneous tissue samples (7). Dry weight was obtained by lyophilizing each individually ground carcasses for eight days. Body fat, nitrogen, and energy content were determined as described previously for the diets.

During weeks 2, 3, and 4 all feces were collected, weighed and analyzed separately for each animal. Feces were not collected during week 1 to avoid excreta from the pre-test diet. Digestibilities during week 1 were assumed to equal the mean of the subsequent three weeks. Feces were dried in a 100^{O}C oven for 48 hours. Gross energy was determined by oxygen bomb calorimetry. Fecal fat was obtained by extracting each sample first with petroleum ether to determine ether soluble fat content and second with a 40:10:1 isopropanol: heptane: 1N sulfuric acid solution to release soap-bound fat (7, 16).

Statistical analysis of the data used the General Linear Models procedure of SAS (17). When interactions were detected ($P< 0.05$), means were compared by the Duncan's Multiple Range Test.

Results

Calcium Supplementation

Dry matter intake was limited and intentionally was greater for animals fed higher amounts of calcium so that intake of other nutrients would remain unchanged (Table 3). Daily gain and feed efficiency were not significantly altered by added calcium. Compared with mice fed the basal diet, mice fed tallow-supplemented diets at all levels of calcium had more fat and less protein ($P<0.02$) in the empty body at the end of the study. Calcium supplementation linearly increased fecal excretion of fat ($P<0.10$) and soap ($P<0.01$).

Digestibilities of added dry matter, energy, and fat were calculated assuming that digestibility of components of the basal diet were not influenced by added tallow or calcium. Digestibilities of added dry matter and energy from tallow plus calcium were linearly ($P<0.01$) reduced by addition of calcium to the diet with a tendency for less effect at higher calcium concentrations (quadratic effects of $P<0.01$ and $P<0.15$). Digestibility of fat was not depressed by added calcium . What nutrient is responsible for the depression in digestibility is not clear. Overall, the digestible energy (DE) content of the diet and DE of the added fat were linearly reduced by added calcium ($P<.01$).

Tallow Supplementation

Feed intakes were increased when tallow and fiber were added intentionally to test the effects of added tallow on digestibility (Table 4). Adding tallow plus fiber to the diet increased ($P<0.01$) fecal output of dry matter, fat and soap and decreased ($P<0.01$) digestibility of dietary dry matter and energy. However, because fat was added, digestible energy content of the basal diet was increased when tallow or tallow plus fiber was added.

Fiber Sources

Among the fiber sources, consumption of the rice bran diet was lowest due to partial rejection of this diet by mice (Table 4). Fiber source did not affect ($P>0.05$) average daily gain, efficiency of feed utilization, or body composition. Among the fiber sources, wheat bran tended to result in the highest body fat and least body protein. Fecal dry matter output of dry matter, fat and soap were increased ($P<0.06$) by added fiber, particularly with added rice bran. Among the fiber sources, fecal fat excretion was elevated most by added rice bran whereas fecal soap excretion was elevated by adding either cellufil or beet pulp to the tallow diet.

Digestibilities of dry matter, fat and energy were decreased ($P<0.01$) by adding fiber to the tallow-supplemented diet. Fiber sources differed in their effects on diet digestibilities. Dry matter and energy digestibilities were depressed greatly by rice bran, more ($P<0.05$) than by other fiber sources tested and by cellufil and wheat bran more ($P<0.05$) than by beet pulp. Fat digestibility was decreased ($P<0.05$) with the addition of rice bran but not by the other fiber sources. Among the fiber sources, rice bran diet depressed intake and digestibility the most (Table 3).

Digestibility of each fiber source tested was calculated by subtracting the amounts of energy and nutrients digested from the tallow diet from those digested with each fiber-supplemented diet which also contained tallow. Digestibilities do not directly match changes in diet digestibility because different amounts of the tallow-supplemented diet and sucrose were replaced by the various sources of fiber. Dry matter digestibility of cellufil, calculated to be negative, indicates that it may have slightly depressed digestion of other components of the diet. Digestibility of this purified cellulose would be expected to be low. Among the other fiber sources, beet pulp had lower ($P<.05$) dry matter and energy digestibilities than rice bran or wheat bran.

Discussion

Elevated calcium levels decreased digestibility of dry matter and energy (Figure 1) partly but not exclusively by increasing excretion of fat and soap in feces. This matches the general concept that supplementing diets containing moderately high amounts of fat with calcium will reduce energy digestibility and increase fat excretion (7, 9, 11, 18). Previously, calcium levels tested have generally not exceeded 1% of the diet. In this study fecal output of both fecal soap and total fecal fat were increased linearly by calcium addition. As the calcium level in the tallow diet increased, dry matter and energy digestibilities of the diet decreased linearly. The caloric value, as estimated from digestible energy of the tallow diet decreased by 1.97% for each 1% increase in the calcium content of diet (Figure 1) although quadratic, and cubic statistical associations also were detected ($P<0.03$). The decrease tended to reach a maximum with 2% calcium in the diet (the tallow diet with an added 1.5% Ca). This decrease in metabolizable energy was due partly to formation of insoluble soaps which rendered the fatty acids unavailable for absorption (10, 11, 19, 20). Calcium, at 1.5 or 2% of the diet, reduced the retained energy value of added tallow from 9.12 kcal/g to 6.79 and 5.87 kcal/g. Adding 1.5% Ca to the tallow diet produced the same reduction in digestibility as adding 10% fiber to the diet.

Adding fiber to the diet decreased digestibilities of dry matter and energy reduced ME content of the diet. The ability of fiber to reduce diet digestibility has been documented previously (5, 18, 21) with fiber provided from a variety of different sources. However, various sources of fiber, as used in this study, differed in their effects on digestion and fecal fat excretion. The greatest increase in fecal fat and the greatest decrease in energy and dry matter digestibilities was observed for animals fed rice bran. Rice bran contained a higher calcium level (1.7%) than other fiber sources. Perhaps this additional calcium or a fiber-calcium interaction is responsible for the greater effects of this fiber source. Greater depression in body weight and in blood triglycerides also have been observed in rats fed rice bran (22).

Digestibilities of dry matter and energy from the fiber sources differed, with energy digestibilities generally being lower for fiber sources higher in dietary fiber content. As expected, contributions of these fiber sources to energy digestibility of the diet were less than the physiological fuel value of 4 kcal/g for carbohydrates, and energy values varied (70 to 97% of 4 kcal/g) with the source of fiber.

In conclusion, the true availability of calories from mixed diets can vary with concentrations of certain dietary components. Hence, caloric value estimated from nutrient content alone may prove inaccurate. The amount of fiber and the calcium level in a diet should be considered because these factors influence digestibility and, consequently, the availability of the energy from the diet.

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Table 1
Description of Diets

	DIET COMPOSITION
Basal	AIN purified diet (13)
Tallow	Basal diet + 4.4 kcal/day from added tallow
1% Ca Tallow	Tallow diet with 0.4% Ca added from $CaCO_3$ (39 mg $CaCO_3$ /day)
1.5% Ca Tallow	Tallow diet with 0.9% added calcium from $CaCO_3$ (88 mg $CaCO_3$ /day)
2% Ca Tallow	Tallow diet with 1.4% added Ca from $CaCO_3$ (144 mg $CaCO_3$ /day)
2.5% Ca Tallow	Tallow diet with 1.9% added Ca from $CaCO_3$ (201mg $CaCO_3$ /day)
Cellufil	Tallow diet with 0.39 g dietary fiber/day from cellufil (0.39 g cellufil/day)
Rice bran	Tallow diet with 0.34 g added dietary fiber/day from rice bran (1.30 g rice bran/day)
Wheat bran	Tallow diet with 0.39 g dietary fiber/day from wheat bran (0.90 g wheat bran /day)
Beet pulp	Tallow diet with 0.38g added dietary fiber/day from beet pulp (0.46 g beet pulp/day)

Table 2
Composition of Diets (%)

	Basal	**Tallow**	**1% Ca Tallow**	**1.5%Ca Tallow**	**2% Ca Tallow**	**2.5%Ca Tallow**	**Cellufil**	**Rice Bran**	**Wheat Bran**	**Beet Pulp**
$CaCO_3$	0	0	1.01	2.31	3.60	4.89	0	0	0	0
Cellufil	6.87	5.89	5.83	5.76	5.68	5.60	15.30	5.63	5.27	5.25
Fiber source	0	0	0	0	0	0	10.00	37.65	22.54	12.06
Sucrose	51.89	44.48	44.03	43.46	42.88	42.31	40.04	9.33	27.84	38.52
Casein	27.49	23.57	23.33	23.02	22.72	22.42	21.21	22.51	21.06	20.98
Tallow	0	14.27	14.13	13.94	13.76	13.57	12.84	13.63	12.76	12.71
Corn Oil	6.87	5.89	5.83	5.76	5.68	5.60	5.30	5.63	5.27	5.25
Mineral Mix	4.81	4.12	4.08	4.03	3.98	3.92	3.71	3.94	3.69	3.67
Vitamin Mix	1.37	1.18	1.17	1.15	1.14	1.12	1.06	1.13	1.05	1.05
Methionine	0.41	0.35	0.35	0.35	0.34	0.34	0.32	0.34	0.32	0.31
Choline Cl	0.27	0.24	0.23	0.23	0.23	0.22	0.21	0.23	0.21	0.21
Ca content, %	0.70	0.60	1.00	1.50	2.00	2.50	0.54	1.70	0.54	0.54

Table 3
Calcium Supplementation Effects on Digestibility

	DIET								Ca Effects		
Diet **Ca added**	**Basal**	**Tallow**	**Tallow 1%**	**Tallow 1.5%**	**Tallow 2 %**	**Tallow 2.5%**	**SE***	**Tallow effect**	**Lin. (P<)**	**Quad (P<)**	**Cubic (P<)**
DM# Intake, g/day	3.48^{d}	3.88^{bc}	3.91^{bc}	3.80^{c}	4.01^{ab}	4.12^{a}	0.04	0.01	0.01	0.01	0.80
Gain, g/day	0.34	0.45	0.49	0.41	0.38	0.46	0.05	0.10	0.71	0.52	0.20
Gain/Feed	0.096	0.114	0.124	0.107	0.096	0.113	0.02	0.34	0.49	0.69	0.21
Body composition, %:											
Dry Matter	39.1	39.9	42.4	39.6	40.7	43.0	1.52	0.25	0.35	0.54	0.18
Protein, % of DM*	47.0^{a}	44.2^{ab}	38.6^{b}	44.0^{ab}	42.2^{ab}	40.2^{b}	1.84	0.02	0.47	0.98	0.06
Fat, % of DM	38.6^{a}	44.0^{ab}	48.9^{a}	42.8^{ab}	45.0^{ab}	48.9^{a}	2.50	0.02	0.45	0.51	0.12
Fecal output											
DM, g/28 days	10.9^{e}	10.8^{e}	13.2^{d}	15.3^{c}	17.8^{b}	19.4^{a}	0.50	0.01	0.01	0.52	0.71
Fat, g/28 days	0.46^{c}	0.93^{ab}	0.70^{bc}	0.87^{ab}	0.96^{ab}	1.08^{a}	0.09	0.01	0.10	0.10	0.27
Soap, g/28 days	0.09^{c}	0.37^{b}	0.37^{b}	0.45^{ab}	0.43^{ab}	0.48^{a}	0.03	0.01	0.01	0.96	0.88
Digestibility, %:											
Total diet:											
Dry Matter	88.9^{ab}	90.1^{a}	87.9^{b}	85.6^{c}	84.1^{d}	83.1^{d}	0.42	0.01	0.01	0.06	0.61
Energy	91.6^{a}	90.9^{ab}	89.8^{b}	88.4^{cd}	87.5^{d}	87.3^{d}	0.48	0.01	0.01	0.19	0.56
Fat	95.2	96.2	97.1	96.4	96.1	96.0	0.64	0.11	0.51	0.54	0.46
Added:											
Dry Matter	-	97.3^{a}	82.6^{b}	68.9^{c}	61.5^{cd}	57.9^{d}	2.64	-	0.01	0.01	0.74
Energy	-	79.3^{a}	73.9^{ab}	66.4^{bc}	63.5^{c}	64.7^{bc}	3.20	-	0.01	0.15	0.54
Fat		86.1	92.8	88.7	88.0	87.2	10.3	-	0.75	0.23	0.19
DE**, kcal/g	-										
Diet	4.13^{e}	4.84^{a}	4.59^{b}	4.57^{b}	4.43^{c}	4.33^{d}	0.03	0.01	0.01	0.04	0.02
Added Fat	-	9.12^{a}	7.16^{b}	6.79^{b}	5.87^{c}	5.23^{d}	0.17	-	0.01	0.01	0.03
Retained, kcal/d	1.15^{b}	1.61^{ab}	1.97^{b}	1.46^{ab}	1.74^{a}	1.99^{a}	0.18	0.01	0.38	0.45	0.17

* Pooled standard error. # Dry matter. ** Digestible energy. ^{a,b,c,d} Means in rows with different superscripts differ ($P < 0.05$)

Table 4
Fiber Supplementation Effects on Digestibility

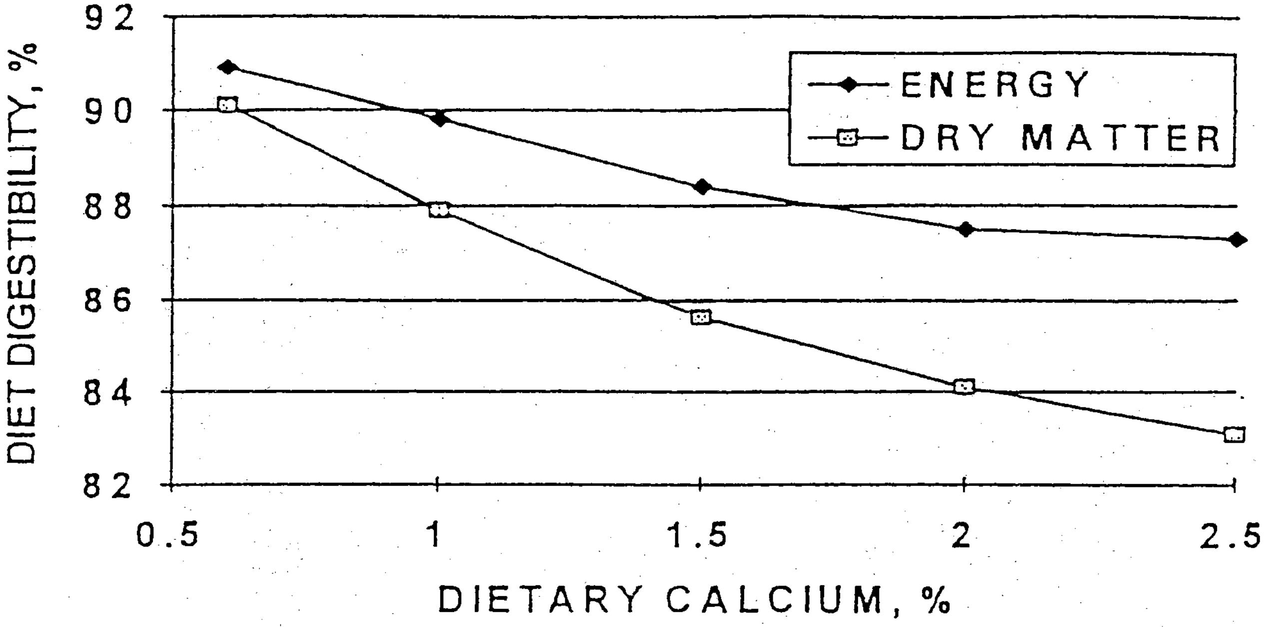
	DIET							Effects	
Diet	**Basal**	**Tallow**	**Cellufil**	**Rice Bran**	**Wheat Bran**	**Beet Pulp**	**SE**	**Tallow (P<)**	**Fiber (P<)**
Dry Matter, g/day	3.48^{b}	3.88^{a}	4.05^{a}	3.50^{b}	4.09^{a}	4.06^{a}	0.08	0.01	0.62
Average Gain, g/day	0.34	0.45	0.43	0.37	0.48	0.38	0.06	0.21	0.64
Gain/Feed	0.096	.114	.107	.104	.117	.093	0.02	0.52	0.59
Body composition, %:									
Dry Matter	39.1	39.9	41.7	39.7	42.3	42.2	2.23	0.42	0.52
Protein	47.0	44.2	41.4	44.2	38.3	45.9	2.91	0.21	0.59
Fat	38.6	44.0	48.3	42.8	48.8	42.3	2.32	0.13	0.72
Fecal Output									
DM, g/28 days	10.9^{d}	10.8^{d}	21.3^{b}	25.7^{a}	20.5^{b}	18.0^{c}	0.65	0.01	0.01
Fecal fat, g/28 days	0.46^{c}	0.93^{b}	1.12^{b}	1.64^{a}	1.26^{b}	1.18^{b}	0.10	0.01	0.01
Fecal soaps, g/28 days	0.09^{d}	0.37^{c}	0.58^{a}	0.42^{bc}	0.38^{c}	0.50^{ab}	0.04	0.01	0.06
Diet digestibility, %									
Dry Matter	88.9^{a}	90.1^{a}	81.2^{c}	73.6^{d}	82.1^{c}	84.2^{b}	0.61	0.01	0.01
Energy	91.6^{a}	90.9^{a}	85.1^{c}	80.6^{d}	86.3^{bc}	87.4^{b}	0.56	0.01	0.01
Fat	95.2^{a}	96.2^{a}	95.1^{a}	90.5^{b}	94.8^{a}	94.7^{a}	0.65	0.21	0.01
DE of Diet, kcal/g	4.13^{e}	4.84^{a}	4.38^{c}	4.28^{d}	4.50^{b}	4.45^{bc}	0.03	0.01	0.01
Digestibility of added tallow or tallow plus fiber*									
Dry Matter	-	97.3^{a}	55.3^{c}	66.4^{b}	73.5^{b}	70.4^{b}	2.38	-	0.01
Energy	-	79.2^{a}	59.6^{c}	73.6^{a}	77.1^{a}	72.2^{a}	2.73	-	0.01
Fat	-	86.1	86.5	85.8	90.2	86.1	2.23	-	0.69
Added DE, kcal/g	-	9.12^{a}	5.21^{b}	4.42^{c}	5.17^{b}	5.40^{b}	0.14	-	0.01
Digestibility of added fiber sources#									
Dry Matter	-	-	-14.5^{c}	54.4^{a}	55.4^{a}	17.9^{b}	4.69	-	0.01
Energy	-	-	25.1^{c}	71.1^{a}	73.6^{a}	51.7^{b}	4.68	-	0.01
Fat	-	-	87.6^{ab}	86.9^{ab}	92.3^{a}	83.5^{b}	2.61	-	0.17
DE, kcal/g	-	-	2.80^{c}	3.74^{a}	3.87^{a}	3.24^{b}	0.09	-	0.01

* Calculated by subtracting contributions of the basal diet from test diets.

\# Calculated by subtracting contributions of the tallow diet from test diets. ^{a,b,c,d} Means in rows with different superscripts differ ($P < 0.05$).

Figure Legend

Figure 1. Impact of calcium concentration on dry matter and energy digestibility of the diet.


92
90
88
86
84
82
DIET DIGESTIBILITY, %
ENERGY
DRY MATTER
0.5
1
1.5
2
2.5
DIETARY CALCIUM, %

CHAPTER V

ADDING CALCIUM REDUCES THE CALORIC VALUE OF PIG DIETS SUPPLEMENTED WITH CORN OIL OR TALLOW

Dania A. Khalil, Christa F. Hanson, and Frederick N. Owens

Abstract

Isocaloric amounts of either sucrose, corn oil or tallow were added to a basal pig feed mixture. The high fat diets were formulated so that diets contained 0.92 or 1.80% calcium. Each of the six diets was limit fed to five individually penned weanling pigs for three weeks starting when pigs were four weeks old and weighed 7.5 kg. Chromic oxide was included as an indigestibility marker. Compared with the sucrose diet, diets with added fat increased the gain to feed ratio ($P<0.01$), gain to consumed metabolizable energy ($P<0.01$), soap content of feces ($P<0.04$), and plasma HDL cholesterol levels ($P<0.1$) without affecting total plasma cholesterol or the HDL to total cholesterol ratio. Compared with corn oil diets, tallow diets caused an increase in fecal energy; however, net energy of tallow was higher than that of corn oil ($P<0.02$). Added calcium depressed the digestibility of dry matter and energy ($P<0.01$). Added dry matter and energy were less digestible with added calcium ($P<0.01$). Average daily gain of pigs was increased more by the addition of calcium with the corn oil than with the tallow (interaction, $P<0.02$). Results indicate that the energy contribution of fat for young pigs varies with fat source and dietary calcium level; these should be considered in feed formulations.

Introduction

Most diet formulations rely on the traditional assumption that fat provides 9 kcal/g (1). This value was obtained assuming that the digestibility of fat was 95%. However, fat digestibility and absorption is not a constant, but rather varies with intrinsic properties of individual fats as well as with other dietary components. One such intrinsic property is the level of saturation in the fat. Saturated fatty acids are less well absorbed than unsaturated fatty acids (2, 3). Another dietary component that may affect fat absorption is the calcium level of the diet. Diets high in calcium bind fatty acids in the gastrointestinal tract to form insoluble soaps, fatty acid - calcium complexes, which render the fatty acids unavailable for absorption (2, 4-7). This experiment was designed to examine the impact of added calcium on fat digestion and absorption by weanling pigs. The objectives were to: 1. compare the caloric value of two sources of fat, animal (tallow) and plant (corn oil), to that of carbohydrate (sucrose); and 2. study the effects of two levels of calcium on fat and energy digestibility of high fat diets for young pigs.

Methods

Animals and Diets

The study involved 32 Yorkshire weanling pigs (weaned at three weeks of age) weighing 7.5 kg initially. Piglets were assigned to six treatments to produce equal weight groups, of five animals each. Two animals were chosen for initial body composition analyses. The six diets were assigned randomly to the six groups and were limit fed in individual pens for four weeks starting when pigs were four weeks old. The compositions of the six diets are shown in Table 1. For the first week all animals were fed 415g/day of a basal diet mixture followed by either the basal mixture alone (basal treatment) or supplemented with isocaloric (metabolizable energy basis) amounts of either sucrose (an additional 110g/day), corn oil (an additional 50g/day), or tallow (an additional 50 g/day) for the remaining three week trial period. The fat supplemented diets contained either 0.92% calcium or were supplemented with $CaCO_3$ (an additional 10g/day; for a 1.8% calcium level). The basal diet contained ground corn grain, soybean meal, dehydrated whey, dried skim milk, menhaden fish meal, dicalcium phosphate, vitamin mineral premix, mecadox, ethoxyquin, copper sulfate, and flavor to meet nutrient needs. Chromic oxide was included in all diets starting day 8 through the end of the trial as a digestibility marker.

All animals were individually caged in iron steel pens with *ad libitum* access to water in a climate controlled room with a 12 hour dark: light cycle. Animals were fed twice daily at 800 and 1600. The protocol and methodology of the study was approved by the Laboratory Animal Use Committee at Oklahoma State University. Statistical analysis of the data used the General Linear Models procedure of SAS (8) with treatment means contrasted using the Duncan's Multiple Range Test. Orthogonal contrasts were employed to examine differences obtained when feeding 1) basal versus energy supplemented diets; 2) sucrose versus fat supplemented diets; 3) corn oil versus tallow supplemented diets; 4) low versus high calcium diets; and 5) the fat level by calcium level interaction.

Measurements

Animals were weighed initially and weekly. Feed refusals were collected weekly for each animal. They were weighed and samples were preserved until the end of the experimental period to calculate actual feed dry matter intake. Diets were oven dried and analyzed for gross energy by oxygen bomb calorimetry (Parr, 1261 Calorimeter Parr Inst. Co. Moline, IL), nitrogen by the AOAC Kjeldahl method (9) utilizing the Tecator Kjeltech instruments for digestion and distillation, and lipid by ether extraction (9). Diet samples also were analyzed for chromic oxide content spectrophotometrically (Gilford Response Series, UV Visible Spectrophotometer, Ciba Corning Diagnostics Corp., Medfield, MS) (10). Laboratory analyses of the diets is presented in Table 1.

Initial body composition was obtained by sacrificing two animals at the initiation of the trial. Final body composition was obtained by sacrificing two pigs from each of the six treatments on day 21 of the experimental period. Each carcass was skinned and de-boned; the head and hooves were removed. The gastrointestinal tract was removed, washed, and placed back with the remaining soft tissues. All parts of each carcass were weighed separately upon removal. Soft tissues, which included all organs and all separable lean and fat tissue, were removed from bone and ground in a meat grinder to achieve homogeneity of sampling. Soft tissue samples were utilized for estimates of nutrient and energy retention. The skin, bones, head, and hooves were not included in any laboratory analysis. Dry weight of soft tissues was obtained by lyophilizing subsamples of the individually ground carcasses for eight days. Tissue fat, nitrogen, and energy content were determined as previously described for the diets. Soft tissue samples also were ashed in an ashing oven at 600°C for eight hours.

Feces were collected throughout the final nine days of the trial during three periods of three days each. Subsamples of the feces were dried in a 100°C oven for 48 hours. Gross energy of feces was determined by oxygen bomb calorimetry. Fecal fat was obtained by first extracting the samples in petroleum ether to determine ether soluble fat content and then further extracted in a 40:10:1 isopropanol: heptane: 1N sulfuric acid solution to release soap bound fat (2, 11). Chromic oxide content of the feces was determined as with the diets as described above.

Blood samples from each pig were obtained on the last day of the trial. Plasma total and HDL cholesterol were manually determined using Sigma Diagnostics ^{R} enzymatic procedures (12).

Results

Treatment means and effects are presented in Table2.

Energy Supplementation

Pigs fed the energy-supplemented diets consumed more feed than those fed the basal diet, with those fed the sucrose diet consuming the most feed per day ($P<0.01$). These differences reflect the design of the experiment with diets being limit fed. Average daily gain also was lowest in the basal diet treatment ($P<0.05$). However, gain per unit of feed was similar between the basal diet and other diets. Animals fed the sucrose diet consumed the most feed per day, but gained the least weight per unit of consumed feed (0.71 vs. 0.74 and 0.90 for corn oil and tallow, respectively) ($P<0.01$) and per unit of metabolizable energy consumed relative to all fat supplemented diets ($P<0.01$). Supplementing the basal diet with either sucrose, corn oil, or tallow resulted in an increase in soft tissue retention of energy, protein, fat, and ash ($P<0.02$). The tallow-fed animals retained more ash in their soft tissues than did the corn oil fed groups (86 vs. 73g) ($P<0.05$). Animals fed the diets supplemented with energy also had higher weights ($P<0.02$) of total tissues, soft tissues, and skin with no treatment effects on weights of total bones or the head and hooves. The skin of the pigs fed the high fat diet weighed more than the skin of pigs fed the high sucrose diet ($P<0.05$); however, no analyses were done on the skin to determine if the diets resulted in differences in skin composition or thickness. Soft tissue analysis revealed that energy supplemented diets resulted in an increase ($P<0.03$) in tissue energy, and percent dry matter, but a decrease ($P<0.01$) in percent ash. The sucrose supplemented diet resulted in a lower ($P<0.02$) dry matter content of the soft tissues than did the fat supplemented diet (24.3 vs. 27.2% for the mean of the low calcium fat supplemented treatments). This can be ascribed to greater fat in dry matter of energy-supplemented pigs. The high sucrose diet, however, provided a greater ($P<0.03$) percent ash in soft tissues than did the fat supplemented diets. The ash content of soft tissues also was different ($P<0.02$) among the different fats, lower for corn oil than for tallow added diets (3.68 vs. 4.06%). Fat or protein content of soft tissues was similar for all treatments. The tissue analyses were performed on two animals/treatment only; therefore, further research involving a larger number of animals is needed to investigate to obtain more powerful statistical comparisons between treatments.

Supplementing the basal diet with energy resulted in more (P<0.01) energy being excreted in feces, with the high tallow diet resulting in more (P<0.01) excretion of energy than the high corn oil diet (4.82 vs. 4.55 kcal/g). Fecal soap content was higher (P<0.04) with the fat supplemented diets than for the sucrose supplemented diet although, ether extractable fat and total fecal fat were similar for all treatments. The basal and the sucrose diets had much lower (P<0.01) fat digestibilities than did the high fat diets. This can be attributed to secretion of metabolic fecal fat. Plasma HDL cholesterol was lowest in animals fed the basal diet (P<0.01). Animals fed the fat supplemented diets had higher HDL cholesterol levels than those fed the sucrose supplemented diet (P<0.08); but neither total plasma cholesterol nor the ratio of HDL cholesterol to total cholesterol were significantly different between treatments. Dry matter and energy digestibilities were similar for the basal and the energy supplemented diets.

The digestible energy of the added nutrients was 3.3, 10.4, and 12.6 kcal/g, for the added sucrose, added corn oil, and added tallow, respectively. By comparison, the gross energy of the added nutrients was 4.2, 9.4, and 9.4 kcal/g; this means that apparent energy digestibilities were 79, 111, and 134% for sucrose, corn oil, and tallow, respectively. Differences from the expected digestibilities of 96% can be attributed to associative effects of these nutrients with other components of the diet. The net energy value of the added sources, based on added energy retention were 1.51, 4.12, and 5.59 kcal/g, for the added sucrose, added corn oil and added tallow respectively (Figure 1). The net energy contribution of the added corn oil was lower (P<0.02) than that of the added tallow.

Calcium Supplementation

Supplementing the high fat diets with calcium had different effects depending on the source of fat added to the diet. Supplemental calcium increased the gain per feed ratio for animals fed the corn oil diet from 0.74 to 0.87, but decreased it for those fed the tallow diet from 0.90 to 0.84. Calcium also increased total wet tissue weight of animals fed corn oil from 12.2 to 14.2 kg, but did not change that for the tallow fed animals (13.4 vs. 13.2 kg, for the low and high calcium level, respectively). The weight of head and hooves was increased from 1.93 to 2.18 kg in the corn oil fed pigs, but was decreased from 2.15 to 1.97 kg in the tallow fed pigs with calcium supplementation.

Calcium supplementation had affected diet and added nutrient digestibility similarly for both corn oil and tallow supplemented treatments, based on fecal analyses. Feces weight increased ($P<0.01$) with the higher level of calcium from 43.5 to 79.1 g/day, and from 53.6 to 72.4 g/day for the high corn oil and high tallow diets, respectively. Fecal energy concentration decreased ($P<0.05$) with added calcium from 4.55 to 4.29 kcal/g, and from 4.82 to 4.66 kcal/g for the corn oil and tallow containing diets, respectively. Fecal soaps concentration was increased ($P<0.02$) from 1.67 to 2.51g/day for the corn oil diets, and from 2.16 to 2.58g/day for the tallow diets, when calcium content of the diet was increased. Diet dry matter and energy digestibilities were reduced ($P<0.01$) with supplemental calcium added to the high fat diets (Figure 2), while diet fat digestibility was not affected by calcium level. Calcium had a similar effect on the digestibility of added nutrients; it decreased ($P<0.01$) the digestibility of added dry matter (from 122.4 to 66.9% for corn oil, and from 103.1 to 75.1% for tallow) and added energy (from 119.7 to

68.8% for corn oil, and from 94.6 to 67.6% for tallow); while it had no effect on digestibility of added fat. Calcium drastically reduced ($P<0.01$) the metabolizable energy value of both added fats from 10.4 and 12.6 to 5.4 and 5.5 kcal/g for corn oil and tallow, respectively (Figure 1). However, increasing the calcium in the diet increased the net energy of added corn oil from 4.12 to 5.02 kcal/g, and that of added tallow from 5.59 to 7.32 kcal/g; this increase was not statistically significant ($P<0.06$).

Discussion

Energy supplementation of a basal diet with sucrose or fat resulted in increased feed intake, weight gain, tissue deposition, tissue weights, HDL cholesterol, fat digestibility, and increased fecal energy. However, isocaloric amounts of fat and sucrose produced different outcomes. The fat supplemented treatments resulted in higher gain to feed values, gain to energy values, greater skin weight, more dry matter and ash in soft tissues, higher HDL cholesterol levels, more soap in feces, and higher apparent fat digestibility than the sucrose supplemented diet. Consequently, the digestible energy of the added fat was 3.15 times that of sucrose for added corn oil and 3.82 times that of sucrose for added. These digestible energy values are higher than the traditionally accepted metabolizable energy ratios of fat being 2.25 times that of sucrose (1). The fact that added energy from fat has an effect on calories beyond that provided by fat itself has been observed by others (2, 13-16). Reid (17) and Dale and Fuller (18) explained this phenomenon by the ability of added fat in to slow the rate of passage of other feed components thus, improving their digestibility (19). The increase in plasma HDL cholesterol levels observed with energy supplementation particularly from fat agrees with observations in humans in which subjects consuming low fat diets had low plasma HDL cholesterol levels (20,21).

Supplementing high fat diets with calcium produced results in concert with others (2, 5, 7, 22). Doubling the calcium level in the diet increased daily fecal excretion, the amount of soaps excreted per day, and reduced dry matter and energy digestibilities of the diet as well as the added fat from both corn oil and tallow. Calcium binds to the fatty acids in the small intestine forming insoluble soaps rendering the fatty acids unavailable for absorption and utilization (6-7, 23,24). The metabolizable energy of the fats were reduced by almost half by the added calcium, from 10.4 to 5.4 kcal/g for corn oil and from 12.6 to 5.5 kcal/g for tallow. The two fat sources also differed in the amount of energy excreted in feces. Regardless of the calcium level, the diets containing tallow resulted in more energy excretion than did the corn oil treatments, also in agreement with results of others (2, 25, 26). Ockner *et al.* (27) and Denke (7) have reported that saturated fatty acids, such as the palmitate and stearate that are predominant in tallow, are less well absorbed than unsaturated fatty acids because they reside for a longer time in the gut and are thus more vulnerable to disruptions, such as those brought upon by the presence of divalent cations like calcium.

The retained or net energy of added sucrose was equal to approximately 38% of gross energy whereas net energy from added corn oil and added tallow were 54 and 68%, respectively, averaged across the two levels of calcium. That these vary can be attributed to differences in both digestibility and utilization of absorbed energy. Fat has a lower heat increment than carbohydrate, and the processes of fat absorption, transport, and deposition are more energetically favorable from fat than from carbohydrate for fat synthesis (17, 18). The fact that net energy as a fraction of digestible energy was greater for fat than for sucrose can be attributed to differences in heat increment; these values were 41% of DE for sucrose, 52% of DE for corn oil, and 93% of DE for tallow, averaged across both levels of calcium inclusion.

In conclusion, evaluating the caloric content of mixed diets based only on macro nutrient content may not provide accurate energy values for growing animals. The level and source of fat, and the calcium level of the diet must also be evaluated, as these factors influence digestibility and consequently the availability of the energy in the diet.

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Table 1. Percentage Composition of Experimental Diets.

	DIET					
	Basal	Sucrose Low Ca	Corn Oil Low Ca	Tallow Low Ca	Corn Oil High Ca	Tallow High Ca
	%					
Ground Corn Grain	31.70	25.04	28.29	28.29	27.67	27.67
Soybean meal, 44% CP	25.00	19.75	22.31	22.31	21.82	21.82
Dehydrated Whey	20.00	15.80	17.85	17.85	17.46	17.46
Dried Skim Milk	15.00	11.85	13.39	13.39	13.09	13.09
Mernhaden Fish Meal	6.00	4.74	5.35	5.35	5.24	5.24
Dicalcium Phosphate	1.10	0.87	0.98	0.98	0.96	0.96
Vitamin-Mineral Premix.	0.74	0.58	0.66	0.66	0.64	0.64
Mecadox	0.25	0.19	0.22	0.22	0.22	0.22
Ethoxyquin	0.25	0.19	0.22	0.22	0.22	0.22
Copper Sulfate	0.10	0.08	0.09	0.09	0.09	0.09
Flavor	0.10	0.08	0.09	0.09	0.09	0.09
Sucrose	-	20.90	-	-	-	-
Corn Oil	-	-	10.75	-	10.53	-
Tallow	-	-	-	10.75	-	10.53
CaCO3	-	-	-	-	2.10	2.10
Analyses of Dry Diets						
Dry matter, %	93.23	93.82	93.77	93.22	93.14	93.86
Protein, %	23.42	21.53	21.06	22.72	22.70	22.35
Energy, kcal/g	4.14	4.05	4.67	4.63	4.58	4.55
Ash	7.94	6.42	7.46	7.46	9.64	9.90

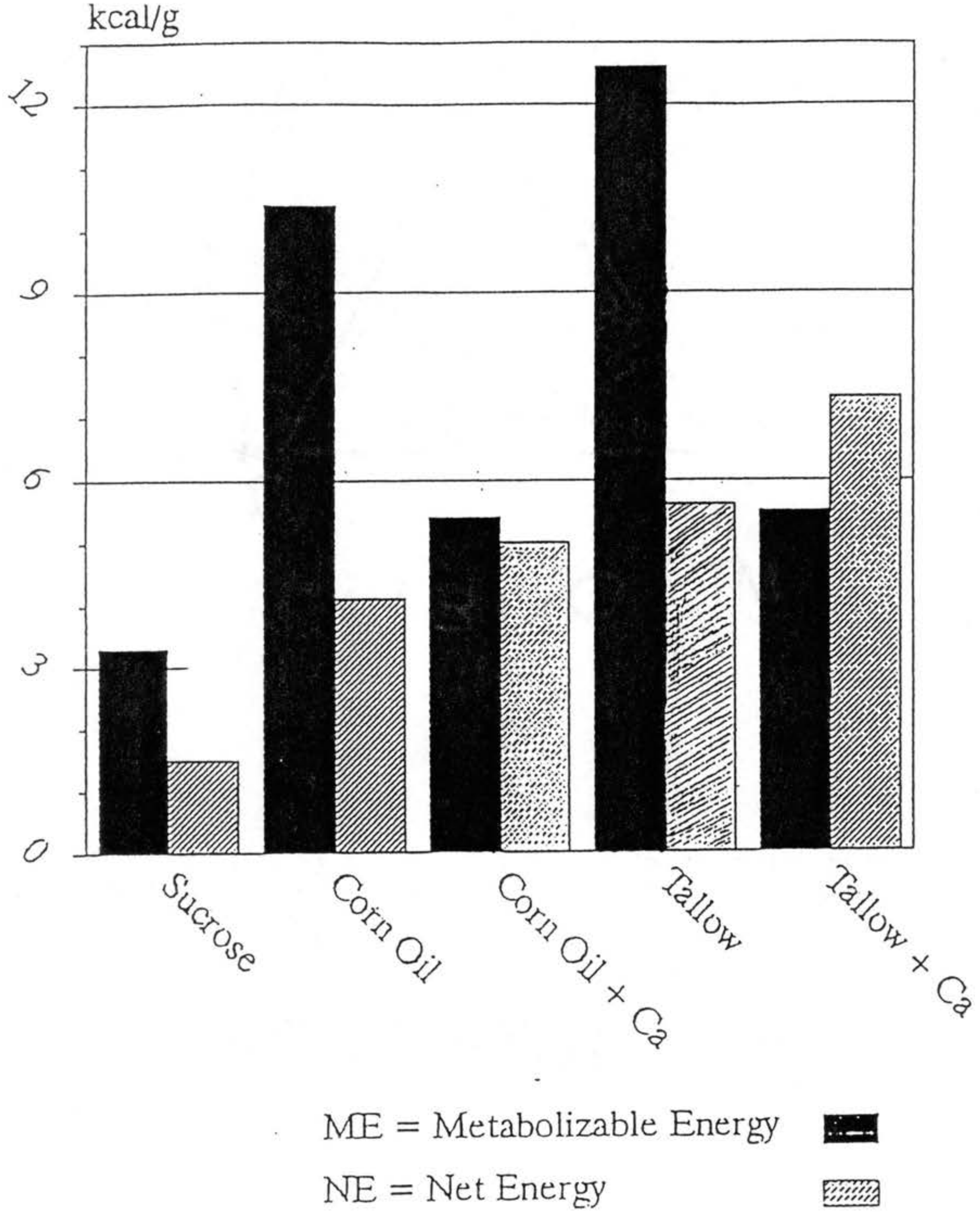
Table 2. Effect of Energy or Calcium Supplementation on Gain and Body Composition of Pigs

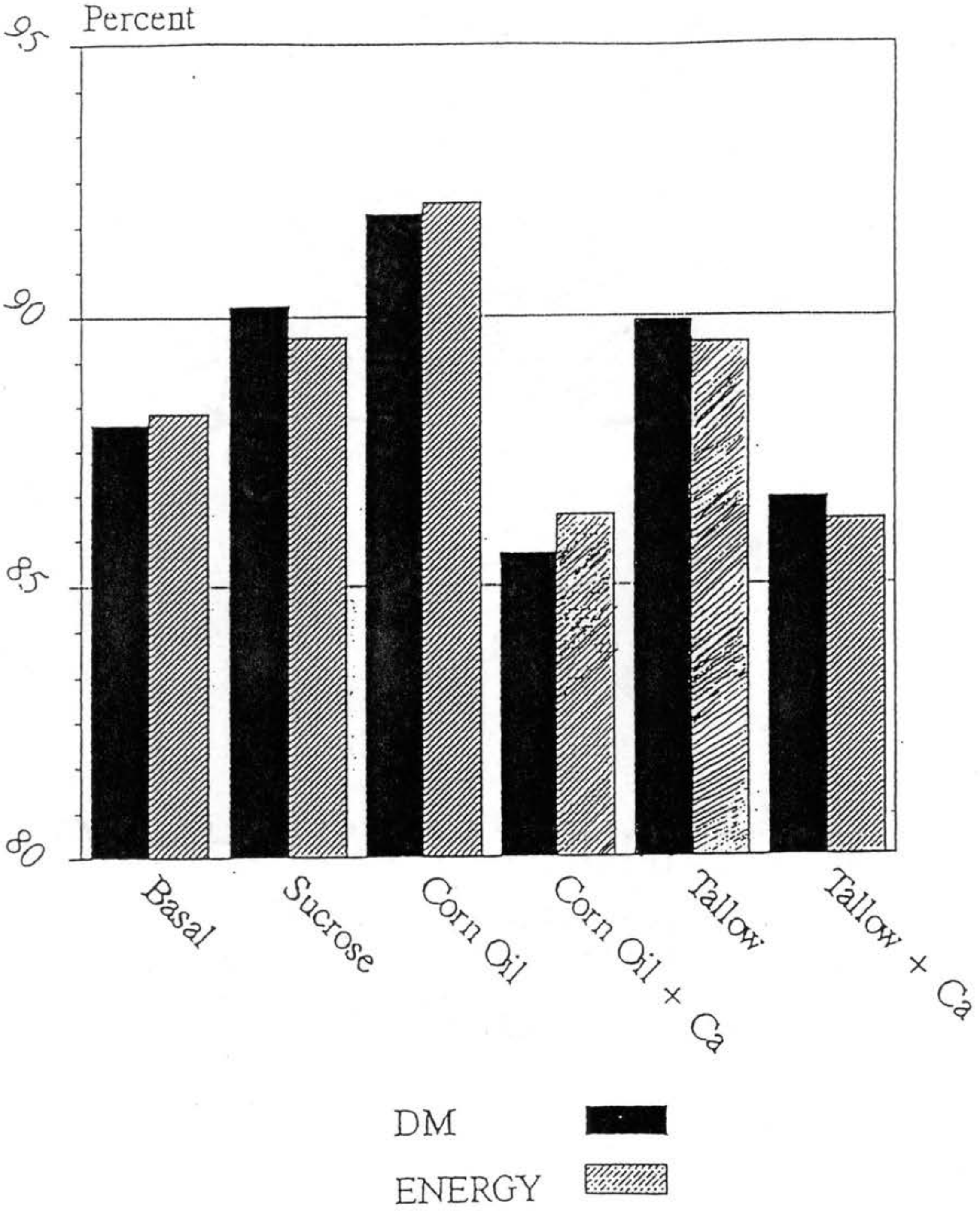
	DIET						CONTRASTS (P<)				
	Basal	**Sucrose**	**Corn Oil Low Ca**	**Corn Oil High Ca**	**Tallow Low Ca**	**Tallow High Ca**	**Basal vs. ALL**	**Sucrose vs. Fat**	**Corn Oil vs Tallow**	**Ca Level**	**Fat By Ca Level**
Live Measurements											
Initial Weight, kg	7.38	7.84	7.42	7.98	7.22	7.18	0.76	0.42	0.28	0.57	0.51
Intake g DM/day	403	498	458	461	434	451	0.01	0.01	0.12	0.35	0.50
Intake kcal GE/day	1668	2017	2139	2111	2009	2052					
Daily Gain, g	319	355	338	400	392	377	0.04	0.40	0.50	0.29	0.10
Gain: Feed	0.79	0.71	0.74	0.87	0.90	0.84	0.66	0.01	0.12	0.46	0.02
Gain: Energy In	0.77	0.65	0.68	0.85	0.83	0.81	0.82	0.01	0.29	0.13	0.06
Soft Tissue Retention											
Energy, Mkcal	8.7	12.1	12.6	14.6	13.6	13.8	0.01	0.11	0.87	0.24	0.32
Protein, g	966	1189	1124	1296	1195	1333	0.02	0.54	0.49	0.09	0.82
Fat, g	364	605	594	731	673	584	0.02	0.62	0.61	0.71	0.15
Ash, g	63.3	81.0	73.0	73.1	85.6	86.1	0.01	0.68	0.03	0.94	0.96
Wet Tissue Weights, kg											
Total	11.2	13.0	12.2	14.2	13.4	13.2	0.01	0.53	0.79	0.04	0.02
Soft Tissues	6.38	7.92	7.20	8.21	7.82	7.80	0.01	0.60	0.71	0.14	0.13
Bone	1.44	1.48	1.55	1.91	1.62	1.52	0.13	0.13	0.15	0.23	0.06
Skin	1.40	1.50	1.46	1.90	1.77	1.85	0.02	0.05	0.22	0.04	0.12
Head/ Hooves	1.92	2.10	1.93	2.18	2.15	1.97	0.12	0.68	0.91	0.67	0.04
Soft Tissue Composition											
Energy, kcal/g	6.09	6.29	6.37	6.41	6.45	6.37	0.03	0.34	0.83	0.85	0.57
Dry Matter, %	22.5	24.3	27.5	27.7	27.0	27.8	0.01	0.02	0.80	0.61	0.75
Protein, % DM	67.1	61.9	56.7	56.9	56.7	61.5	0.06	0.32	0.54	0.50	0.53
Fat, % DM	25.4	29.1	30.0	32.1	31.8	29.4	0.19	0.70	0.90	0.97	0.53
Ash, % DM	4.40	4.21	3.68	3.26	4.06	3.98	0.02	0.03	0.02	0.17	0.33
Feces											
Feces DM, g/day	56.4	58.0	43.5	79.1	53.6	72.4	0.56	0.63	0.83	0.01	0.29
Energy, kcal/g	4.05	4.38	4.55	4.29	4.82	4.66	0.01	0.08	0.01	0.05	0.62
Soap, g/day	1.59	1.57	1.67	2.51	2.16	2.58	0.06	0.04	0.25	0.02	0.37
Ether Extract, g/day	2.90	3.25	4.25	9.30	6.56	5.11	0.23	0.25	0.65	0.40	0.13
Total Fat, g/day	4.50	4.82	5.92	11.80	8.73	7.68	0.18	0.18	0.76	0.28	0.13
Diet Digestibility, %											
Dry Matter	88.0	90.2	91.9	85.6	89.9	86.6	0.55	0.25	0.71	0.01	0.27
Energy	88.2	89.6	92.1	86.3	89.5	86.2	0.72	0.53	0.37	0.01	0.42
Fat	68.4	64.4	91.5	83.7	85.7	88.1	0.01	0.01	0.86	0.48	0.18
Added Source Digestibility											
Dry Matter, %		97.3	122.4	66.9	103.1	75.1		0.68	0.64	0.01	0.26
Energy, %		92.3	119.7	68.8	94.6	67.6		0.75	0.32	0.01	0.37
Fat, %		43.4	271.7	177.2	216.9	212.5		0.01	0.78	0.16	0.20
Energy of Added Source											
ME/ DM, kcal/g		3.3	10.4	5.4	12.6	5.5		0.03	0.56	0.01	0.60
NE/ DM, kcal/g		1.51	4.12	5.02	5.59	7.32		0.01	0.02	0.06	0.45
Plasma Cholesterol											
Total Cholesterol, mg/dl	83.4	87.8	83.3	117.3	115.4	112.6	0.21	0.20	0.35	0.29	0.22
HDL Cholesterol, mg/dl	28.85	37.70	45.71	42.16	45.47	49.22	0.01	0.08	0.48	0.98	0.45
HDL / Total Cholesterol	0.37	0.46	0.56	0.41	0.46	0.49	0.19	0.74	0.85	0.43	0.23

Legends for Figures

Figure 1. Metabolizable energy (solid bars) and net energy (diagonally hatched bars) of added energy source in the energy supplemented diets.

Figure 2. Percent dry matter digestibility (solid bars) and energy digestibility (diagonally hatched bars) of the six diets.


kcal/g
12
9
6
3
0
Sucrose
Corn Oil
Corn Oil + Ca
Tallow
Tallow + Ca
ME = Metabolizable Energy
NE = Net Energy


Percent
95
90
85
80
Basal
Sucrose
Corn Oil
Corn Oil + Ca
Tallow
Tallow + Ca
DM
ENERGY

CHAPTER VI

ASSOCIATION BETWEEN CARDIOVASCULAR RISK FACTORS AND DIETARY FAT, FIBER AND CALCIUM AMONG MEN AND WOMEN

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Abstract

Four day food records, one week food frequency questionnaires, and physical measures of health of young middle-aged men (n=26) and women (n=23) were analyzed to investigate the potential relationships between cardiovascular risk factors and dietary fat, fiber, and calcium. Combined across men and women, subjects consuming diets with more than 30% of calories from fat had lower ($P<0.05$) fiber intakes but higher ($P<0.05$) % body fat, serum total cholesterol, triglycerides, apolipoprotein A1, and apolipoprotein B than subjects consuming less fat. Subjects consuming 1 or more g fiber/100 kcal were more ($P<0.05$) were more likely to have low fat diets and at least 67% of the RDA for vitamin E than subjects consuming lower fiber diets. Subjects consuming at least 800 mg of calcium daily had lower ($P<0.05$) systolic blood pressure than those with lower calcium intake. Regression analyses revealed that subjects with greater dietary intake of fat, saturated fatty acids, and cholesterol had less desirable values for health status parameters, whereas those with a greater intake of calcium had more desirable indices of cardiovascular disease risk factors.

Introduction

Current dietary recommendations emphasize reducing dietary fat, particularly saturated fat, in order to reduce disease risk (1,2). Fat intake has been linked to coronary heart disease, the leading cause of morbidity and mortality in American men and women (3,4). Fat promotes several cardiovascular risk factors such as elevating serum concentrations of total cholesterol, low density lipoprotein (LDL) cholesterol, and triglycerides (5,6). Excess dietary fat is also associated with indices of obesity such as excess body fat (7) and high body mass index (8,9). Obesity and abdominal fat deposition such as that precipitating high waist to hip ratio are considered culprits in increased risk for hypertension and cardiovascular disease (10-12). Dietary components, such as dietary fiber, aimed at reducing the detrimental effects of dietary lipids on health have been studied extensively (13,14). A daily consumption of 20-25 g of dietary fiber is recommended to produce protective health effects (15). Fiber produces some of its beneficial effects by binding to dietary cholesterol, fatty acids, and bile acids preventing their absorption and utilization by the body (16). However, another dietary component which may produce similar effects on absorption is calcium. Calcium binds long chain fatty acids and bile acids in the intestine to produce calcium soaps which, if insoluble, will render the fatty acids and bile acids unavailable for absorption (17-19). If saturated fatty acids are not absorbed, then the body will probably be spared the health risks associated with their consumption. The aim of this study was to examine the diets and parameters of cardiovascular disease risk in middle aged men and women. Increases in cardiovascular risk factors and accumulation of excess weight are observed with advances in age (4). Our objectives were to investigate: 1) the nutrient adequacy of diets containing the recommended amounts of either fat, fiber, or calcium versus the nutrient adequacy of diets either too high in fat, too low in fiber, or too low in calcium, respectively; 2) the impact of diets containing the recommended amounts of either fat, fiber, or calcium versus diets containing inferior intakes of these nutrients on factors implicated in heart disease risk; 3) the correlation between cardiovascular disease risk factors and dietary fat, fiber, and calcium in men and in women.

Methods

Subjects

Subjects included 23 women and 26 men between 32 and 54 years of age recruited from the Stillwater campus of Oklahoma State University through campus mail. Participating subjects were members of the faculty and staff as well as spouses of faculty and staff at the university. In order to qualify, subjects had to be healthy and not on a restricted or special diet or taking any medication for a chronic disorder, such as diabetes mellitus, heart disease, gout, high blood cholesterol, high blood triglycerides, or high blood pressure. Subjects were visited twice. The first visit was to obtain the subjects' consent, to collect anthropometric measurements, and to be instructed on dietary four day record keeping and filling out a one week food frequency questionnaire. The food frequency questionnaire used in this study was adapted and modified from Willett's 1-year food frequency questionnaire (20,21). Subjects also reported frequency and duration of physical activity performed in a typical week period. The second visit, which was at least one week after the initial visit, was to obtain an overnight-fasting blood sample, measure blood pressure, and collect the completed four day food records and one week food frequency questionnaires from each subject. The study was approved by the Institutional Review Board at Oklahoma State University.

Dietary Analysis

Four day food records and one week food frequency questionnaires from each subject were analyzed using ESHA's Food Processor^R for windows^{TM} software (22), averaged and then divided by four or seven, respectively. Subjects were then sorted by level of dietary fat, fiber, or calcium intake. Subjects were classified into intake groups with each subject belonging to three of these groups depending on his or her intake of fat, fiber, and calcium. The six groups, of both men and women, were as follows: "High Fat", if the caloric contribution from fat exceeded 30% of total daily calories; "Low Fat", if the caloric contribution from fat equaled or was below 30% of total daily calories; "High Fiber", if daily dietary fiber equaled or exceeded 1g/100 kcal; "Low Fiber", if daily dietary fiber was below 1g/100 kcal; " High Calcium", if daily dietary calcium equaled or exceeded the 1989 RDA (15) value for adults over 25 years of age set at 800 mg; or "Low Calcium", if daily dietary calcium was below 800 mg. Adequacy of nutrient intake by subjects was determined by comparing each subject's average daily dietary intake, from either the four day record or from the food frequency questionnaire data, to recommended intakes. Protein, vitamin and mineral intake, except for that of calcium, was considered acceptable if average daily intake equaled or exceeded 67% of the 1989 RDA or ESADDI (15). Calcium intake was considered adequate if average daily intake equaled or exceeded 100% of the 1989 RDA (15). Fat intake was considered adequate if the average daily caloric contribution from fat did not exceed 30% of total calories (15). Fiber intake was considered adequate if average daily intake was 1 g or more of dietary fiber/100 kcal.

Measurements

Anthropometric measurements obtained from the subjects included height, weight, waist circumference measured at the narrowest area below the rib cage and above the umbelicus (23), hip circumference measured at the point of greatest circumference around the hips or buttocks with the subject standing (23), and bioelectrical impedance for body composition and % body fat determination using the Biodynamics Model 310 Body Composition Analyzer (24). Body mass index (BMI) was then calculated from height and weight data, and waist to hip ratio (WHR) was calculated from the waist and hip circumference data. The means of these data for our subjects are presented in Table1.

Clinical measurements included systolic and diastolic blood pressure and serum analyses. Serum samples were analysed using Sigma Diagnostics^R reagents and procedures (25) for automated determination employing the Roche ^{R} Cobas Fara automatic analyzer. Serum was analyzed for: total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol (by difference), apolipoprotein A1 (Apo A1), and apolipoprotein B (Apo B). The means of the clinical measurements of the subjects are presented in Table 1.

Statistical Analysis

Anthropometric, dietary, clinical and biochemical data were analyzed using the General Linear Models procedure of the 4th edition of SAS (26). Interactions between gender and fat, fiber, or calcium level of intake as well as interactions between level of fat and level of calcium intake were examined using the Least Squares Means procedure of SAS for the anthropometric, clinical and biochemical data. Stepwise regression analysis was performed on the dependent variables Apo A1, Apo B, total serum cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, diastolic blood pressure, systolic blood pressure, BMI, WHR, and % body fat by gender, fat level, fiber level, and calcium level against nutrient intake as determined by the four day food record method or by the food frequency questionnaire method.

Results

The two different tools, the four day food records and the one week food frequency questionnaire, that were used to assess dietary intake produced dissimilar results when examining nutrient intake or correlation between diet and health indices. However, it was not our aim in this study to compare the two data collection methods or to chose one over the other; others have compared different methods of dietary data collection and their reproducibility (27, 28). Dietary supplements were not included in nutrient analysis.

Nutrient Adequacy

Results of the four day food records analysis for adequacy of nutrient intake, as based on the consumption of at least 67% of the current recommended intakes, are presented in Table 2. Each subject in the study had adequate (acceptable) intakes of protein, vitamin A, vitamin D, vitamin B_{12}, folic acid, niacin, riboflavin, thiamin, and phosphorous. Most subjects (over 88% of individual sets) were adequate in vitamin C, vitamin B_6, iron, magnesium, and selenium.

The only significant difference between men and women was that more women, than men, had acceptable vitamin E intakes. (91 vs. 65%; $P<0.05$). A higher proportion of subjects consuming low fat diets had acceptable fiber ($\geq$ 1 g/100 kcal) intakes (62 vs. 26%). Half the subjects ate adequate amounts of fiber, although, all subjects in the high fiber group had adequate intakes of iron compared to only 73% of subjects consuming less fiber ($P<0.05$). Similarly, those consuming higher fiber diets had more diets with acceptable levels of vitamin E (91vs. 67% being adequate) and fat (73 vs. 37% consuming $\leq$ 30% of calories from fat). Individuals consuming more calcium had more diets with acceptable amounts of vitamin C (100 vs. 83%), vitamin B_6 (97 vs. 78%), pantothenate (45 vs. only 6%), magnesium (90 vs. 67%), and iodine (45 vs. 0%) ($P<0.05$). However, numbers reflecting the intake of pantothenic acid could be misleading since the tool used to analyze the diets lacked the pantothenic acid content of many foods; also, the intake of iodized salt was not quantified in this study.

Results of the one week food frequency questionnaire analysis for accepted nutrient intake are presented in Table 3. Generally, intakes judged by the one week food frequency questionnaire were similar to those judged from the four day food records even though fewer significant differences among groups were detected. The major exceptions were that based on the one week questionnaire, those consuming higher fiber diets more, rather than less, than 30% of calories from fat; they also tended to have iron intakes as adequate as those consuming low fiber diets. No specific reasons for these discrepancies are obvious.

Anthropometric, Clinical and Biochemical Assessment

Table 1 lists means for the obtained anthropometric, clinical, and biochemical measurements for all subjects and for subjects stratified by gender. Women were shorter, weighed less, had a smaller waist to hip ratio, had more body fat, lower diastolic blood pressure, higher HDL cholesterol, lower serum triglycerides, more apolipoprotein A1, and less apolipoprotein B than men ($P<0.05$). Eight men and ten women had serum total and LDL cholesterol above the currently desirable values of 200 and 130 mg/dl, respectively. Nonetheless, none of the listed means for either men or women were outside acceptable values for these health indices.

Tables 4 and 5 list the means of these health indices for subjects as stratified by fat, fiber, or calcium intake based on four day food records and one week food frequency questionnaires, respectively. Based on four day food records (Table 4) subjects who ate higher fat diets had more body fat, higher total serum cholesterol, apolipoprotein A1, and apolipoprotein B levels than those consuming diets with fat supplying 30% or less of daily calories (29.0 vs. 27.2%, 222 vs. 191 mg/dl, 154.3 vs. 138.7 mg/dl, and 75.0 vs. 64.2 mg/dl, respectively) ($P<0.05$). However, differences between gender and fat level were detected for apolipoprotein A, apolipoprotein B, systolic pressure, diastolic pressure, HDL cholesterol, and LDL cholesterol (Table 6). This difference reflected the fact that females consuming high fat diets had higher apolipoprotein A1 ($P<0.02$), systolic blood pressure ($P<0.01$), diastolic blood pressure ($P<0.1$), and HDL cholesterol ($P<0.1$) than females consuming low fat diets (172.6 vs.144.2 mg/dl, 139 vs.118 mm Hg, 81 vs.73 mm Hg, and 61 vs. 48 mg/dl, respectively); while for men, higher fat diets did not significantly impact these four risk factors. However, men consuming high fat diets had higher apolipoprotein B ($P<0.05$) and higher LDL cholesterol levels ($P<0.1$) than men consuming low fat diets (93.6 vs.72.2 mg/dl and 171 vs.135 mg/dl, respectively) while higher fat intakes by women did not significantly alter these two factors.

Based on the food frequency questionnaires (Table 5), trends were similar to those of food records. Subjects in the high fat group had more body fat and higher serum triglycerides and apolipoprotein B levels than subjects in the low fat group (31.8 vs. 27.3%, 127 vs. 91 and 85.1 vs. 67.9 mg/dl, respectively) ($P<0.05$) (Table 5). In this case, however, serum triglycerides were significantly higher for individuals consuming the higher fat diets. Interactions by gender were detected for the variables % body fat and serum apolipoprotein B (Table 7). In this case, only men but not women with higher fat intakes had more body fat ($P<0.1$) and higher serum triglycerides ($P<0.05$) than their counterparts with low fat diets (29.7 vs. 24.7% and 96.0 vs. 70.8 mg/dl, respectively).

Subjects with different intakes of dietary fiber did not differ significantly in any of these measured variables nor were fiber intake by gender interactions detected.

Averaged across gender, subjects consuming 800 mg or more of calcium daily had lower ($P<0.05$) systolic blood pressure than subjects consuming less daily calcium (122 vs. 131 mm Hg, respectively) (Table 5). However, again an interaction by gender was detected suggesting that calcium level had an effect only on male subjects. Males with a high level of calcium intake had lower systolic blood pressure than males consuming less than the 1989 RDA for calcium (124 vs. 135 mm Hg) ($P<0.05$). A calcium level by gender interaction also was detected for diastolic pressure ($P<0.1$), only the male subjects consuming adequate calcium had lower levels (83 vs. 90 mm Hg).

Two and three way interactions were examined and were detected between calcium and fat for the variables apolipoprotein B ($P<0.01$), total serum cholesterol ($P<0.1$), serum triglycerides ($P<0.05$), systolic blood pressure ($P<0.05$), % body fat ($P<0.1$), and BMI ($P<0.05$). Subjects with high calcium and low fat diets had lower apolipoprotein B, total serum cholesterol, triglycerides, % body fat, and BMI levels than their high fat consuming counterparts (66.8 vs.93.0 mg/dl, 193 vs. 228 mg/dl, 88 vs. 141 mg/dl, 27.4 vs. 32.4%, and 24.4 vs. 28.8, respectively). Effects of fat level on these measurements were detected among the subjects consuming low calcium diets. Furthermore, among the subjects consuming a high fat diet, those that consumed high calcium diets had lower systolic blood pressure than those consuming low calcium diets (122 vs. 132 mm Hg) ($P<0.05$); calcium level did not have an effect on systolic blood pressure among subjects consuming low fat diets.

Regression Analysis

Results of stepwise regression analysis of health indices against dietary intake are presented in tables 8, 9, and 10; only regressions with a significance level below $P<0.05$ are presented. Because of a high incidence of gender by diet interactions, correlations were conducted within rather than across gender. Marked differences between men and women and between the four day records and the food frequency questionnaire in correlations between dietary factors and risk factors were detected.

Among these relationships, the only effects similar for men and women is the response to higher carbohydrate intake with decreased serum cholesterol and LDL concentrations as based on the four day food record (Table 8). Of the individual lipid components, increase in apolipoprotein A1 and HDL cholesterol for women appears to be associated most closely with intake of polyunsaturated fatty acids whereas the increases in systolic and diastolic blood pressure appear to be related most closely to intake of cholesterol. For men, the association of an increased apolipoprotein B could be attributed to intake of saturated fatty acids whereas the elevated LDL cholesterol seems to match with elevated intakes of monounsaturated fatty acids. The relationship of higher fat intake with greater body fat and higher blood triglycerides based on the food frequency questionnaire among men might be attributed to greater intakes of lauric and myristic acids, respectively. Effects of exercise on risk factors was evident only for men, in which case increased physical activity was associated with greater HDL cholesterol concentrations. Greater alcohol and higher carbohydrate diets appeared related to body composition in women, alcohol to blood pressure only for men, and primary effects of fiber only for men. Higher sodium intakes were negatively associated with waist to hip ratio for men, and higher calcium intakes were associated with a lower BMI and lower systolic and diastolic blood pressures for men (Table 8).

Dietary Lipids. To examine relationships within groups of men and women with low or high fat intake, according to four day food records, regressions of intake of various nutrients and exercise on measurements of health status were calculated. Significant regressions are noted in Table 9. Responses were quite diverse. However, for women, regardless of fat intake, increased intakes of polyunsaturated fatty acids were associated with increased apolipoprotein A1 concentrations in blood. With either men or women consuming high fat diets, increased calcium intakes were associated with lower diastolic blood pressures. Higher calcium intakes were associated with lower BMI and WHR in women and men, respectively, consuming high fat diets. Other than these relationships, responses for men and women differed. This may be due to the low number of subjects within the groups, to metabolic differences due to gender or level of fat intake, or to haphazard relationships among these diverse groups.

First, for women with low fat diets, increases in fat intake were associated with lower diastolic blood pressure (Table 9). Elevations in dietary cholesterol, for these women was accompanied by elevations in total serum cholesterol and diastolic blood pressure (Table 9). For women with higher fat intakes, higher cholesterol intakes were associated with higher values for apolipoprotein B, total serum cholesterol, and systolic blood pressure (Table 9). Among the factors recommended to improve health status, increased physical activity appeared to increase apolipoprotein A1 among men consuming low fat diets. Increasing the caloric contribution of alcohol to the diet decreased LDL among women with low fat diets and increased apolipoprotein B among men consuming high fat diets. Saturated fat intake was negatively related to apolipoprotein A1 among men consuming low fat diets. Intakes of mono and polyunsaturated fatty acids were negatively related to serum total cholesterol among men consuming high fat diets though they differed in their relationship to body fat, with monounsaturated fatty acids increasing body fat and polyunsaturated fatty acids decreasing body fat. In contrast, higher intakes of polyunsaturated fatty acids were associated with increased body fat of women consuming high fat diets.

Among the individual nutrients, consistently desirable effects in cases where more than one response was noted, were observed for elevated intakes of alcohol, omega 3 fatty acids, lauric acid, and calcium. Polyunsaturated fatty acid intake was desirable for most measurements. Consistently undesirable effects on health status indices were noted with increased intakes of erucic acid and cholesterol.

Discussion

Subjects consuming more than 30% of their calories from fat generally were fatter and had higher levels of circulating total cholesterol, triglycerides, apolipoprotein A1, and apolipoprotein B than subjects consuming diets in accordance with recommended national guidelines (1,2). Men consuming high fat diets had elevations in body mass index and % body fat coinciding best with elevations in the caloric contribution from total fat (Table 9), and reductions in HDL cholesterol with elevations in the caloric contribution from saturated fatty acids (Table 10). Women with high fat diets had elevations in total serum cholesterol (Table 10), apolipoprotein B, and systolic blood pressure (Table 9) accompanying elevations in dietary cholesterol. These observations are in accord with previous findings implicating fat, saturated fat, and cholesterol with an increased chronic disease risk (5,6,10-12). Among the saturated fatty acids, the fatty acids lauric, myristic, and palmitic are the most atherogenic (10, 29). These fatty acids were negatively associated with increases in the measured cardiovascular disease risk factors for women with high fat diets and men with low fat intakes for which increased palmitate was related to decreased HDL concentrations. We propose that the overall nutrient intake of these two groups, such as the higher vitamin E intake of most our female subjects and the low fat diet of this group of male subjects, could impart some protective effects despite elevated intakes of these proposed atherogenic fatty acids.

Our results suggest that overall nutrient intake, instead of fat intake alone, may provide a better understanding of the relationship linking diet to disease risk. For example, we observed that subjects with high fat diets had diets that were less adequate in fiber intake. Fiber is proposed to have hypocholesterolemic and hypotriglyceridemic properties (13,14); although, factors other than dietary fiber tended to be more closely related to serum cholesterol in our study (Tables 9 and 10). Ascherio *et al.* (30) suggested that fiber intake is a stronger predictor of coronary disease risk than fat, saturated fat, or cholesterol intake since many studies implicating saturated fat or cholesterol intake with increased cardiovascular disease risk often have been confounded by fiber intake (31, 32). We observed a very strong correlation ($r = -1.0$) (Table 9) between serum triglycerides and insoluble fiber among male subjects with high fat diets; insoluble fiber intake was inversely correlated with body mass index in male subjects (Table 10). A negative correlation also was detected between fiber intake and blood pressure in women with high fat diets. However, fiber intake did not seem beneficial for all the examined risk factors. Increases in fiber intake were correlated with increases in diastolic blood pressure in men; systolic blood pressure, diastolic blood pressure, and body mass index in men with high fat diets; diastolic blood pressure in women with high fat diets; and body mass index in women with low fat diets. We speculate that many high fiber foods consumed by our subjects were also high in fat, such as whole grain muffins or cookies, and that an increase in fiber intake in some cases was accompanied by an increase in fat intake, particularly for subjects with high fat diets. An analysis of the types of ingested foods was not included in this paper, but such an analysis may be warranted.

Dietary calcium consistently had protective effects against cardiovascular disease risk. Subjects consuming at least 800 mg of calcium had lower systolic blood pressure levels than subjects with a lower calcium intake (Table 5). Men with adequate calcium diets also had lower diastolic pressure than men consuming less calcium. Furthermore, interactions were detected with fat level and calcium level in the diet. Only when subjects had diets with adequate amounts of calcium, did consuming a low fat diet, in accord with current recommendations for fat intake (15), present better apolipoprotein B, total serum cholesterol, serum triglycerides, %body fat, and body mass index values than consuming a high fat diet. Increases in calcium intake were observed alongside reductions in diastolic blood pressure, systolic blood pressure, and body mass index in women (Table 8); diastolic blood pressure and waist to hip ratio in men with high fat diets; and diastolic blood pressure, body mass index, and triglycerides in women with high fat diets (Tables 9, 10). Calcium was, therefore, associated with reductions in certain disease risk factors especially in subjects consuming diets which do not agree with current dietary recommendations, i.e. high in fat, implying a calcium role in reducing the disease risk associated with such diets. The protective effect of calcium on blood pressure observed in our study is in agreement with previous observations (33). Our findings are also in concert with findings by Denke *et al.* (34) who observed that high calcium diets were effective in reducing total and LDL cholesterol in a small sample of men with moderate hypercholesterolemia. However, that study found no effect of calcium on HDL cholesterol. In contrast, our study showed a weak but negative correlation between calcium intake and HDL cholesterol in men with high fat diets; and a positive correlation between increases in dietary calcium and elevations in HDL cholesterol in women with high fat diets. This discrepancy may be explained by the following differences in the design of the two studies: 1) the diet consumed by subjects in that study was compliant with dietary guidelines for fat intake (15), whereas, we observed calcium effects on HDL cholesterol among subjects whose diets were high in fat; 2) unlike our subjects, subjects in that study were all males (others (35,36) have reported gender differences in the lipoprotein response to diet); and 3) subjects in that study were all hypercholesterolimic while the majority of our subjects were not.

We conclude that calcium may be beneficial in reducing cardiovascular disease risk. We support recommendations for a high level of calcium intake along with other dietary recommendations for the reduction of cardiovascular disease risk factors (2) as was suggested by Denke *et al.* (34). Further studies examining the overall nutrient contribution of foods associated with the most reductions in disease risk are needed to elucidate the effects of calcium on disease prevention or reduction. Artaud-Wild *et al.* (37) found that even after adjusting for cholesterol and saturated fat intake, calcium intake was still related to increased coronary heart disease rates in countries with diets rich in dairy products and low in plant foods; this further emphasizes the value of examining food intake and the overall diet rather than individual nutrients alone.

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Table 1. Anthropometric and Biochemical Means of Participating Subjects.

	All Subjects	**Female Subjects**	**Male Subjects**
N	49	23	26
Age (years)	42.2 ± 5.8	42.9 ± 5.2	41.6 ± 6.3
Height (inches)	67.9 ± 3.3	65.6^{a} ± 2.6	70.0^{b} ± 2.3
Weight (pounds)	164.1 ± 32.7	144.4^{a} ± 30.0	181.4^{b} ± 24.4
BMI (kg/m^2)	24.9 ± 4.0	23.6 ± 4.4	26.0 ± 3.2
Waist: Hip Ratio	0.81 ± 0.08	0.74^{a} ± 0.05	0.87^{b} ± 0.04
%Body Fat	28.0 ± 5.7	31.0^{a} ± 6.0	25.4^{b} ± 3.9
Systolic Blood Pressure (mmHg)	124 ± 11	120 ± 12	126 ± 9
Diastolic Blood Pressure (mmHg)	80 ± 9	74^{a} ± 8	85^{b} ± 7
Total Serum Cholesterol (mg/dl)	195 ± 37	189 ± 32	201 ± 40
HDL Cholesterol (mg/dl)	45 ± 12	50^{a} ± 12	40^{b} ± 10
LDL Cholesterol (mg/dl)	131 ± 35	122 ± 31	139 ± 37
Serum Triglycerides (mg/dl)	96 ± 44	83^{a} ± 28	108^{b} ± 52
Apolipoprotein A1 (mg/dl)	140.6 ± 19.3	147.9^{a} ± 16.1	134.2^{b} ± 19.9
Apolipoprotein B (mg/dl	69.3 ± 17.4	63.1^{a} ± 12.4	74.7^{b} ± 19.5

Means of female and male subjects with different superscripts are different at $P<0.05$

Table 2. Percent of Subjects With Acceptable Nutrient Intakes Using Data from Four Day Food Records.

	Males	**Females**	**High Fat**	**Low Fat**	**High Fiber**	**Low Fiber**	**High Calcium**	**Low Calcium**
N	26	23	23	26	27	22	18	31
				%				
Adequacy								
Protein	100	100	100	100	100	100	100	100
Fat	50	57	0	100	73^{x}	37^{y}	48	61
Fiber	46	43	26^{1}	62^{2}	100	0	48	39
Vitamin A	100	100	100	100	100	100	100	100
Vitamin C	92	96	96	92	100	88	100^{a}	83^{b}
Vitamin D	100	100	100	100	100	100	100	100
Vitamin E	65^{A}	91^{B}	83	73	91^{x}	67^{y}	77	78
Vitamin K	35	52	43	42	64	26	48	33
Vitamin B_{12}	88	100	96	92	100	88	93	94
Folic acid	96	100	96	100	95	100	97	100
Vitamin B_{6}	92	87	91	88	95	85	97^{a}	78^{b}
Niacin	100	100	100	100	100	100	100	100
Riboflavin	96	100	100	96	100	96	100	94
Thiamin	96	100	100	96	100	96	100	94
Pantothenate	38	22	26	35	32	30	45^{a}	6^{b}
Zinc	50	65	65	50	59	56	64	44
Iron	85	86	83	88	100^{x}	73^{y}	90	78
Magnesium	73	91	83	81	91	74	90^{a}	67^{b}
Phosphorous	100	100	100	100	100	100	100	100
Calcium	69	56	70	58	68	59	100	0
Manganese	31	35	30	35	41	26	32	33
Iodine	23	35	26	31	27	30	45^{a}	0^{b}
Selenium	77	91	83	84	91	77	83	83

Means with different superscripts are different at $P< 0.05$.

High Fat defines subjects with more than 30% of caloric intake from fat.

Low Fat defines subjects with 30% or less of caloric intake from fat.

High Fiber defines subjects with adequate fiber intake ($\geq$1g fiber / 100 kcal).

Low Fiber defines subjects with less than adequate fiber intake (<1g fiber / 100 kcal).

High Calcium defines subjects with daily calcium intake of 800 mg (1989 RDA) or more.

Low Calcium defines subjects with daily calcium intake less than the 1989 RDA of 800mg.

Adequacy of vitamins and minerals (except for calcium) is set at intake $\geq$ 67% RDA or ESADDI (1989). Adequacy of calcium is set at $\geq$ 100% RDA (1989). Adequacy of fiber is set at intake $\geq$1g fiber/100kcal/day. Adequacy of fat is set at $\leq$30% of kcal from fat.

Table 3. Percent of Subjects With Acceptable Nutrient Intakes Using Data from One Week Food Frequency Questionnaires.

	Males	Females	High Fat	Low Fat	High Fiber	Low Fiber	High Calcium	Low Calcium
N	26	23	13	35	15	33	23	25
				%				
Adequacy								
Protein	92	100	100	94	94	100	100	92
Fat	73	73	0	100	64^{x}	93^{y}	78	68
Fiber	35	27	8^{1}	40^{2}	100	0	39	24
Vitamin A	73	86	77	80	76	87	87	72
Vitamin C	96	95	92	97	94	100	100	92
Vitamin D	100	100	100	100	100	100	100	100
Vitamin K	65	91	77	77	73	87	91	64
Vitamin B_{12}	96	100	100	97	97	100	100	96
Folic acid	96	100	100	97	97	100	100	96
Vitamin B_{6}	85	95	92	89	85	100	96	84
Niacin	96	95	100	94	94	100	96	96
Riboflavin	88	100	92	94	91	100	100	88
Thiamin	92	100	92	97	94	100	100	92
Pantothenate	62	54	54	60	48	80	87^{a}	32^{b}
Zinc	50	86	69	60	61	67	74	52
Iron	96	86	100	89	91	93	96	88
Magnesium	88	95	92	91	88	100	100	84
Phosphorous	100	100	100	100	100	100	100	100
Calcium	46	50	38	51	42	60	100	0
Manganese	62	59	54	63	54	73	74	48
Iodine	35	32	31	34	33	33	70^{a}	0^{b}
Selenium	100	100	100	100	100	100	100	100

Means with different superscripts are different at $P < 0.05$.
High Fat defines subjects with more than 30% of caloric intake from fat.
Low Fat defines subjects with 30% or less of caloric intake from fat.
High Fiber defines subjects with adequate fiber intake (≥1g fiber / 100 kcal).
Low Fiber defines subjects with less than adequate fiber intake (<1g fiber / 100 kcal).
High Calcium defines subjects with daily calcium intake of 800 mg (1989 RDA) or more.
Low Calcium defines subjects with daily calcium intake less than the 1989 RDA of 800mg.
Adequacy of vitamins and minerals (except for calcium) is set at intake ≥ 67% RDA or ESADDI (1989). Adequacy of calcium is set at ≥ 100% RDA (1989). Adequacy of fiber is set at intake ≥1g fiber/100kcal/day. Adequacy of fat is set at ≤30% of kcal from fat.

Table 4. Means of Anthropometric and Biochemical Data of Subjects Classified Into Six Groups Based on Acceptable Intakes of Either Fat, Fiber, or Calcium from Four Day Food Records.

	High Fat	**Low Fat**	**High Fiber**	**Low Fiber**	**High Calcium**	**Low Calcium**
BMI (kg/m^2)	25.6 ± 4.8	24.2 ± 3.0	24.7 ± 4.2	25.0 ± 3.8	24.4 ± 3.1	25.7 ± 5.1
WHR	0.82 ± 0.09	0.80 ± 0.07	0.82 ± 0.09	0.81 ± 0.08	0.81 ± 0.08	0.83 ± 0.09
Body Fat (%)	29.0^{a} ± 6.4	27.2^{b} ± 4.9	27.5 ± 5.1	28.5 ± 6.1	28.3 ± 5.6	26.1 ± 5.8
Systolic Pressure (mmHg)	126 ± 13	121 ± 8	122 ± 9	125 ± 12	123 ± 11	126 ± 11
Diastolic Pressure (mmHg)	81 ± 11	78 ± 8	78 ± 9	81 ± 9	79 ± 9	82 ± 9
Total Cholesterol (mg/dl)	222^{a} ± 36	191^{b} ± 31	193 ± 36	197 ± 38	195 ± 34	197 ± 39
HDL Cholesterol (mg/dl)	47 ± 14	42 ± 10	42 ± 12	47 ± 11	45 ± 12	44 ± 13
LDL Cholesterol (mg/dl)	144 ± 35	119 ± 31	133 ± 38	130 ± 33	131 ± 30	131 ± 40
Triglycerides (mg/dl)	102 ± 52	91 ± 35	90 ± 39	102 ± 48	95 ± 48	106 ± 36
Apolipoprotein A1 (mg/dl)	154.3^{a} ± 19.8	138.7^{b} ± 17.6	136.6 ± 49.1	144.0 ± 21.0	141.2 ± 20.7	136.0 ± 16.5
Apolipoprotein B (mg/dl)	75.0^{a} ± 20.5	64.2^{b} ± 12.5	68.3 ± 15.6	70.0 ± 19.0	69.2 ± 18.2	70.3 ± 15.8

Means with different superscripts are different at $P<0.05$

Table 5. Means of Anthropometric and Biochemical Data of Subjects Classified Into Six Groups Based on Acceptable Intakes of Either Fat, Fiber, or Calcium from One Week Food Frequency Questionnaires.

	High Fat	**Low Fat**	**High Fiber**	**Low Fiber**	**High Calcium**	**Low Calcium**
BMI (kg/m^2)	26.6 ± 4.6	24.3 ± 3.6	24.2 ± 3.3	25.2 ± 4.3	24.8 ± 3.3	25.0 ±
WHR	0.83 ± 0.08	0.80 ± 0.08	0.82 ± 0.08	0.81 ± 0.09	0.81 ± 0.08	0.82 ± 0.09
Body Fat (%)	31.8^{a} ± 7.0	27.3^{b} ± 5.0	26.8 ± 6.0	28.5 ± 5.9	28.1 ± 5.0	27.1 ± 6.0
Systolic Pressure (mmHg)	127 ± 13	123 ± 10	122 ± 9	125 ± 12	122^{x} ± 8	131^{y} ± 12
Diastolic Pressure (mmHg)	82 ± 7	79 ± 10	81 ± 8	79 ± 10	78 ± 9	81 ± 10
Total Cholesterol (mg/dl)	208 ± 34	192 ± 36	194 ± 39	198 ± 35	192 ± 34	200 ± 37
HDL Cholesterol (mg/dl)	50 ± 11	43 ± 12	46 ± 12	45 ± 12	45 ± 11	46 ± 13
LDL Cholesterol (mg/dl)	138 ± 29	130 ± 36	130 ± 35	134 ± 34	129 ± 30	135 ± 37
Triglycerides (mg/dl)	127^{a} ± 39	91^{b} ± 34	92 ± 40	98 ± 46	95 ± 48	97 ± 41
Apolipoprotein A1 (mg/dl)	150.4 ± 16.8	137.6 ± 19.1	136.1 ± 20.6	143.3 ± 18.4	140.5 ± 18.9	141.6 ± 19.9
Apolipoprotein B (mg/dl)	85.1^{a} ± 23.4	67.9^{b} ± 14.6	69.4 ± 14.4	69.7 ± 18.8	69.2 ± 19.5	70.0 ± 15.6

Means with different superscripts are different at $P<0.05$

Table 6. Differences Between Gender and Fat Intake, Based on Four Day Food Records, on Clinical Measurements.

Gender	***Women***	***Women***	***Men***	***Men***
Fat intake	***Low***	***High***	***Low***	***High***
WHR	0.74^{b}	0.75^{b}	0.87^{a}	0.89^{a}
Body Fat (%)	30.4^{a}	34.9^{a}	25.0^{b}	28.8^{ab}
Systolic Pressure (mmHg)	118^{c}	139^{a}	127^{b}	121b^{c}
Diastolic Pressure (mmHg)	73^{b}	81^{a}	84^{a}	86^{a}
Total Cholesterol (mg/dl)	185^{b}	213^{ab}	197^{ab}	231^{a}
HDL Cholesterol (mg/dl)	48^{a}	61^{a}	41^{b}	33^{b}
LDL Cholesterol (mg/dl)	121^{b}	134^{ab}	135^{ab}	171^{a}
Triglycerides (mg/dl)	82^{b}	90^{ab}	105^{ab}	136^{a}
Apolipoprotein A1 (mg/dl)	144.2^{b}	172.6^{a}	134.0^{b}	136.0^{b}
Apolipoprotein B (mg/dl)	68.6^{bc}	62.3^{c}	72.2^{b}	93.6^{a}

Means with different superscripts are different at $P<0.05$

Table 7. Differences Between Gender and Fat Intake, Based on One Week Food Frequency Questionnaires, on Clinical Measurements.

Gender	***Women***	***Women***	***Men***	***Men***
Fat intake	***Low***	***High***	***Low***	***High***
WHR	0.77^{b}	0.73^{b}	0.87^{a}	0.90^{a}
Body Fat (%)	30.3^{a}	34.7^{a}	24.7^{b}	29.6^{ab}
Systolic Pressure (mmHg)	120	125	126	129
Diastolic Pressure (mmHg)	73^{b}	81^{ab}	84^{a}	86^{a}
Total Cholesterol (mg/dl)	189	205	196	227
HDL Cholesterol (mg/dl)	50	49	40	42
LDL Cholesterol (mg/dl)	123	139	136	153
Triglycerides (mg/dl)	81^{b}	82^{b}	99^{b}	161^{a}
Apolipoprotein A1 (mg/dl)	150^{a}	141^{ab}	132b	146^{ab}
Apolipoprotein B (mg/dl)	63^{b}	71^{b}	71^{b}	96^{a}

Means with different superscripts are different at $P<0.05$

Table 8. Correlation Between Dietary Intake, From Either Four Day Records or One Week Food Frequency Questionnaires, and Clinical Measurements in Female and Male Subjects at P<0.05.

	Females		Males	
	Four Day Records	**Food Frequency Questionnaire**	**Four Day Records**	**Food Frequency Questionnaire**
Fatty Acids				
Saturated			ApoB r =+ 0.50, Triglycerides r =+0.42	
Monounsaturated	WHR r-+0.34, BMI r=+0.34		LDL r=+0.33, WHR r=+0.37	LDL r=+ 0.37
Polyunsaturated	ApoA1 r=+0.50, HDL r=+0.52			
Trans		Systolic Pressure r=+0.48		
Omega 3	Diastolic Pressure r=-0.43, %Body Fat r=-0.37, BMI r=-0.39	Systolic Pressure r=-0.27		
Omega 6		Diastolic Pressure r=+0.53		
Lauric				WHR r=+0.48, BMI r=+0.50
Myristic				Triglycerides r=+0.42
Oleic		WHR r=+0.60		
Erucic		Diastolic Pressure r=+0.43, Systolic Pressure r=+0.56		
Cholesterol	Diastolic Pressure r=+0.59, Systolic Pressure r=+0.68		HDL r=-0.30	
Exercise		WHR r=+0.47	HDL r=+0.48	HDL r=+0.48
Protein			WHR r=+0.45, triglycerides r=+0.46	
Alcohol	LDL r=-0.42		Diastolic Pressure r=+0.36, Systolic Pressure r=+0.63	Systolic Pressure r=+0.58
Carbohydrate	Total cholesterol r=-0.50, LDL r=-0.50, %Body fat r=-0.56		Total cholesterol r=-0.54, LDL r=-0.49	ApoA1 r=-0.48
Fiber				
Total			Systolic Pressure r=+0.31	
Soluble			HDL r=-0.45	
Insoluble			BMI r=-0.47	
Sodium		ApoB r=-0.54		WHR r=-0.34
Calcium				

Table 9. Correlation Between Dietary Intake, From Four Day Records, or Lifestyle and Clinical Measurements in Female and Male Subjects Consuming Diets High or Low in Fat at P<0.05.

	Females		**Males**	
	High Fat	**Low Fat**	**High Fat**	**Low Fat**
Fat		Diastolic Pressure r=-0.37		%Body fat r=-0.46
Saturated				ApoA1 r=-0.48
Monounsaturated			Total cholesterol r=-0.40, %Body fat r=+0.33	WHR r=+0.47
Polyunsaturated	ApoA1 r=+0.81, % Body fat r=+0.50	ApoA1 r=+0.50	Total cholesterol r=-0.55, LDL r=-0.39, %Body fat r=-0.45, WHR r=-0.76	
Trans				ApoB r=-0.44
Omega 3	HDL r=+0.48			
Linolenic	ApoA1 r=-0.63			
Linoleic		WHR r=-0.42		
Stearic		WHR r=+0.81		Diastolic Pressure r= -0.57, BMI r=-0.44
Palmitic				HDL r=-0.42
Lauric	ApoB r=-0.36, LDL r=-0.67, Total cholesterol r=-0.71, , BMI r=-0.54			
Myristic				BMI r=-0.77
Oleic			Total cholesterol r=+0.47	
Erucic	Total cholesterol r=+0.56, LDL r=+0.39			
Cholesterol	ApoB r=+0.50, Systolic Pressure r=+0.79	Total cholesterol r=+0.62, Diastolic Pressure r=+0.49		Diastolic Pressure r=+0.62, BMI r=+0.37
Exercise	LDL r=+0.54			ApoA1 r=+0.65
Protein	LDL r=+0.32	Systolic Pressure r=+0.60	Total cholesterol r=-0.33	Triglycerides r=+0.53
Alcohol		LDL r=-0.66	Systolic Pressure r=+0.37	Triglycerides r=+0.37
Carbohydrate	HDL r=+0.35			Systolic Pressure r=+0.81
Sugars		Systolic Pressure r=+0.42	ApoA1 r=+0.49, HDL r=+0.45	
Total Fiber	Systolic Pressure r=-0.33		BMI r=+0.53	
Soluble Fiber			Diastolic Pressure r=+0.35	
Insoluble Fiber	Diastolic Pressure r=+0.44	BMI r=+0.39	Triglycerides r=-1.0	
Sodium		Triglycerides r=-0.43	%Body fat r=+0.59	
Calcium	Diastolic Pressure r=-0.53 (P<0.1), BMI r=-0.33	ApoB r=-0.51	Diastolic Pressure r=-0.74, WHR r=-0.49	ApoA1 r=+0.36, HDL r=-0.35

Table 10. Correlation Between Dietary Intake, From One Week Food Frequency Questionnaires, and Clinical Measurements in Female and Male Subjects With Either a High or Low Fat Intake at P<0.05.

	Females		Males	
	High Fat	**Low Fat**	**High Fat**	**Low Fat**
Fat		ApoA1 r=-0.52, Total cholesterol r=-0.58, LDL r=-0.50, BMI r=-0.50	%Body fat r=+0.94, BMI r=+0.93	ApoB r=+0.66
Saturated			HDL r=-0.75	
Monounsaturated				Total cholesterol r=+0.40
Polyunsaturated	ApoB r=-0.55			%Body fat r=-0.44
Trans		ApoA1 r=+0.44, ApoB r=-0.51		ApoB r=+0.33
Omega 3	LDL r=+0.55			
Omega 6	Diastolic Pressure r=+0.82, %Body fat r=+0.90			Triglycerides r=-0.45
Linolenic				ApoA1 r=-0.34
Palmitic	WHR r=-0.58			
Lauric		Diastolic Pressure r=+0.39		HDL r=-0.61, LDL r=+0.40, WHR r=+0.56
Myristic				WHR r=-0.39, BMI r=-0.59
Oleic	ApoA1 r=-0.38	%Body fat r=-0.49		
Erucic	Systolic Pressure r=+0.93	Systolic Pressure r=+0.53		
Cholesterol	Total cholesterol r=+0.83			
Exercise		BMI r=+0.35		
Protein				%Body fat r=-0.37
Alcohol			ApoB r=+0.43, WHR r=+0.49	ApoA1 r=+0.64, Systolic Pressure r=+0.66, Triglycerides r=+0.51
Carbohydrate	Systolic Pressure r=-0.35		ApoB r=-0.89	HDL r=-0.53, %Body fat r=+0.44
Sugars	LDL r=-0.81		Diastolic Pressure r=-0.77	BMI r=+0.44
Total Fiber			Systolic Pressure r=+0.52	
Insoluble Fiber		Triglycerides r=+0.49		
Calcium	HDL r=+0.97, Triglycerides r=-1.0			

CHAPTER VII

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Summary

This research evaluated the effects of fat, calcium and fiber on fat digestibility, caloric contribution of fat, energy retention, and physical measures of health in mice, pigs, and people. Diets high in isocaloric amounts of sucrose, beef tallow, or corn oil were fed to mice and pigs. Mice were fed the high corn oil diets at two levels of calcium intake (0.6 %, equivalent to the calcium content of the sucrose diet, or 1.5% of the diet), and the high tallow diets at five levels of calcium intake (0.6, 1.0, 1.5, 2.0, or 2.5 % of the diet) or with one of four different sources of dietary fiber (cellufil, rice bran, wheat bran, and beet pulp). Pigs were fed the high fat diets at two levels of calcium intake, one equivalent to that of the high sucrose diet (0.92 % of the diet) and one higher (1.80 % of the diet). Comparisons were, therefore, performed between effects of: 1)added sucrose, added beef tallow, and added corn oil in mice and, similarly, in pigs; 2) high and normal levels of calcium in high corn oil or high tallow diets in mice and, similarly, in pigs; 3) the four levels of calcium in high tallow diets fed to mice; and 4) the four sources of dietary fiber fed to mice. We also examined the level of dietary fat, fiber, and calcium and measured body mass index, waist to hip ratio, % body fat, systolic and diastolic blood pressures, serum total cholesterol, serum HDL cholesterol, serum LDL cholesterol, serum triglycerides, serum apolipoprotein A1, and serum apolipoprotein B in forty nine men and women. We divided our human subjects into groups of high and low intakes of fat, fiber, or calcium in order to examine any relationship between diet (particularly fat, fiber and calcium) and anthropometric, clinical, or biochemical values. Chapter I lists the objectives and null hypotheses developed for this research. Each of the hypotheses is individually addressed below.

The null hypothesis H1 stated that supplemental isocaloric amounts from the different energy sources sucrose, corn oil, or beef tallow produce equal effects on fat and energy digestibility in mice or pigs and similar cholesterol values in pigs.

In mice, different results were observed when supplementing a basal diet with isocaloric amounts (Atwater values (15)) of carbohydrate vs. fat and beef tallow vs. corn oil. Supplementing the basal diet with sucrose resulted in lower fecal dry matter, fecal soaps, % fat digestibility of the diet, digestible energy of the diet, and digestible energy of the added energy source; but higher digestibilities of added dry matter and energy than when supplementing with isocaloric Atwater equivalent ME amounts of fat (beef tallow or corn oil). Supplementing the mouse diet with beef tallow resulted in greater fecal soap and body protein, but lower fat energy digestibility, body dry matter content, % body fat, daily diet energy retention, diet energy retention of digested calories, retention of added energy per day, retention of added energy per gram of added fat fed, and efficiency of energy retention than feeding mice the same amount of supplemental corn oil. Based on these results, the null hypothesis H1 is rejected in mice.

In pigs, supplementing the basal diet with sucrose did not produce the same effects as supplementing with fat, and further, supplementing with beef tallow produced different results than when supplementing with corn oil. Pigs fed the sucrose supplemented diet gained less weight per calorie consumed; these pigs had less skin weight and dry matter content of soft tissues, lower fat digestibility of the diet, lower HDL cholesterol levels, and more ash in soft tissues than their fat supplemented counterparts. Supplementing with beef tallow resulted in a higher soft tissue retention of ash and more ash in soft tissues, and more fecal energy excretion, but higher net energy of added fat per dry matter weight than when supplementing with an equivalent amount of corn oil. Based on these findings, the null hypothesis H1 is, also, rejected in pigs.

The null hypothesis H2 stated that supplementing high beef tallow or high corn oil diets with calcium does not affect the digestibility of either the fat or the diet in mice or pigs.

Increasing the calcium level in the diet affected the measured outcomes in mice. Mice fed a high corn oil diet with 1.5% calcium had higher fecal dry matter excretion and lower % dry matter digestibility of the diet, dry matter digestibility of fat, digestible energy of the diet and digestible energy of the added corn oil than mice fed a high corn oil diet with 0.6% calcium. Mice fed a high tallow diet with 1.5% calcium had higher fecal dry matter and lower % dry matter digestibility of the diet, % energy digestibility of the diet, dry matter digestibility of fat, energy digestibility of fat, digestible energy of diet, and digestible energy of added tallow than mice fed a high beef tallow diet with 0.6% calcium. Furthermore, increasing the calcium content of the high beef tallow diet to 2.0

and 2.5% produced a further linear increase in fecal dry matter output and a decrease in digestibility of added dry matter from tallow, digestible energy of the diet, and digestible energy of the added tallow. Therefore, the null hypothesis H2 is rejected in mice.

Supplementing the high fat diets of pigs with calcium affected the digestibility of the fat and the diet. Feeding the high corn oil diet with double the amount of calcium to pigs resulted in higher total tissue weight, higher skin weight, fecal dry matter content, and fecal soap content, with lower fecal energy, % dry matter digestibility of the diet, % energy digestibility of the diet, % dry matter digestibility of the added corn oil, % energy digestibility of the added corn oil, and metabolizable energy per dry matter fed than when feeding the high corn oil diet without supplemental calcium. Feeding the high beef tallow diet with supplemental calcium produced higher skin weight, fecal dry matter, and fecal soap content with lower fecal energy, % dry matter digestibility of the diet, % energy digestibility of the diet, % dry matter digestibility of the added tallow, % energy digestibility of the added tallow, and metabolizable energy per dry matter fed than when feeding the high tallow diet without supplemental calcium. Consequently, the null hypothesis H2 is rejected in pigs.

The null hypothesis H3 stated that addition of fiber to a high beef tallow diet does not affect the digestibility of the tallow or the diet of mice.

Supplementing the high tallow diet of mice with fiber increased fecal dry matter output and decreased % dry matter digestibility of the diet, % energy digestibility of the diet, % fat digestibility of the diet, digestible energy of the diet, dry matter digestibility of the added tallow (or tallow plus fiber source), energy digestibility of added tallow (or tallow plus fiber source), and added digestible energy than when feeding the high tallow diet without added fiber. Therefore, the null hypothesis H3 is rejected.

The null hypothesis H4 stated that the nutrient adequacy of the diets of human subjects is the same regardless of gender or the level of fat, fiber, or calcium in the diet.

Examining the dietary intake of our subjects, we found that more women consumed at least 67% of the recommended intake of vitamin E than men, more subjects consuming low fat diets were also consuming acceptable amounts of fiber than subjects with high fat diets, more of the subjects consuming a high fiber diet were also consuming a low fat diet and at least 67% of the recommendations for vitamin E and iron than subjects with low fiber intake, and more of the subjects consuming diets adequate in calcium consumed at least 67% of the recommendations for vitamin C, vitamin B_6, and magnesium than subjects consuming diets lower in calcium. Therefore, diets with different amounts of either fat, fiber, or calcium differ in other nutrients as well. Hence, the null hypothesis H4 is rejected.

The null hypothesis H5 stated that fat, fiber, or calcium intake by human subjects does not affect anthropometric measures or indices of cardiovascular disease risk factors.

Analyzing the dietary intake and physical measures of health in young middle aged subjects revealed several associations between diet and the measured indices. Subjects consuming high fat diets had higher % body fat, serum total cholesterol, triglycerides, apolipoprotein A1, and apolipoprotein B than subjects consuming low fat diets. Subjects consuming adequate calcium had lower systolic blood pressure than subjects with lower daily calcium intake. Body mass index was positively correlated with % calories from fat in men with high fat diets. Waist to hip ratio was inversely correlated with % of calories from polyunsaturated fatty acids and calcium intake in men eating high fat diets, and positively correlated with intake of stearic acid in women with low fat diets. Percent fat in the body was positively correlated with the % of calories from fat in men with high fat diets. Systolic blood pressure was positively correlated with intake of dietary cholesterol in women with high fat diets. Diastolic blood pressure was inversely correlated with calcium intake in men eating high fat diets. Serum total cholesterol was positively correlated with dietary cholesterol in women with high fat diets. HDL cholesterol was inversely correlated with % calories from saturated fatty acids in men with high fat diets and positively correlated with calcium intake in women with high fat diets. Apolipoprotein A1 was positively correlated with calcium in women with high fat diets. Serum apolipoprotein B was inversely correlated with calcium in women with low fat diets. These results indicate that fat, fiber, and calcium intake are associated with changes in values of the obtained anthropometric as well as cardiovascular disease risk factor measurements. Therefore, the null hypothesis H5 is rejected.

Conclusions

Conclusions that were derived from feeding mice diets supplemented with isocaloric amounts from either sucrose, beef tallow (at two levels of calcium, 0.6 or 1.5 %), or corn oil (at two levels of calcium, 0.6 or 1.5%) were as follows. The average metabolizable energy values for the different energy sources were 4.01 kcal/g for sucrose, 7.96 kcal/g for corn oil, and 8.94 kcal/g for tallow. But, the average energy retention per gram of fed tallow was 1.6 times that per gram of sucrose while the average energy retention per gram of corn oil was 4.11 times that from sucrose; both values are very different from 2.25, the ratio of energy from fat to that from sucrose proposed by the traditional Atwater values (15). Calcium supplementation depressed dry matter and energy digestibilities of both corn oil and tallow, but the effect was more pronounced with tallow than with corn oil. Therefore, estimating the caloric content of diets from standard Atwater numbers for protein, carbohydrate, and fat alone may not be accurate. The source of fat and the level of calcium in the diet should be considered since these factors influence the availability of energy from mixed diets.

The conclusions that were drawn from feeding mice a high beef tallow diet with either one of five levels of calcium (0.6, 1.0, 1.5, 2.0, and 2.5%) or four fiber sources (cellufil, rice bran, wheat bran, or beet pulp) were as follows. Added calcium linearly increased the excretion of fat and the amount of soap in the feces. Also, added calcium linearly depressed dry matter digestibility, energy digestibility, and calculated metabolizable energy values. For every 1% increase in calcium, there was a 2% decrease in metabolizable energy, but cubic and quadratic effects were detected; the decrease reached a maximum when calcium level was at 2% of the diet. The decrease in energy retention was drastic; the retained energy value of added tallow decreased from 9.12 kcal/g, when calcium level was at 0.6%, to 6.79 kcal/g when calcium level was at 1.5%, and further to 5.87 kcal/g when calcium level was at 2% of the diet. Moreover, when the high beef tallow diet contained 2% calcium, the reductions in digestibility where equivalent to adding 10% fiber to that diet. Fiber in the diet reduced dry matter digestibility, energy digestibility and metabolizable energy value of the diet. However, the various sources of fiber used in this study produced varying results. Of the four sources of fiber fed to the mice, mice fed the rice bran containing diet had the lowest dry matter digestibility, energy digestibility, and fat digestibility of the diet. Cellufil appeared to slightly depress the digestion of the other components in the diet. Beet pulp had lower dry matter digestibility and energy digestibility than rice bran or wheat bran. Consequently, the true availability of calories for mice fed mixed diets can vary with the level of calcium or source of fiber in the diet.

Feeding pigs isocaloric amounts from either sucrose, beef tallow, or corn oil provided the following conclusions. More soap was excreted in the feces for pigs consuming high fat diets than when feeding high sucrose diets although overall fat excretion was similar for all three energy sources. Fat digestibility of the sucrose diet was lower than that of either high fat diet. The obtained metabolizable energy values were 3.3 kcal/g for added sucrose, 10.4 kcal/g for added corn oil, and 12.6 kcal/g for added beef tallow. The net energy values were 1.51 kcal/g for added sucrose, 4.12 kcal/g for added corn oil, and 5.59 kcal/g for added tallow. However, increasing the calcium level of the high fat diets from 0.92 to 1.80% drastically reduced the metabolizable energy values to 5.4 kcal/g (from 10.4 kcal/g) for corn oil and to 5.5 kcal/g (from 12.6 kcal/g) for tallow. The increase in calcium level caused more fecal soap excretion with the high tallow diet than with the high corn oil diet. Hence, different energy sources produce different effects on diet digestibility in pigs. The metabolizable energy value varies with the fat source and is greatly affected by the level of calcium in pigs diets.

The conclusions that were derived from studying the dietary intake and the health measurements in human subjects were the following. Subjects who ate high fat diets, also ate low fiber diets. Subjects who ate low fiber diets also ate high fat diets. Therefore, fat level in the diet may not be the only factor explaining differences in either % body fat, serum total cholesterol, triglycerides, apolipoprotein A1, or apolipoprotein B. Subjects who consumed high calcium diets had better systolic blood pressure than subjects with low calcium diets, however, more subjects eating high calcium diets were adequate in vitamin C, B_6, pantothenic acid, magnesium, and iodine. Regression analyses revealed that, overall, undesirable measurements of health indices were obtained with increased intakes of fat, especially, stearic acid, palmitic acid, and cholesterol; whereas desirable values for the health indices were observed with increased intakes of calcium.

Recommendations

Further animal studies are needed in order to accurately estimate the true caloric value of different fat sources in diets. Experiments should be conducted on a per species basis, since tallow had a lower energy value than corn oil in mice but not in pigs. Diets should also be evaluated with various levels of calcium inclusion and with different fiber sources. Since linear effects were observed upon the addition of calcium to the high beef tallow diet in mice, the same treatments should be tested in pigs and also with high corn oil diets . Such valuable experiments would allow for the development of regression equations for predicting the caloric value of a diet instead of using the 4,4, and 9 kcal/g numbers that are currently used in the estimation of energy content of diets and feeds.

Results of the human study described in this manuscript indicated that diets differ not only in their fat content, but also in the level of intake of other nutrients; these may have a bearing on overall health. Studies in humans would be needed to evaluate the overall diet and its total nutrient content, especially when examining the impact of nutrients on the etiology of cardiovascular disease and its risk factors. Further studies in humans are warranted to examine the influence of calcium on the digestibility and ultimately the caloric contribution of fat in diets. Future research with human subjects should investigate the impact of high calcium intakes, whether from supplements or from food, on energy contribution, weight gain, and cardiovascular disease risk factors.

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APPENDIX

INSTITUTIONAL REVIEW BOARD APPROVAL FORM

OKLAHOMA STATE UNIVERSITY
INSTITUTIONAL REVIEW BOARD
HUMAN SUBJECTS REVIEW

Date: 12-12-95 **IRB#:** HE-96-027

Proposal Title: EATING HABITS AND NUTRIENT ADEQUACY

Principal Investigator(s): Christa F. Hanson, Dania A. Khalil

Reviewed and Processed as: Expedited

Approval Status Recommended by Reviewer(s): Approved

ALL APPROVALS MAY BE SUBJECT TO REVIEW BY FULL INSTITUTIONAL REVIEW BOARD AT NEXT MEETING.
APPROVAL STATUS PERIOD VALID FOR ONE CALENDAR YEAR AFTER WHICH A CONTINUATION OR RENEWAL REQUEST IS REQUIRED TO BE SUBMITTED FOR BOARD APPROVAL.
ANY MODIFICATIONS TO APPROVED PROJECT MUST ALSO BE SUBMITTED FOR APPROVAL.

Comments, Modifications/Conditions for Approval or Reasons for Deferral or Disapproval are as follows:

Provisions received and approved.

Signature: John H. Wyckoff Date: January 29, 1996

Chair of Institutional Review Board

VITA

Dania Agha Khalil

Candidate for the Degree of

Doctor of Philosophy

Thesis: EFFECTS OF DIETARY CALCIUM AND FIBER ON DIGESTIBILITY OF FAT AND ENERGY AND ON HEALTH INDICES OF CARDIOVASCULAR DISEASE

Major Field: Human Environmental Sciences-Nutritional Sciences

Biographical:

Personal Data: Born in Beirut, Lebanon, on the 22nd day of September, 1966, the daughter of Mohamad Agha and Nahile Abu-Khadra Agha. Wife of Ibrahim Khalil and mother of Sarah and Ryan Ahmad Khalil.

Education: Graduated from Rawdah High School, Beirut, Lebanon, June 1984; received Bachelor of Science degree in Medical Laboratory Technology from the American University of Beirut, Beirut, Lebanon in June 1987; received a Master of Science degree in Food Nutrition and Institution Administration from Oklahoma State University, Stillwater, Oklahoma in December 1990; earned the Registered Dietitian title in May 1993; completed the requirements for the Doctor of Philosophy degree at Oklahoma State University in December 1998.

Professional Experience: Laboratory Medicine internship, American University Medical Center, Beirut, Lebanon, 1986-1987; graduate work at the Department of Food Technology and Nutrition, American University of Beirut, Beirut, Lebanon, 1988-1989; graduate research assistant at the Department of Food Nutrition and Institution Administration/Nutritional Sciences Department, Oklahoma State University, during various times between 1989-present; teaching assistant at the Department of Nutritional Sciences, Oklahoma State University,1992-present.

Professional Organizations: American Dietetic Association; Oklahoma Dietetic Association; Sigma Xi.