NATIONAL DAIRY COUNCIL®

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November 20, 2003

Division of Dockets Management (HFA-305)
Food and Drug Administration
5630 Fishers Lane, rm. 1061
Rockville, MD 20852

Dear Sir or Madam:

On behalf of the NATIONAL DAIRY COUNCIL ® (NDC) I would like to thank you for the opportunity to present at the Public Meeting on Obesity on October 24, 2003. Enclosed is a copy of my presentation along with some scientific support for dairy's role in weight management.

Enclosed documents include:

- Obesity& Weight Management: Presentation (text version)
- Dairy's Role in Weight Management: Fact sheet
- Supporting research

NDC commends the FDA Obesity Working Group for undertaking this important initiative, as obesity is one of the key health issues facing Americans today. As you work toward solutions to the problems of obesity, please do not hesitate to contact us if you would like additional information.

Sincerely,

Gregory Miller, PhD
Senior Vice President
Nutrition Research and Scientific Affairs

2003N-0338

TS17

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Oral Presentation by Gregory Miller, 10-12 minutes

Good afternoon. I'm Dr. Greg Miller, Senior Vice President, Nutrition & Scientific Affairs, for the National Dairy Council.

We commend the FDA and the Obesity Working Group for undertaking such an important initiative, as obesity is one of the key health issues facing Americans today. For more than 85 years the National Dairy Council has worked to advance the state of scientific knowledge on the role and value of dairy foods in promoting and enhancing human nutrition and health, and we look forward to assisting you in any way possible to help consumers build healthy diets that promote health, prevent disease, and help maintain ideal body weight.

You asked for comments on 6 questions specific to developing solutions to the obesity problem in America. Before I address some of those questions, I have a few overarching comments that I would like to make.

First, though there are many tools available today to help consumers make better diet decisions, including the Dietary Guidelines and USDA's Food Guide Pyramid, Americans are not following the government's nutrition recommendations. Only 1-3 percent of Americans are actually following the Pyramid[1]; however, this does not necessarily mean that the tools are ineffective. It illustrates that Americans need more help turning the information in those guidelines into action for better health.

One way to do this is to simplify consumer education materials by including consistent information. For example, if the Food Guide Pyramid, Dietary Guidelines and food labels - including the nutrition facts panel, used the same serving size references, you could project that American's could more easily build a pyramid-based diet by using the information on the nutrition facts panel of the foods they purchase. Today that's not possible.

[1] 2001 study in the Journal of Nutrition

Consistency in information, like serving sizes, might promote behavior change and help to close the large gap between recommendations and compliance.

Second, in Tommy Thompson's recent remarks at the National Food Policy Conference, he said, "So many of our chronic, debilitating illnesses can be *prevented* through lifestyle choices."

The staggering statistics demonstrate that Americans do not fully comprehend how what they eat, and what they do or don't do over a period of time, translates into their weight. Helping Americans and especially children understand energy balance, and how to select foods to build a nutritionally adequate diet that is appropriately balanced for the level of energy expended, could go a long way toward prevention of obesity and its many related diseases.

Today, food labels focus on energy in, but not the other half, how to balance it with energy out. Labels could be an important tool in the prevention of obesity and related diseases by helping consumers understand the concept of energy balance, so they can more easily

select foods to build a nutritionally sound diet that is appropriately balanced for the level of energy expended.

Finally, there will be many great ideas that come out of today's meeting and subsequent written comments for FDA consideration. But we know there is no single, easy answer. We commend you for your use of a scientific, evidence-based approach to energy balance, weight loss and weight management, and for leading this initiative to ensure that the best, most accurate health information is being delivered to Americans.

We also commend your continued enforcement of fraudulent weight loss claims, which will help reduce consumer confusion, directing them toward positive, life long changes for weight loss and overall better health.

Now, I'd like to address some of your questions, as they relate specifically to dairy.

In response to Question 3 on the available evidence to guide public efforts to prevent and treat obesity:

A growing body of evidence suggests that milk, cheese and yogurt may play a role in weight management efforts when coupled with a balanced, reduced-calorie diet. As the nation focuses on preventing obesity and weight gain, it is important for consumers to understand that dietary calcium, especially from dairy products, may play an important role in the regulation of energy metabolism, resulting in a reduction in body fat and an acceleration of weight and fat loss during caloric restrictions. A number of studies over the past five years have looked at this connection.

The current science indicates that increasing calcium intakes to adequate levels can enhance the effectiveness of a balanced reduced calorie diet for weight and body fat loss, but the impact is more dramatic when the calcium is delivered along with the package of nutrients found in dairy foods. While more research continues to

unfold, this science is important as it relates to prevention and treatment of obesity.

I'd like to address Questions 4 and 5 together - changes in food labeling to develop and promote lower calorie foods and opportunities that exist for development of healthier foods.

Science and history show that one-dimensional strategies, such as low calorie or low fat, do not provide a magic bullet for the development of better diets for weight management. We've already undergone years of the low-fat craze, and yet Americans have gained more weight than ever. Promotion and development of low calorie foods alone will not prevent or reduce the rates of obesity.

It is scary to think - but if you take a low-calorie focus to the extreme, individuals could eat low calorie foods and still suffer from a host of chronic diseases - precisely because they are not getting the nutrients they need to promote health or prevent disease. One could project that this approach could continue to distort consumer behavior rather than

help educate consumers on the right balance of foods and physical activity for a healthy weight.

People eat food, not calories. It is not the number of calories on the nutrition facts panel, or the energy density of the individual food, that builds a nutritious diet. The overall nutrition and the health benefits that those calories deliver is what really matters, balanced with appropriate physical activity. Dairy foods have been shown to be important for bone health; and as I mentioned a minute ago, we are learning that nutrients in dairy that are good for bones may also be good for weight management. Clinical trials have shown that the *calcium in dairy* may play a role in helping to reduce weight. Additionally, studies have shown that people who follow moderate fat diets have better compliance and success with weight management[2]. Nutritious foods like dairy – that science shows can help control body fat and deliver a variety of important nutrients – are part of the solution. This is important for consumers to know.

[2] 2003 PREMIER study in the Journal of American Medical Association

Food labels and other educational tools can help consumers build healthier - not just lower calorie - diets that optimize personal energy balance and help manage weight.

I will briefly address Question 6 about the most important things FDA could do to make a significant difference in the obesity effort.

I'm sure we all agree that physical activity should be a main area of focus. Forty percent of adults over 18 engage in NO leisure-time physical activity and only 23 percent report regular, vigorous exercise 3 or more days a week[3]. When you combine Americans low energy output with high energy intake - and tack on the gap between nutrition recommendations and consumer compliance - it paints a grim picture.

Properly regulated through a scientific evidence-based process, the FDA's on-label Qualified Health Claims will create more awareness of emerging science and help consumers make more informed decisions about the foods they choose.

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[3] Healthy People 2010

We might begin tackling the obesity epidemic with the following implementation considerations:

- consistent information across educational tools, such as serving sizes;
- a focus on prevention by helping consumers understand the concept of energy balance on labels, so they can turn it into an action plan suitable to their individual lifestyles;
- a communications plan to convey the information in a consumer-relevant way with multiple touch-points, from labels to marketing to government nutrition guidelines;
- scientific, evidence-based solutions for selecting foods that are part of the solution to weight management; and
- a pilot test to determine effectiveness and feasibility of any proposed plan before serving it up to Americans.

The combination of these things could start to make a sizeable difference in the prevention and treatment of obesity.

As you work toward solutions to the problems of obesity, please do not hesitate to contact me if you would like additional information. Thank you for your consideration.

DAIRY'S ROLE IN WEIGHT MANAGEMENT

The Supporting Evidence Continues to Grow

A growing body of research suggests that milk, cheese and yogurt may play a role in weight management efforts when coupled with a balanced reduced-calorie diet. Additional research is being conducted in this exciting area of nutrition.

Adults

- This research review concluded that dietary calcium may play an important role in the regulation of energy metabolism and may result in a reduction of body fat and an acceleration of weight and fat loss during caloric restriction. This review also concluded that dairy sources of calcium demonstrate substantially greater effects than supplemental or fortified sources.
  Zemel, MB. Role of dietary calcium and dairy products in modulating adiposity. *Lipids*. 2003; 38(2):139-146.

- Dietary calcium may play a role in regulating body weight, supporting the hypothesis that increasing dietary calcium or dairy intake may reduce future weight gain.
  Parikh SJ, et al. Calcium intake and adiposity. *American Journal of Clinical Nutrition*. 2003; 77:281-287.

- A research review concluded that nutrients found in dairy, including calcium, may contribute to the reduction of body weight, body fat and insulin resistance syndrome.
  Teegarden D, et al. Symposium: Dairy product components and weight regulation. *Journal of Nutrition*. 2003; 133: 243S-256S.

- Data from over 550 women was reevaluated to assess the effects of calcium on weight gain. While calcium is only one factor that potentially affects obesity, findings from this reanalysis of data suggest that increasing calcium intakes to recommended levels may reduce the incidence of overweight and obesity by 60-80% in a population. This is an estimate and the conclusion is based on data projection.
  Heaney RP, et al. Normalizing calcium intake: Projected population effects for body weight. *Journal of Nutrition*. 2003; 133:268S-270S.

- Low daily calcium intake was associated with greater body fat and body weight, particularly in women.
  Jacqmain M, et al. Calcium intake, body composition, and lipoprotein-lipid concentrations in adults. *American Journal of Clinical Nutrition*. 2003; 77:1448-1452.

- In a study involving 35 non-obese, healthy adults, a higher dietary calcium intake over a 24-hour period was associated with burning significantly more body fat, even during sleep.
  Melanson EL, et al. Relation between calcium intake and fat oxidation in adult humans. *International Journal of Obesity*. 2003; 27: 196-203.

- Consuming a diet high in fruit, vegetables, reduced-fat dairy and whole grains, and low in red and processed meat, fast food and soda, was associated with smaller gains in body mass and waist circumference.
  Newby PK, et al. Dietary patterns and changes in body mass index and waist circumference in adults. *American Journal of Clinical Nutrition*. 2003; 77:1417-1425.

- Obese people who consumed three to four servings of milk, yogurt or cheese while on a balanced, reduced calorie diet, lost significantly more weight and fat than those who consumed equivalent amounts of calcium through supplements, or who consumed one or fewer servings of milk, yogurt or cheese per day.
  Zemel MB, et al. Dietary calcium and dairy products accelerate weight and fat loss during energy restriction in obese adults. *American Journal of Clinical Nutrition*. 2002; 75(2S):342S. Abstract.

– more –

- Among overweight young adults, increased dairy consumption may protect overweight individuals from the development of obesity and insulin resistance syndrome and may reduce the risk of type-2 diabetes and cardiovascular disease. Obesity is one of the risk factors of insulin resistance syndrome.
  Periera MA, et al. Dairy consumption, obesity, and the insulin resistance syndrome in young adults: The CARDIA Study. *Journal of the American Medical Association.* 2002; 287:2081-2089.

- Women who consumed higher levels of calcium, the majority of which came from dairy products, had lower body weights than women who consumed less calcium. Results from this study indicated that women weighed an average of 17.6 pounds less for every 1,000 mg of calcium consumed.
  Davies KM, et al. Calcium intake and body weight. *Journal of Clinical Endocrinology & Metabolism.* 2000; 85(12): 4635-4638.

Children and Adolescents

- Adolescent boys who drank three servings of milk daily while participating in a standardized strength training program had significantly greater increases in bone mineral density and a better overall nutrient profile with significantly higher intakes of vitamin A, vitamin D, riboflavin, calcium and phosphorous, than boys who drank juice. The author noted that although not statistically significant, there were trends for the milk group to lose more body fat after training.
  Volek JS, et al. Increasing fluid milk favorably affects bone mineral density responses to resistance training in adolescent boys. *Journal of the American Dietetic Association.* 2003; 103:1353-1356.

- Girls ages 9-14 who consumed diets rich in calcium weighed less and had less abdominal fat than girls who consumed less calcium. For every 300 milligrams of calcium consumed, girls were, on average, 1.9 pounds lighter.
  Novotny R, et al. Higher dairy intake is associated with lower body fat during adolescence. *FASEB Journal.* 2003; 17(4):A453.8. Abstract.

- Dairy consumption in adolescent girls is not associated with a higher body mass index (BMI) or an increase in percentage of body fat.
  Phillips SM, et al. Dairy food consumption and body weight and fatness studied longitudinally over the adolescent period. *International Journal of Obesity.* 2003; 27(9):1106-1113.

- In this study, a children's diet rich in calcium and dairy foods was associated with lower body fat than a children's diet with lower calcium and dairy product intakes.
  Carruth BR, et al. The role of dietary calcium and other nutrients in moderating body fat in preschool children. *International Journal of Obesity.* 2001; 25:559-566.

Additional resources include the www.healthyweightwithdairy.com Web site, the Dairy Council Digest titled "Weight Control: An Emerging Beneficial Role for Dairy" and the Healthy Weight Health Education Kit available at www.nationaldairycouncil.org.

Call (312) 240-2880 for more information.

Role of Dietary Calcium and Dairy Products in Modulating Adiposity

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ABSTRACT: Dietary calcium plays a pivotal role in the regulation of energy metabolism. High-calcium diets attenuate adipocyte lipid accretion and weight gain during overconsumption of an energy-dense diet and increase lipolysis and preserve thermogenesis during caloric restriction, thereby markedly accelerating weight loss. Our studies of the *agouti* gene demonstrate a key role for intracellular Ca^{2+} in regulating adipocyte lipid metabolism and TG storage. Increased intracellular Ca^{2+} resulting in stimulation of lipogenic gene expression, and lipogenesis and suppression of lipolysis resulting in adipocyte lipid filling and increased adiposity Moreover, we recently demonstrated that the increased calcitriol produced in response to low-calcium diets stimulates adipocyte Ca^{2+} influx and, consequently, promotes adiposity. Accordingly, suppressing calcitriol levels by increasing dietary calcium is an attractive target for obesity intervention. In support of this concept, transgenic mice expressing the *agouti* gene specifically in adipocytes (a human-like pattern) respond to low-calcium diets with accelerated weight gain and fat accretion, whereas high-calcium diets markedly inhibit lipogenesis, accelerate lipolysis, increase thermogenesis, and suppress fat accretion and weight gain in animals maintained at identical caloric intakes. Further, low-calcium diets impede body fat loss, whereas high-calcium diets markedly accelerate fat loss in transgenic mice subjected to caloric restriction. Dairy sources of calcium exert markedly greater effects in attenuating weight and fat gain and accelerating fat loss. This augmented effect of dairy products is likely due to additional bioactive compounds in dairy that act synergistically with calcium to attenuate adiposity. These concepts are confirmed by both epidemiological and clinical data, which demonstrate that increasing dietary calcium results in significant reductions in adipose tissue mass in obese humans in the absence of caloric restriction and markedly accelerates the weight and body fat loss secondary to caloric restriction, whereas dairy products exert significantly greater effects. These data indicate an important role for dairy products in both the prevention and treatment of obesity.

Paper no. L9245 in *Lipids 38*, 139–146 (February 2003).

A substantial body of evidence has emerged over the last 3 yr to support what may appear to be a very unlikely concept: that dietary calcium may play a role in the regulation of energy metabolism and in modulating obesity risk. We first observed an "antiobesity" effect of dietary calcium accidentally, during the course of a study investigating the antihypertensive effect of dairy products in African Americans. We noted that adding calcium-rich dairy foods (yogurt) to the daily diet resulted in significant reductions in body fat and circulating insulin (1) as well as a sustained reduction in intracellular calcium and an antihypertensive effect (2,3). Twelve months of yogurt supplementation, sufficient to raise daily calcium intake from approximately 400 to approximately 1,000 mg/d, resulted in significant decreases in both serum insulin (from 22 ± 3 to 14 ± 4 μU/mL, $P < 0.03$) and body fat (from 32.3 ± 2.6 to 27.4 ± 3.1 kg fat, $P < 0.01$ by repeated measures comparison). Although these data were inexplicable to us at the time, our recent studies of the mechanism of action of the agouti gene in obesity and insulin resistance have provided a compelling mechanism that has now been confirmed in a series of studies described in this review. These data demonstrate a key role for intracellular Ca^{2+} in the regulation of both murine and human adipocyte metabolism, resulting in modulation of adipocyte TG stores, as described below. Since intracellular Ca^{2+} can clearly be modulated by calcitrophic hormones, including 1,25-dihydroxy-vitamin D [1,25-$(OH)_2$-D] and parathyroid hormone, these data provide a theoretical framework that may explain our earlier clinical trial observations (1). The next portion of this review is devoted to a portrayal of these findings.

AGOUTI, INTRACELLULAR CALCIUM, AND OBESITY

Most of the data describing the role of intracellular Ca^{2+} in human adipocyte metabolism derives from our recent studies of the mechanism of action of *agouti*, the first of the obesity genes to be cloned (4). The C-terminal region of agouti protein, which retains full functional activity relative to the intact protein in an *in vitro* assay system (5), exhibits a striking spatial homology in both number and spacing of cysteine residues to spider and snail venoms (ω-conotoxins, plectoxins), which target Ca^{2+} channels (6). Accordingly, the C-terminus may form a 3-D structure that is functionally similar to these venoms and may thereby serve to modulate Ca^{2+} transport. Indeed, we have reported that obese *agouti* mutant mice (viable yellow, A^{vy}) exhibit increases in both steady-state intracellular Ca^{2+} and Ca^{2+} influx in several tissues (7,8). This increase in intracellular Ca^{2+} was closely correlated with both

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Abbreviations: ACE, angiotensin-converting enzyme; FAS, fatty acid synthase; 1,25-(OH_2)-D, 1,25-dihydroxy-vitamin D; RAS, renin-angiotensin system; SUR, sulfonylurea receptor; UCP2, uncoupling protein 2.

the degree of ectopic *agouti* expression and body weight (8), suggesting the possibility of a causal mechanism between intracellular Ca^{2+} and obesity in these animals. Since A^{vy} mice exhibit elevated rates of adipocyte lipogenesis and increased adipocyte size relative to lean controls (9,10), we explored the links between *agouti*, intracellular Ca^{2+}, and regulatory enzymes in lipid metabolism.

INTRACELLULAR CALCIUM REGULATES ADIPOCYTE LIPID METABOLISM

Recombinant agouti protein directly increased Ca^{2+} influx and steady-state intracellular Ca^{2+} in a variety of cell types, including both murine and human adipocytes (7,8). This regulation occurs in response to physiologically meaningful concentrations of *agouti* (EC_{50} of 18–62 nM, depending on cell type), and, although studies in HEK-293 cells demonstrate the dependence of this effect on the presence of intact melanocortin receptors, it is not dependent on melanocortin receptor antagonism (7). The role of these increases in Ca^{2+} in lipogenesis has been explored using fatty acid synthase (FAS), as this multifunctional enzyme is highly regulated by nutrients and hormones and is a key enzyme in *de novo* lipogenesis. FAS expression and activity are markedly increased in A^{vy} relative to control mice (11), and nanomolar concentrations of agouti protein stimulate *ca.* twofold increases in FAS gene expression and activity and TG accumulation in 3T3-L1 adipocytes as well as in human adipocytes, similar to the maximal increases stimulated by insulin (11). These increases are mediated by a distinct *agouti*/Ca^{2+} response sequence in the FAS promoter (12). This sequence maps to the –435 to –415 region of the FAS promoter and is upstream of the insulin response element, which maps to –67 to –52, consistent with the observed additive effects of *agouti* and insulin on FAS gene transcription (12). Further, we recently reported that *agouti* exerts a regulatory effect on human FAS expression *in vivo*, and that there is a strong correlation between *agouti* expression and FAS expression in adipose tissue obtained from normal volunteers (13). This *agouti* modulation of FAS transcription appears to be mediated *via* intracellular Ca^{2+}, as it can be inhibited by Ca^{2+} antagonism (11,14) and can be mimicked in the absence of *agouti* by either receptor- or voltage-mediated Ca^{2+} channel activation (15).

In addition to activating lipogenesis, recent data also indicate that increasing intracellular Ca^{2+} may also contribute to increased TG stores by inhibiting lipolysis. Increasing Ca^{2+} influx with either arginine vasopressin or epidermal growth factor was reported to inhibit lipolysis in rat adipocytes in a Ca^{2+} dose-responsive fashion (16). Further, we have shown that the *agouti* gene product similarly inhibits lipolysis in human adipocytes *via* a Ca^{2+}-dependent mechanism (17). This inhibition can also be mimicked in the absence of *agouti* by either receptor- or voltage-mediated Ca^{2+} channel activation (17). The antilipolytic effect of intracellular Ca^{2+} is due to a direct activation of phosphodiesterase 3B, resulting in a decrease in cAMP and, consequently, reduced ability of agonists to stimulate phosphorylation and activation of hormone- sensitive lipase (13). Thus, *agouti* regulation of adipocyte intracellular Ca^{2+} appears to promote TG storage in human adipocytes by exerting a coordinated control of lipogenesis and lipolysis, serving to stimulate the former and inhibit the latter simultaneously.

However, it is important to note that *agouti* interaction with insulin is required for the full expression of *agouti*-induced obesity. *Agouti* and insulin exert independent, additive effects on FAS transcription and lipogenesis (12). Since increased intracellular Ca^{2+} is the proximate signal for insulin release, and *agouti* regulates Ca^{2+} in several cell types (7), it is reasonable to speculate that *agouti* may stimulate insulin release as well. Indeed, we recently found that *agouti* is expressed in human pancreas and stimulates Ca^{2+} signaling in rat, hamster, and human pancreatic β cells (18). Further, hyperplasia of β cells precedes the development of obesity in *agouti* mutant mice, suggesting that hyperinsulinemia may be a direct effect of *agouti* acting on the pancreas and that the combination of this hyperinsulinemia and *agouti*-stimulated adipocyte Ca^{2+} influx may lead to obesity. In support of this concept, transgenic mice expressing *agouti* at high levels in adipose tissue under the control of the aP2 promoter become obese if they are also hyperinsulinemic as a result of either exogenous insulin or a high-sucrose diet, whereas hyperinsulinemia was without effect in nontransgenic littermate controls (19–21). Since humans exhibit a similar pattern of adipocyte *agouti* expression (22), similar *agouti*/insulin/Ca^{2+} interactions may result in excessive adipocyte TG storage.

Taken together, these data indicate that regulation of adipocyte and pancreatic intracellular Ca^{2+} may be an important target for the development of therapeutic strategies for the prevention and treatment of obesity (14). This concept is summarized in Figure 1.

To further evaluate this hypothesis, *agouti*-expressing transgenic mice were treated with high doses of a Ca^{2+} channel antagonist, nifedipine. This treatment resulted in an 18% reduction in fat pad mass and completely normalized the *agouti*-induced hyperinsulinemia over a 4-wk treatment period in the transgenic mice, but was without effect in the nontransgenic littermate controls (23). Thus, adipocyte and/or pancreatic β-cell Ca^{2+} appears to be a reasonable therapeutic target for the treatment and/or prevention of obesity.

We recently extended this concept by demonstrating that human adipocytes express a sulfonylurea receptor (SUR) that exerts a regulatory effect on the Ca^{2+} channel and, consequently, modulates adipocyte lipid accumulation (15,24). Compounds acting on the pancreatic SUR to increase (e.g., glinbeclamide) or decrease (e.g., diazoxide) intracellular Ca^{2+} (indirectly, *via* a K^+-ATP channel) cause corresponding increases and decreases in weight gain, although these effects have previously been attributed to the effects of these compounds on circulating insulin. However, the identification of SUR expression in human adipocytes (15) suggests that it may modulate adipocyte Ca^{2+} flux and thereby regulate lipid metabolism. Indeed, the SUR agonist glinbeclamide increases human adipocyte intracellular Ca^{2+} and thereby causes

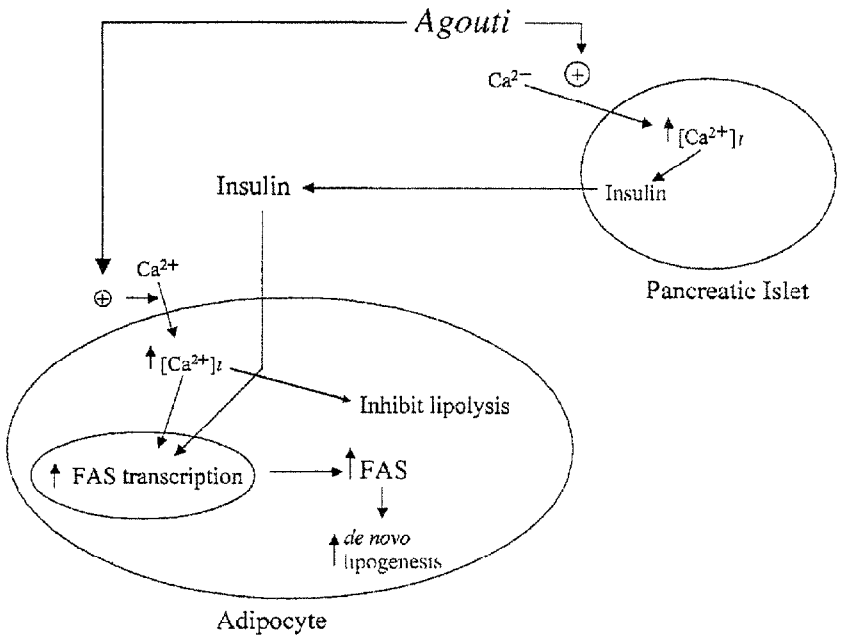

FIG. 1. Ca^{2+}-mediated mechanisms of *agouti* regulation of adiposity. FAS, fatty acid synthase.

marked increases in lipogenic enzyme activity and inhibition of lipolysis. Moreover, inhibition of the adipocyte SUR-regulated Ca^{2+} channel with diazoxide completely prevents each of these effects. Accordingly, the adipocyte SUR may represent a new target for the development of pharmacological interventions in obesity (15). In support of this concept, diazoxide has been demonstrated to exert significant antiobesity effects in both obese Zucker rats and hyperinsulinemic obese adults (25–27). Although this effect was attributed to actions on pancreatic β-cell insulin release, we subsequently found diazoxide treatment significantly suppresses adipose tissue FAS and lipoprotein lipase in obese Zucker rats (24).

ROLE OF 1,25-$(OH)_2$-D IN REGULATING ADIPOCYTE Ca^{2+} AND LIPID METABOLISM

Based on these findings and on our earlier observations of dietary calcium-induced reductions in adiposity, we proposed that the elevations in 1,25-(OH_2)-D that occur in response to low-calcium diets may stimulate adipocyte Ca^{2+} influx and thereby increase adiposity. Indeed, we recently reported that 1,25-(OH_2)-D stimulates significant Ca^{2+} influx and sustained dose-responsive increases in steady-state intracellular Ca^{2+} in primary cultures of human adipocytes (1). Moreover, treatment of human adipocytes with 1,25-(OH_2)-D resulted in a coordinated activation of FAS and inhibition of lipolysis, similar to the action of *agouti* on these cells (1,28). Consequently, suppression of 1,25-(OH_2)-D with high-calcium diets would be anticipated to reduce adipocyte intracellular Ca^{2+}, inhibit FAS, and activate lipolysis, thereby exerting an antiobesity effect. This concept is summarized in Figure 2.

DIETARY CALCIUM MODULATION OF ADIPOSITY

This concept was confirmed in transgenic mice expressing *agouti* in adipose tissue under the control of the aP2 promoter. Mice placed on low-calcium (0.4%)/high-fat/high-sucrose diets for 6 wk exhibited marked increases in adipocyte lipogenesis, inhibited lipolysis, and accelerated increases in body weight and adipose tissue mass. However, high-calcium (1.2%) diets reduced lipogenesis by 51% and stimulated lipolysis three- to fivefold, resulting in 26–39% reductions in body weight and adipose tissue mass (1). The magnitude of these effects depended on the source of dietary calcium, with dairy sources of calcium exerting significantly greater effects than calcium carbonate.

These data are consistent with our observation that 12 mon of yogurt supplementation, sufficient to raise daily calcium intake from approximately 400 to 1000 mg/d, resulted in a 4.9-kg reduction in body fat in obese African Americans without an accompanying reduction in caloric intake. The relevance of this finding at the population level was assessed *via* analysis of the National Health and Nutrition Examination Survey; odds ratios for percent body fat as a function of calcium intake were estimated by logistic regression, with age, race/ethnicity, activity level, and caloric intake as covariates. The odds of being in the highest quartile of body fat were reduced from 1.0 for the first quartile of calcium intake to 0.75, 0.40, and 0.16 for the second, third, and fourth quartiles of calcium intake, respectively, for women (1). The regression model for males similarly demonstrated a significant inverse relationship between dietary calcium and body fat, although the same simple dose–response relationship found in women was

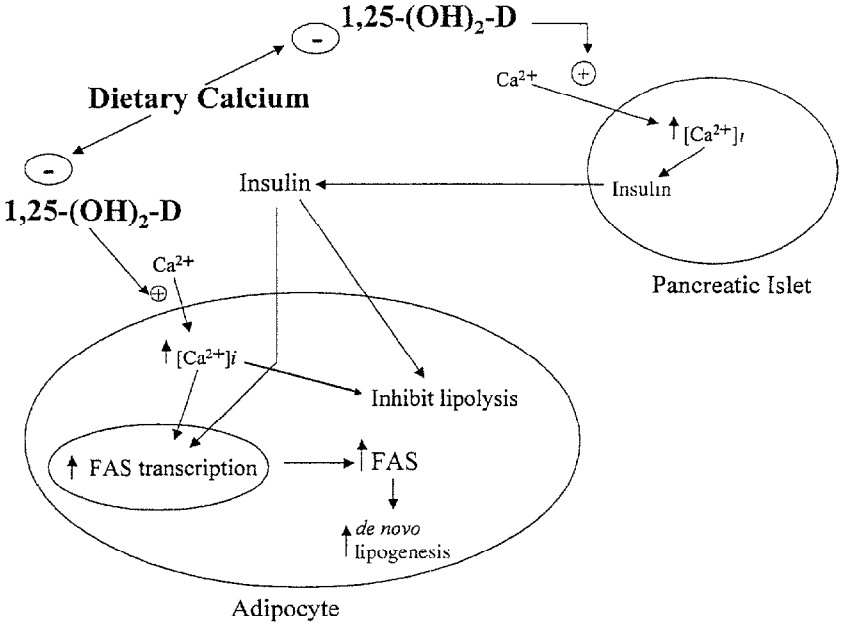

FIG. 2. Dietary calcium modulation of adiposity is mediated *via* 1,25-dihydroxy-vitamin D [1,25-(OH_2)-D] regulation of Ca^{2+} flux

not evident (1). Notably, the strength of the relationship was improved for both genders when dairy product intake was included in the analysis compared to when total calcium intake was evaluated without consideration of calcium source.

These data have significant implications for the prevention or attenuation of diet-induced obesity but do not directly address the issue of whether high-calcium diets will exert any effect on established obesity. Accordingly, we next conducted a study to extend these findings by determining whether dietary calcium and calcium-rich dairy products reduce metabolic efficiency and accelerate fat loss secondary to caloric restriction in the same mouse model following dietary induction of obesity (29). Mice (aP2-*agouti* transgenic) similar to those in the studies described above were used. They were fed the same basal low (0.4%)-Ca/high-fat/high-sucrose diet for 6 wk as in the preceding study.

Administration of the low-calcium (0.4%), high-fat, high-sucrose diet to aP2-*a* mice for 6 wk resulted in a ~100% increase in adipocyte $[Ca^{2+}]_i$ (128 ± 18 vs. 267 ± 15 nM, $P < 0.001$), with a corresponding body weight gain of 29% ($P < 0.001$) and twofold increase in total fat pad mass ($P < 0.001$), demonstrating that diet-induced dysregulation of adipocyte $[Ca^{2+}]_i$ is associated with increased adiposity in aP2-*a* mice.

All three calcium diets, including the high-calcium diet (1.2% Ca derived from $CaCO_3$), the medium-dairy diet (1.2% Ca derived from nonfat dry milk replacing 25% of protein), and the high-dairy diet (2.4% Ca derived from nonfat dry milk replacing 50% of protein), caused a 50% decrease in adipocyte $[Ca^{2+}]_i$ ($P < 0.001$). In contrast, $[Ca^{2+}]_i$ in adipocytes from mice maintained on the energy-restricted basal low-calcium diet remained at the same elevated level as that of animals fed *ad libitum*.

Energy restriction resulted in a body weight loss of 11% ($P < 0.001$), compared to the *ad libitum* group. However, markedly greater weight reductions of 19, 25, and 29% were observed in the high-calcium, medium-dairy, and high-dairy groups, respectively ($P < 0.01$ vs. basal energy-restricted group). Consistent with this result, energy restriction caused only 8% lower fat pad mass (not significant), compared to the basal diet *ad libitum* group, whereas the high-calcium diet caused a 42% decrease ($P < 0.001$), which was further reduced by 60 and 69% by the medium- and high-dairy diets ($P < 0.001$ vs. basal energy-restricted group), respectively.

The high-calcium diet caused a 35% decrease in FAS activity ($P < 0.05$ vs. basal energy-restricted group), which was further reduced by 63 and 62% by the medium- and high-dairy diets ($P < 0.05$), respectively; FAS mRNA followed a similar trend. Increasing dietary calcium caused a corresponding increase in lipolysis. Although the basal energy-restricted diet did not affect adipocyte lipolysis, the high-calcium diet caused 77% stimulation in lipolysis ($P < 0.05$), which was further increased in the medium- and high-dairy diet groups ($P < 0.05$ vs. basal energy-restricted group). Increased lipolysis, coupled with decreased lipogenesis, may represent a metabolic state in which the efficiency of energy metabolism is shifted from energy storage to energy expenditure.

This shift in energy metabolism was further confirmed by a dietary calcium-induced increase in core temperature. All three high-calcium diets exerted stimulatory effects on core temperature, with 0.48, 0.57, and 0.67°C increases on the

high-calcium, medium-dairy, and high-dairy diets, respectively ($P < 0.05$), whereas the basal energy-restricted diet did not affect core temperature. A possible physiological basis underlying the increased core temperature is that the expression of uncoupling protein 2 (UCP2), which has been implicated in thermogenesis, was upregulated in white adipose tissue, with an 80% increase in all three high-calcium diets ($P < 0.05$). However, the role of UCP2 in thermogenesis is not clear, and further study is required to address the precise mechanism whereby dietary calcium regulates UCP2 expression. Although this upregulation of UCP2 expression may result directly from inhibition of $[Ca^{2+}]_i$, it is also possible that it is merely a result of increased substrate (FA) flux secondary to increased lipolysis. However, we recently found that 1,25-$(OH)_2$-D directly suppresses UCP2 expression in isolated human adipocytes and that this effect is independent of FA flux (30).

Collectively, these data demonstrate that high-calcium diets suppress adipocyte $[Ca^{2+}]_i$ and thereby reduce energy storage and increase thermogenesis during energy restriction, with greater effects exerted by dairy products than by elemental calcium. Recent findings from other laboratories support a beneficial role for calcium in weight control. In a 2-yr prospective study of 54 normal-weight women participating in an exercise intervention, the dietary calcium/energy ratio was a significant negative predictor of changes in both body weight and body fat (31); moreover, increased total calcium and dairy calcium intakes predicted fat mass reductions independently of caloric intake for women at lower energy intakes (below the mean of 1876 kcal/d) (31). A similar beneficial effect of dietary calcium on body fat mass accumulation has been demonstrated in growing children, as a significant inverse relationship between dietary calcium and body fat was recently reported in a 5-yr longitudinal study of preschool children ($R^2 = 0.51$) (32).

Davies *et al.* (33) conducted a series of calcium intervention studies designed with primary skeletal end points, and have recently re-evaluated these data with a body weight end point. The re-analysis involved 780 women who participated in five clinical trials (i.e., four observational and one double-blind, placebo-controlled, randomized trial). They noted significant negative associations between calcium intake and body weight for all age groups (third, fifth, and eighth decades of life), and an odds ratio for being overweight of 2.25 for young women in the lower half vs. the upper half of calcium intake (33). Data from the randomized controlled trial demonstrated a calcium treatment effect of 0.325 kg weight loss per year over 4 yr with no intentional change in caloric intake; overall, the relationships derived from this re-analysis indicate that a calcium intake increase of 1,000 mg/d is associated with an 8-kg reduction in body weight (33).

We recently studied the efficacy of a calcium-fortified breakfast cereal, alone or with a small amount of milk, in attenuation of weight and fat gain in the aP2-*a* transgenic mouse (34). Male mice placed on a basal low-calcium (0.4%)/high-fat (25 energy %)/high-sucrose diet for 6 wk exhibited *ca.* twofold increases in $[Ca^{2+}]_i$ and both visceral and subcutaneous fat mass. However, addition of a calcium-fortified breakfast cereal sufficient to increase dietary calcium to 1.2% with macronutrient adjustments to ensure identical carbohydrate, protein, and fat levels with the basal diet resulted in a 41% decrease in adipocyte $[Ca^{2+}]_i$ ($P < 0.001$) and 25–30% decreases in weight gain ($P < 0.03$) and total fat pad mass compared to the basal diet ($P < 0.001$), whereas food consumption was unaffected. Comparable decreases were found in both subcutaneous and visceral fat compartments. A second control group, which received the basal diet supplemented with the same amount cereal without calcium fortification (with macronutrient adjustment) was not significantly different from the basal control group.

We also found the calcium-fortified cereal to have similar effects in markedly accelerating weight and fat loss secondary to caloric restriction in these mice. Interestingly, addition of sufficient nonfat dried milk to bring the calcium content of the calcium-fortified cereal diet from 1.2 to 1.3% (with macronutrient adjustment) resulted in substantial amplification of these effects. Thus, a calcium-fortified breakfast cereal is effective in reducing adiposity and accelerating fat loss during caloric restriction in this model of obesity, whereas addition of a small amount of milk significantly amplifies this effect further.

We recently confirmed the utility of calcium-rich diets in accelerating fat loss during a 6-mon clinical trial in obese patients (35). Obese adults ($n = 32$) were maintained for 24 wk on balanced deficit diets (500 kcal/d deficit) and were randomized to control (0–1 serving/d and 400–500 mg Ca/d supplemented with placebo), high-calcium (control diet supplemented with 800 mg Ca/d), or high dairy (3–4 servings of low-fat dairy products/d, total calcium intake of 1200–1300 mg/d). Control patients lost 6.4 ± 2.5% of their body weight, which was increased by 26% on the high-calcium diet and 70% (to 10.9 ± 1.6%) on the high-dairy diet ($P < 0.01$). Fat loss (*via* dual X-ray absorptiometry) followed a similar trend, with the high-calcium and high-dairy diets augmenting the fat loss found on the low-calcium diet by 38 and 64%, respectively ($P < 0.01$).

An unexpected finding was a marked change in the distribution of body fat loss (35). Patients on the low-calcium diet lost 5.3 ± 2.3% of their trunk (abdominal region) fat on the low-calcium diet. This was increased to 12.9 ± 2.2% on the high-calcium diet and 14.0 ± 2.3% on the high-dairy diet ($P < 0.025$ vs. low-calcium and high-calcium diets). Consequently, fat loss from the abdominal region represented 19.0 ± 7.9% of the total fat lost on the low-calcium diet, and this was increased to 50.1 ± 6.4% of the fat lost on the high-calcium diet ($P < 0.001$) and 66.2 ± 3.0% on the high-dairy diet ($P < 0.001$). Thus, increasing dietary calcium not only accelerates weight and fat loss secondary to caloric restriction but also shifts the distribution of fat loss to a more favorable pattern, with more fat lost from the abdominal region on the high-calcium diet. Moreover, dairy products exert a substantially greater effect on both fat loss and fat distribution compared to an equivalent amount of supplemental calcium.

Consistent with this, Melanson *et al.* (36) recently reported that higher calcium intakes are associated with higher rates of whole-body fat oxidation measured in a whole-room calorimeter, with significant effects noted over a 24-h period, during sleep, and during light exercise.

ROLE OF ADDITIONAL DAIRY-DERIVED BIOACTIVE COMPOUNDS

Data accumulated from experimental animal and human studies clearly support a beneficial role for dietary calcium in weight management, but markedly greater effects are evident from dairy products vs. nondairy sources of calcium. Although the additional components of dairy products responsible for the differential effects between calcium and dairy products are not yet know, work is under way to determine their identity. At present, preliminary data suggest that this additional activity resides in the whey fraction of milk. Whey is recognized as a rich source of bioactive compounds (37) that may act independently or synergistically with the calcium to attenuate lipogenesis, accelerate lipolysis, and/or affect nutrient partitioning between adipose tissue and skeletal muscle. Notably, whey proteins have recently been reported to contain significant angiotensin-converting enzyme (ACE) activity (38,39). Although ACE inhibitory activity may appear to be more relevant to an antihypertensive effect of dairy than to an antiobesity effect, recent data demonstrate that adipocytes have an autocrine/paracrine renin–angiotensin system (RAS), and that adipocyte lipogenesis is regulated, in part, by angiotensin II (reviewed in Ref. 40). Thus, activation or suppression of the adipocyte RAS may exert corresponding effects on adipocyte lipid metabolism independently of the circulating RAS. Indeed, inhibition of the RAS mildly attenuates obesity in rodents, and limited clinical observations support this concept in hypertensive patients treated with ACE inhibitors (40). Thus, it is possible that whey-derived ACE-inhibitory activity may contribute to the antiobesity effect of dairy products (41). However, it is also possible that other whey bioactive compounds may contribute or, alternatively, that a synergistic effect of multiple factors, along with the aforementioned effects of the calcium, are responsible. For example, Layman (42) has recently proposed that the rich concentration of leucine in whey protein may play a significant anabolic role in skeletal muscle and thereby contribute to greater maintenance of skeletal muscle mass during weight loss. Accordingly, the high concentration of leucine and other branched-chain amino acids in dairy products may also be an important factor in the repartitioning of dietary energy from adipose tissue to skeletal muscle.

CONCLUSION

A growing body of evidence now clearly demonstrates a beneficial role for dietary calcium in the partitioning of dietary energy, resulting in reductions in body fat and an acceleration of weight and fat loss during energy restriction. Interestingly, dairy sources of calcium exert substantially greater effects than supplemental or fortified sources of calcium. There is a strong theoretical framework in place to explain the "anti-obesity" effects of dietary calcium; however, the mechanism whereby dairy products augment this anti-obesity effect is not yet clear, although it may be mediated by whey peptides. These data have important implications for the prevention of both pediatric and adult obesity, especially in light of the marginal calcium intakes exhibited by the majority of the population (Fig. 3) and the population-based data indicating protection from obesity and the insulin resistance syndrome in populations consuming greater amounts of calcium and dairy products (1,43,44).

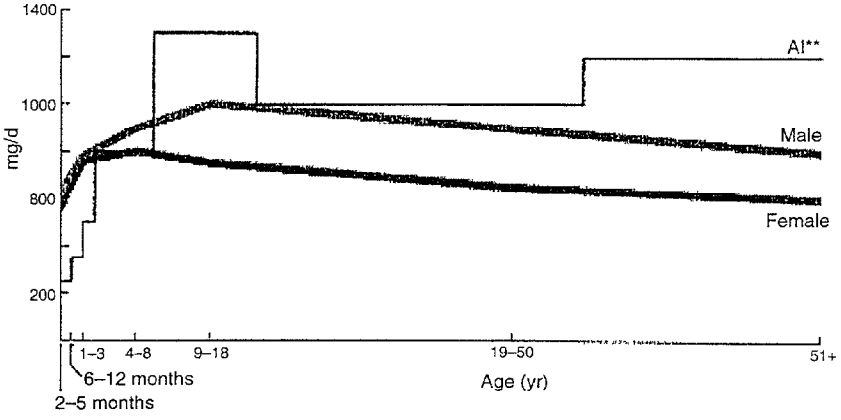

FIG. 3. Median calcium intake from food of the U.S. population, 1988–1994 **AI, adequate intake. 1997 Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine, National Academy of Sciences. *Source*: Centers for Disease Control/National Center for Health Statistics (CDC/NCHS), National Health and Nutrition Examination Survey (NHANES III) 1988–1994 Figure used by permission of the National Dairy Council.

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Review Article

Calcium intake and adiposity[1–3]

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ABSTRACT Limited epidemiologic and experimental data support the possibility that dietary calcium intake plays a role in human body weight regulation. The aim of this review was to present the data from human studies that link calcium and dairy intake to body weight, describe the existing evidence for an effect of calcium intake on body weight from animal models of obesity, present evidence of a role for intracellular calcium in the regulation of lipogenesis and lipolysis, elucidate the potential suggested relation between dietary calcium intake and intracellular calcium concentrations, and outline the effects of calcium supplementation on dietary fat absorption. We suggest that these data support the need for large, population-based clinical trials to assess the effects of supplemental calcium and other components of dairy products on human body weight. *Am J Clin Nutr* 2003;77:281–7.

KEY WORDS Calcium, dairy products, body weight, fatty acid synthase, fat absorption

INTRODUCTION

The prevalence of obesity in the United States has been steadily rising since the 1960s (1). In the past decade, the percentage of adults aged 20–74 y who are overweight or obese has increased to 61% (2). The total costs attributable to obesity-related disease approaches $100 billion annually in the United States (3), and this cost, like the prevalence of increased body mass, is rising at an alarming rate.

Although the characterization of several important obesity genes over the past 10 y has resulted in a quantum leap of insight into the pathophysiology of obesity (4), these studies have not led to any significant improvements in our ability to prevent or treat overweight. Genetic factors, it seems, have largely played only a secondary role in the rising prevalence of obesity. Rather, environmental factors affecting diet and activity appear likely to have been the most important determinants of the increasing adiposity of the US population over the past 30 y (5, 6). Studies seeking epidemiologic explanations for the phenomenon of rising adiposity have identified dietary calcium intake as one factor that is negatively correlated with body mass index (BMI; in kg/m^2) (7–12). In the following sections, we review data from human studies that link calcium and dairy intake to body weight, describe evidence of an effect of calcium intake on body weight from animal models of obesity (9, 13–16), outline the effects of calcium supplementation on dietary fat absorption (17–20), and present evidence of a role for intracellular calcium in regulating lipogenesis and lipolysis (21–23). In addition, we tried to elucidate the potential suggested relation between dietary calcium intake and intracellular calcium concentrations (9, 11, 21).

HUMAN STUDIES LINKING CALCIUM INTAKE TO BODY WEIGHT

Many epidemiologic studies have identified strong inverse correlations between adiposity and calcium intake (7–10, 12). The US Department of Agriculture's Nationwide Food Consumption Survey from 1987 to 1988 showed that the average dietary calcium intake in the United States (24) was far below the suggested optimal calcium intake (1000 mg/d for adults and 1200 mg/d for children and young adults aged 11–24 y) (25) and that persons with the lowest calcium intakes tended to have the highest body weight. When stratified by ethnic group, the non-Hispanic black population, which has one of the highest prevalences of obesity in the United States, was also found to have a lower mean daily calcium intake (592 mg/d) than either the Hispanic white population (653 mg/d) or the non-Hispanic white population (765 mg/d). Using data from the first National Health and Nutrition Examination Survey (NHANES I), McCarron (26) found a statistically significant inverse association between calcium intake and body weight. More recently, Zemel et al (9) found a strong inverse association between the relative risk of obesity and calcium intake for participants of NHANES III (**Figure 1**). Zemel et al's analysis controlled for physical activity and energy intake. They examined the relative risk of being in the highest quartile of body fat for 4 different quartiles of dietary calcium intake. The relative risk of high body adiposity was found to be greatest in those with the lowest calcium intake and was progressively lower as calcium intake increased; the relative risk was 0.75 for the 2nd quartile,

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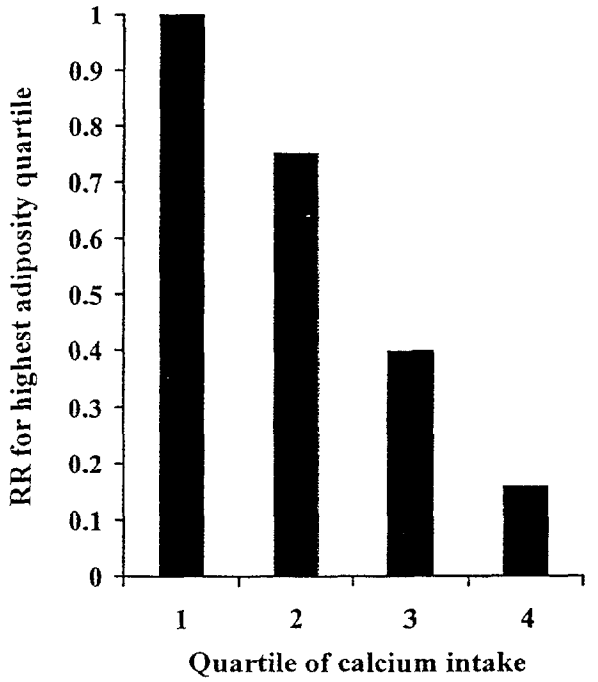

FIGURE 1. Relative risk (RR) of being in the highest adiposity quartile on the basis of the quartile of dietary calcium intake in participants of the third National Health and Nutrition Examination Survey. Adapted from reference 9.

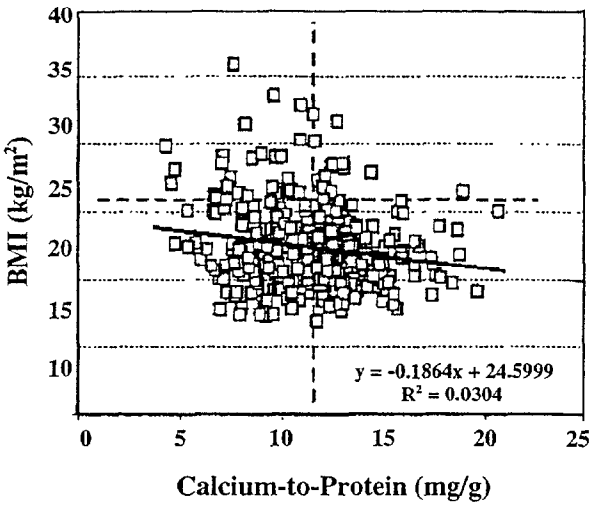

FIGURE 2. Relation between baseline BMI and the ratio of dietary calcium to protein intake in 348 women aged 30–40 y. The horizontal dashed line represents the boundary between normal and overweight, and the vertical line represents the median intake ratio. Reprinted with permission from reference 29.

0.40 for the 3rd quartile, and 0.16 for the 4th quartile of calcium intake for women ($n = 380$; $P < 0.0009$). A similar inverse relation was noted in men ($n = 7114$; $P < 0.0006$). Inverse associations between calcium (or dairy) intake and adiposity have also been reported in children (7, 8), Canadian women (27), and lactose-tolerant and lactose-intolerant African American women (28).

Davies et al (29) reviewed results from 5 clinical studies (30–33) of calcium intake, including an ongoing unpublished study (RP Heaney, Osteoporosis Research Center, Creighton University, Omaha), all of which were designed to assess the effects of dietary calcium on bone mineral. Depending on the study, age ranged from the third to the eighth decades of life. Taken together, the total population was 780 women. Significant negative associations between calcium intake and weight were found for all age groups, and the odds ratio for being overweight (BMI > 26) was 2.25 for women in the lower half of calcium intakes in their respective study groups ($P < 0.02$). For young women in the third decade of their lives, a significant negative association was found when baseline BMI was plotted against the ratio of calcium to dietary protein intake (**Figure 2**).

The 2 longitudinal observational studies mentioned above also enabled Davies et al (29) to examine how the change in body weight (in kg/y) was related to the initial dietary calcium intake. In each study the slope was significantly negative. In pooled data from the 2 studies (**Figure 3**), weight change was negatively related to calcium intake ($P = 0.008$). Another trial (34), not reviewed by Davies et al (29), noted that of 54 normal-weight young women participating in a randomized exercise intervention trial, subjects with higher dairy calcium intakes corrected for total energy intake gained less weight and body fat over a 2-y period.

To date, there have been no large trials designed primarily to examine the effects of dietary calcium supplements on body weight change. However, in one large trial of the effect of calcium supplementation on bone, in which elderly women were randomly assigned to take either placebo or 1.2 g elemental Ca/d as carbonate (33), the data have been retrospectively analyzed for changes in body weight (29). Although both study groups lost some weight over the nearly 4 y of observation, the mean (± SEM) weight change, weighted for duration of the study, was greater in the calcium-supplemented group (−0.671 ± 0.112 kg/y) than in the placebo-control group (−0.325 ± 0.110 kg/y), for an estimated calcium treatment difference of 0.346 kg/y ($P < 0.025$). This change in body weight was consistent with the predicted change found in the longitudinal observational studies reviewed by Davies et al (29). Because body-composition studies were not reviewed in this

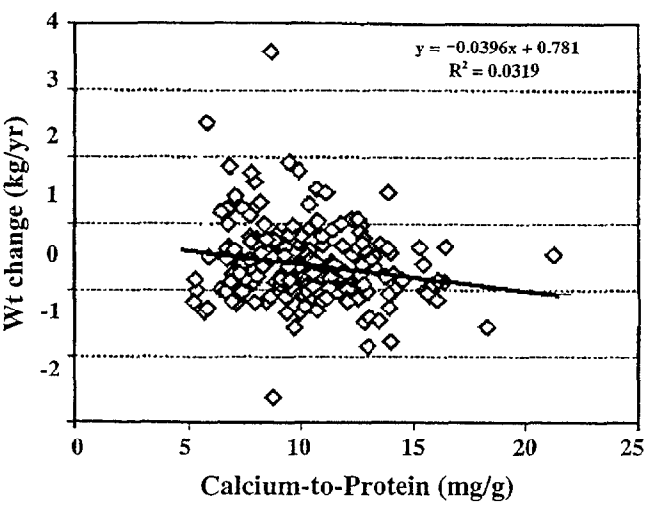

FIGURE 3. Weight (Wt) change versus the average ratio of dietary calcium to protein intake in 2 longitudinal observational studies in 216 middle-aged women. Reprinted with permission from reference 29.

study, it is unknown whether changes in body weight reflect changes primarily in fat or lean body mass.

Davies et al's (29) retrospective analysis of human studies suggests that calcium intake could explain as much as 3% of the variability in adult body weight. Although the reviewed studies predicted that body weight changes relatively little with differences in calcium intake, ≈0.35 kg/y, such effects could become substantial over time. A change in body weight of this magnitude is comparable with the yearly weight gain of ≈0.37 kg/y usually observed during midadulthood (35, 36).

Two recent experimental studies have reached opposite conclusions about the effects of calcium supplementation on weight loss during energy-restricted diets. In one study, presented in abstract form, 32 obese women given an energy-restricted diet (2.1 MJ/d energy deficit) were randomly assigned to a diet low in elemental calcium (400–500 mg/d) or to 1 of 2 diets high in elemental calcium (1300 mg/d; calcium supplemented or dairy supplemented) and were followed for a period of 24 wk (37). Women randomly assigned to either of the high-calcium diets had a significantly greater weight loss (largely due to the loss of fat mass) than did the control group. (The effects of diets high in dairy products are discussed in the following section.) By contrast, another randomized, controlled study that examined both weight and bone mineral changes in obese women during energy restriction (energy intake up to 6.1 MJ/d diet) found no significant differences in weight change over a 3-mo period between a high-calcium-supplemented group ($n = 25$; total calcium intake: 1800 mg/d) and a lower-calcium control group ($n = 27$; total calcium intake: 800 mg/d) (38). The reason for discordance between the results of these 2 studies is not clear, but could be explained by the presence of a threshold value below which insufficient dietary calcium affects body weight gain or possibly by differences in the experimental design, such as the method used for calcium supplementation.

DAIRY INTAKE AND ADIPOSITY

Some recent findings in animals (9, 15, 16) and in humans (37) suggest that there may be greater effects on body weight from dairy-containing foods than might be predicted from their calcium content alone. Although a full discussion of these data are beyond the scope of this review, a few selected epidemiologic and human experimental studies supporting these findings are presented. A recently published multicenter, population-based, prospective observational study (12) found that increased dairy consumption had a strong inverse association with the 10-y cumulative incidence of obesity (ie, BMI = 30) and with the insulin-resistance syndrome in overweight adults (BMI ≥ 25 at baseline; $n = 923$). The odds of obesity, abnormal glucose homeostasis, and elevated blood pressure were ≈20% lower at each additional daily occasion of dairy consumption, whereas the odds of developing the insulin-resistance syndrome were lower by 21%. A recent study, published in abstract form (37), compared the relative effects of supplemental calcium and dairy products for 24 wk on weight loss during energy restriction in 32 obese adults. Body weight loss was 26% greater in the high-calcium group (control diet: 400–500 mg Ca/d supplemented with 800 mg elemental Ca/d) but was 70% greater in the high-dairy group (total elemental calcium intake: 1200–1300 mg/d) than in the placebo control group (total elemental calcium intake: 400–500 mg/d) ($P < 0.01$). When compared with the low-calcium diet, fat loss (by dual-energy X-ray absorptiometry) with the high-calcium and the high-dairy diets was augmented by 38% and 64%, respectively ($P < 0.01$). The subjects who consumed the high-calcium diet and the high-dairy diet also showed a significantly greater ($P < 0.001$) fat loss in the trunk area than did those who consumed the low-calcium diet. Another abstract (39) reported that women with the greatest intake in dietary calcium (primarily in the form of dairy products) had significantly greater weight losses than did those with lower calcium (dairy) intakes as a result of a 6-mo behavioral weight-loss program ($n = 181$ overweight women aged 24–45 y). The mechanisms explaining the greater effects of dairy products relative to calcium supplementation remain unclear. The bioavailability of calcium from dairy sources is not considered to be greater than that of calcium supplied as nondairy foods, except for calcium from a few plant sources with a high phytate or high oxalate content, which can interfere with calcium absorption (40, 41). It is therefore possible that dairy products contain other components unrelated to calcium that affect body weight (11, 34). Thus, future studies should determine the effects on body weight of the components of dairy products other than calcium.

EFFECTS OF DIETARY CALCIUM ON BODY WEIGHT AND ADIPOSITY IN ANIMAL MODELS

Studies in the 1980s in spontaneous hypertensive rats found a lower net weight gain in the rats fed a high-calcium diet (2.8%, wt:wt) than in the rats fed a low-calcium diet (0.4%, wt:wt): 9.1 ± 1.8 and 27 ± 2 g, respectively (13). Shortly afterward, it was observed that diets high in both dietary calcium and sodium induced favorable changes in the total body fat content of spontaneous hypertensive rats and its normotensive genetic control, Wistar-Kyoto rats (14). More recently, Zemel et al (9, 15, 16) studied transgenic mice with an overexpression of the agouti gene (42), specifically in adipocytes. In one of these studies (9), Zemel et al examined the effects of various calcium intakes on weight gain over 6 wk (**Figure 4**). Weight change in the low-calcium group (0.4% Ca) was compared with that in 3 calcium-supplemented groups in which calcium was given as either dietary calcium carbonate (1.2% Ca) or as dairy products (nonfat dry milk, either 1.2% or 2.4% Ca). Weight gain and fat-pad mass were reduced by 26% with the 1.2%-Ca diet ($P < 0.04$ compared with the 0.4%-Ca diet), by 29% with the 1.2%-Ca diet ($P < 0.04$ compared with the 0.4%-Ca diet), and by 39% with the 2.4%-Ca diet ($P < 0.04$ compared with all other diets). These data suggest that, at least for mice expressing excess agouti protein in the adipocytes, an increase in dietary calcium decreases body weight gain. When adipocyte function in fat cells isolated from such transgenic animals was examined, high-calcium diets were associated with a 51% inhibition of adipocyte fatty acid synthase (EC 2.3.1.85) expression and activity ($P < 0.002$) and a 3.4- to 5.2-fold ($P < 0.015$) augmentation of basal lipolysis. When energy-restricted transgenic mice with an overexpression of the agouti gene were studied (15), a significantly greater reduction in body weight, fat-pad mass, and basal intracellular calcium concentrations in the adipocytes was seen after consumption of a high-calcium or high-dairy diet; the decrease in total body weight and fat pad mass was greater with the high-dairy diet. The rate of lipogenesis was suppressed, whereas that of lipolysis was stimulated, more in the high-dairy group than in the high-calcium group, whereas the results in both groups were significantly different from those in the basal energy-restricted group.

Although these data relate only to animals with an overexpression of the agouti gene in their adipocytes, the observed changes

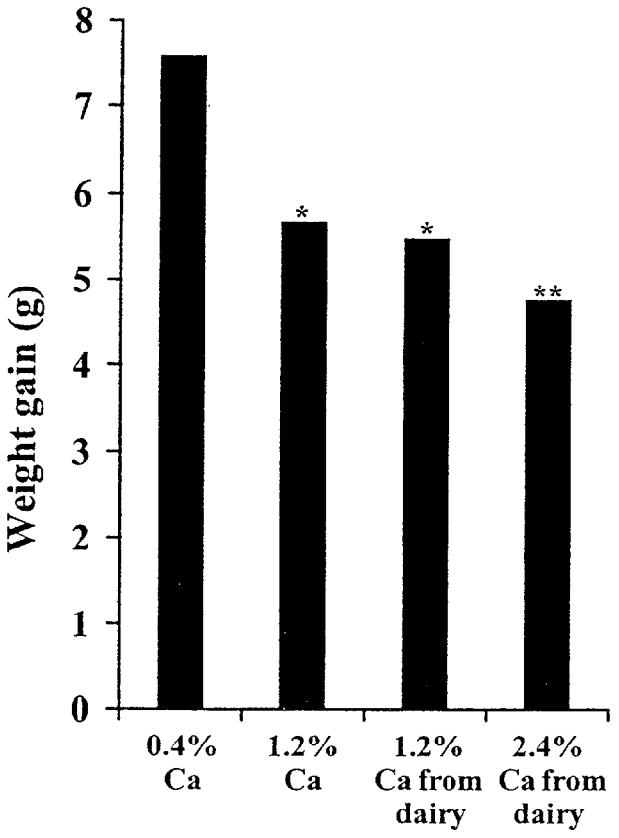

FIGURE 4. Weight gain in transgenic mice expressing the agouti gene, specifically in adipocytes, who were fed diets with different calcium contents. *Significantly different from the 0.4%-Ca diet, $P < 0.04$. **Significantly different from all other diets, $P < 0.04$. Adapted from reference 9.

in intracellular calcium in their adipocytes suggest that changes in intracellular calcium concentrations may be an important part of the effect of calcium and dairy intakes on adiposity in humans. A brief summary of some of the actions of intracellular calcium in adipocytes is given in the next section.

ROLE OF INTRACELLULAR CALCIUM IN HUMAN ADIPOCYTE LIPID METABOLISM

Intracellular calcium ($[Ca^{2+}]i$) concentrations are determined by complex interactions between the flux through voltage-dependent and receptor-stimulated calcium channels, by sequestration with binding proteins, by storage of free Ca^{2+} in intracellular compartments such as the endoplasmic reticulum, and by active gradient-maintaining ion pumps (43). $[Ca^{2+}]i$ appears to play an important role in the metabolic derangements associated with obesity, hypertension, and insulin resistance (21, 44, 45). Factors important in obesity, such as insulin (44) and the agouti protein (22, 46)—normally expressed in human adipocytes (47)—have been shown to trigger an increase in $[Ca^{2+}]i$ in human adipocytes (**Figure 5**). Obese persons have a greater $[Ca^{2+}]i$ than do nonobese age- and sex-matched control persons (44). $[Ca^{2+}]i$ was also found to regulate both lipogenesis and lipolysis in human adipocytes (21). High $[Ca^{2+}]i$ stimulates the expression and activity of fatty acid synthase, a key enzyme in de novo lipogenesis (21). When

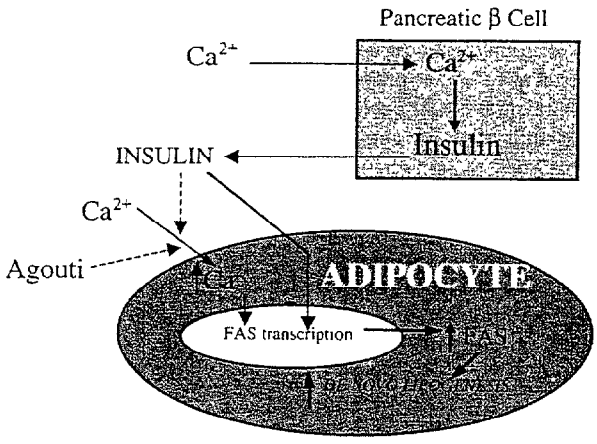

FIGURE 5. Suggested role for the agouti protein, insulin, and intracellular calcium in lipogenesis in human adipocytes. FAS, fatty acid synthase (EC 2.3.1.85). Adapted from reference 21.

potassium chloride is used to increase $[Ca^{2+}]i$ in human adipocytes, agonist-stimulated lipolysis is inhibited through activation of phosphodiesterase 3B (EC 3.1.4.17), thereby reducing cyclic AMP concentrations and thus decreasing hormone-sensitive lipase phosphorylation (23).

With regard to calcium homeostasis, the calcium-regulating hormones vitamin D and parathyroid hormone (PTH) have both been shown to stimulate a significant and sustained increase in $[Ca^{2+}]i$ concentrations in primary cultures of human adipocytes (9). In addition 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$] treatment results in a marked (83%) inhibition of forskolin-stimulated lipolysis in human adipocytes and a 35% reduction in basal lipolysis (48). Shi et al (48) suggested that there are rapid nongenomic actions of 1α,25$(OH)_2D_3$ via a putative membrane vitamin D receptor that play a significant role in vitamin D–induced increases in $[Ca^{2+}]i$. Shi et al found that 1α,25-dihydroxylumisterol$_3$, a specific agonist for the membrane vitamin D receptor, increased $[Ca^{2+}]i$, fatty acid synthase activity and glycerol-3-phosphate dehydrogenase (EC 1.1.1.94) expression and inhibited lipolysis in human adipocytes, whereas the specific membrane vitamin D receptor antagonist 1β-hydroxyvitamin D_3 blocked vitamin D–stimulated increases in $[Ca^{2+}]i$. PTH treatment, although it does increase $[Ca^{2+}]i$, exerts little effect on lipolysis, possibly as a result of the concurrent activation of adenylate cyclase (EC 4.6.1.1) by PTH (9).

RELATION BETWEEN DIETARY AND INTRACELLULAR CALCIUM

The 2 preceding sections outlined the diametrically opposed effects of increases in $[Ca^{2+}]i$ and increases in dietary calcium. Greater $[Ca^{2+}]i$ stimulates lipogenesis and inhibits lipolysis. Greater dietary calcium appears to have opposite effects. If the mechanism through which dietary calcium affects body weight is primarily related to the actions observed within the adipocytes of agouti-expressing mice exposed to greater dietary calcium intakes, there must be a physiologic basis for the dissociation between $[Ca^{2+}]i$ concentrations and dietary calcium intake. One possible explanation that would link greater dietary calcium to less $[Ca^{2+}]i$ is the effect of dietary calcium on the hormones regulating calcium balance. Dietary calcium supplementation in humans has

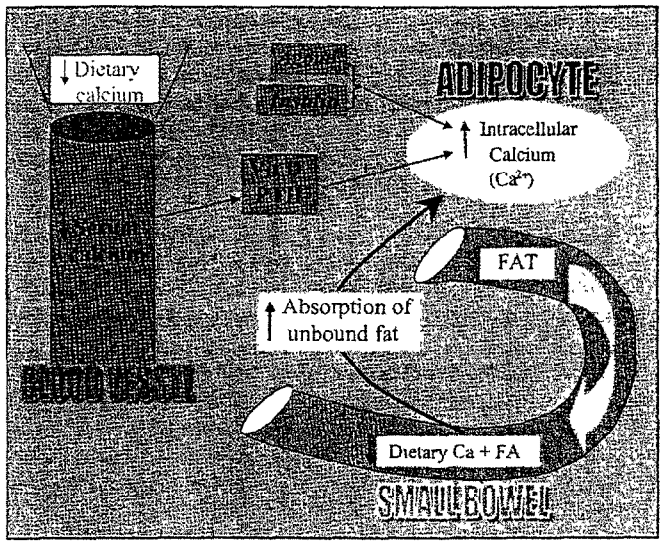

FIGURE 6. Proposed mechanisms through which decreased dietary calcium may increase body weight. Low dietary calcium may increase intraadipocyte calcium concentrations via stimulation of calcitropic hormones such as vitamin D (Vit D) and parathyroid hormone (PTH). Decreased dietary calcium also decreases saponification of fatty acids (FAs) in the gut bound to calcium and thus may increase body fat stores by increasing the absorption of fat.

been shown to cause significant suppression of intact PTH, $1\alpha,25(OH)_2D_3$, and the $[Ca^{2+}]i$ in erythrocytes and platelets (49). Thus, increased calcium intakes lower blood concentrations of calcitropic hormones, such as 1,25-dihydroxyvitamin D [$1,25(OH)_2$] and PTH, whereas, as is well known, diets deficient in calcium stimulate the production and release of $1,25(OH)_2$ and PTH. Bell et al (50) previously reported significantly higher concentrations of immunoreactive PTH and $1,25(OH)_2$ in obese (106 ± 6 kg; $n = 12$) white subjects than in normal-weight (68 ± 2 kg; $n = 14$) control subjects, whereas elevated concentrations of these hormones were previously reported in obese children compared with age-matched control subjects (51). Thus, lower dietary calcium intakes (as found epidemiologically for obese subjects) can lead to increased concentrations of $1,25(OH)_2$ and PTH, which in turn may increase adipocyte $[Ca^{2+}]i$ (**Figure 6**). These elevated intraadipocyte calcium concentrations might then increase the rate of lipogenesis and inhibit lipolysis, consequently leading to increased adiposity. An increased dietary calcium intake would be proposed to prevent this cascade from developing by keeping the calcitropic hormone concentrations low, therefore lowering $[Ca^{2+}]i$ and ultimately the lipid content in adipocytes.

RELATION BETWEEN DIETARY CALCIUM AND DIETARY FAT ABSORPTION

A second mechanism by which dietary calcium intake might affect body adiposity is an effect on the absorption of triacylglycerol from the gastrointestinal tract. Denke et al (18) studied the effect of dietary calcium on fecal fatty acid excretion and serum lipids in a randomized, single-blind, metabolic study of 13 men with moderate hypercholesterolemia. In this study, a low-calcium diet (410 mg elemental Ca/d) was compared with a high-calcium diet (2200 mg elemental Ca/d) using calcium citrate maleate as a source for the supplemental calcium for 10 d. Calcium fortification increased the percentage of dietary saturated fat excreted in 72-h fecal collections from 6% to 13% per day. The high-calcium diet also significantly reduced total cholesterol by 6%, LDL cholesterol by 13%, and apolipoprotein B concentrations by 7% when compared with the low-calcium diet ($P < 0.05$). A 1-y randomized controlled trial in postmenopausal women using 1 g elemental Ca (as calcium citrate) also found a 19% increase ($P = 0.0009$) in the HDL-LDL ratio compared with the placebo group (52). This suggests that the effects of increased calcium intake on lipids may be long lasting. Welberg et al (19) studied the effects of calcium supplementation on quantitative and qualitative fecal fat excretion in 24 subjects consuming a controlled diet (1450–1880 mg Ca/d) that was supplemented with 0, 2, or 4 g elemental Ca/d (given as calcium carbonate). Calcium increased fecal fatty acids in a dose-dependent fashion. Total fat excretion increased from $6.8 \pm 0.9\%$ of total fat intake with no calcium supplementation to $7.4 \pm 1.0\%$ with 2 g Ca and $10.2 \pm 1.4\%$ with 4 g elemental Ca ($P = 0.03$). Increased fat excretion was due to greater fatty acid excretion; the excretion of neutral fat remained changed. Other studies found similar effects (20).

These studies of calcium's effects on fecal fat excretion predict small effects on total-body lipid flux. The degree of fecal fat loss induced by 2 g elemental Ca in Welberg et al's (19) study is only $\approx$3% of that induced by lipase inhibitors such as orlistat (53–55). A person consuming a 2500-kcal diet containing one-third of energy from fat who took an additional 2 g elemental Ca/d as calcium carbonate might be expected to excrete an additional 1% of energy from fat per day and would be anticipated to lose $\approx$12.6 MJ/y (3010 kcal/y) in the stool. Because a 14.64-MJ (3500 kcal) excess or deficit is often quoted as the energy gained or lost when body weight changes by 0.45 kg (1 lb), this amount of lost energy might indeed explain a change in body weight of ≈ -0.4 kg/y. Thus, these data suggest that supplemental calcium–induced fecal fatty acid excretion may have accounted for much (if not all) of the observed weight loss in the calcium-supplemented subjects of Davies et al's randomized trial (29). However, the effects of calcium on fat excretion are not sufficient to explain the much greater weight differences suggested by some animal and human studies, particularly those supplying calcium in the form of dairy products (9, 15, 37).

SUMMARY

In this article, we reviewed the evidence supporting a role for dietary calcium and possibly dairy intake in the regulation of body adiposity. With regards to dietary calcium, epidemiologic and limited experimental data from some studies suggest that differences in calcium intake may be associated with changes in body weight of $\approx$0.35 kg/y (29). The binding of fatty acids in the gut by dietary calcium can decrease fat absorption sufficiently to account for a similar weight change. More recent data, however, point to a much greater magnitude of weight loss with the calcium supplementation of energy-restricted adults, especially when calcium supplementation is achieved through dairy sources (37). Such findings may indicate an independent effect of another component of dairy products (11, 34), but the mechanism for the augmented weight losses from dairy consumption remains unclear.

Limited data from the animal models of obesity described above also suggest that dietary calcium intake may conceivably affect the regulation of lipogenesis and lipolysis within adipocytes.

Dietary calcium might alter lipid flux by lowering plasma concentrations of calcitropic hormones (vitamin D and PTH), which are known to modulate human intraadipocyte calcium concentrations and thereby affect the rate of lipogenesis and lipolysis.

Regardless of the actual mechanism involved, most of the available cross-sectional, longitudinal, observational, and small controlled trials in humans and the available animal studies support the conclusion that dietary calcium may play a role in body weight regulation and lend credence to the hypothesis that increasing dietary calcium or dairy intake may diminish future weight gain. So far, only small clinical trials designed specifically to examine the effects of either dietary calcium or dairy intake on body weight or adiposity have been done. Given the increasing prevalence of obesity along with its significant medical consequences, the importance of environmental factors in the rapid rise in the prevalence of obesity, and the relative cost-effectiveness and safety profile of calcium and dairy supplementation, we believe that well-designed, population-based clinical trials should be carried out to determine whether the body weight of overweight adults can be altered by either dietary calcium or dairy product supplementation.

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Symposium: Dairy Product Components and Weight Regulation

Calcium Intake and Reduction in Weight or Fat Mass[1]

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ABSTRACT Obesity is a growing epidemic with subsequent health consequences leading not only to reduced quality of life but also to increased medical costs. Growing evidence supports a relationship between increased calcium intakes and reductions in body weight specific to fat mass. Since the first observations in rats >10 y ago, several recently published clinical studies support this relationship as well. The impact of calcium intake on weight loss or prevention of weight gain has been demonstrated in a wide age range of Caucasian and African-Americans of both genders. This review focuses on the results of clinical trials that have investigated the impact of calcium and dairy products on prevention of weight gain, weight loss or development of the insulin resistance syndrome. The implications of these results are that calcium may play a substantial contributing role in reducing the incidence of obesity and prevalence of the insulin resistance syndrome. J. Nutr. 133: 249S–251S, 2003.

KEY WORDS: • *calcium* • *weight* • *fat mass* • *review* • *dairy*

The incidence of obesity has rapidly increased in the last 20 y and has become a national and global epidemic. It is a risk factor for chronic diseases such as heart disease, cancer, stroke and diabetes and weight loss is known to reduce the risk for some of these diseases. Although much effort has been devoted to studying the effects of macronutrients on weight control, the role of micronutrients has not been as well studied. Although energy balance is the most critical factor in weight regulation, recent studies suggest that calcium metabolism and perhaps other components of dairy products may contribute to shifting the energy balance and thus play a role in weight regulation. This review discusses the impact of calcium intake on body composition measures presented in clinical studies.

Although the focus of this review is on the impact of calcium in clinical trials, our interest in the area was piqued by two animal studies published >10 y ago (1,2). In 1988 Metz et al. (1) demonstrated a reduction in body fat mass in two strains of hypertensive rats' higher calcium intakes (in conjunction with a higher sodium intake). In addition, in 1989, an abstract by Bursey et al. (2) reported that increasing calcium in the diet from 0.1 to 2.0% resulted in a reduced weight gain in both lean and fatty Zucker rats. However, until recently this suggested relationship between calcium intake and body fat remained unexplored. After the presentation of two abstracts (3,4) at the same national meeting in 1999, substantial data have emerged to support this interesting and unexpected relationship between calcium intake and body fat mass.

The original studies in rats from the 1980s prompted our laboratory to take advantage of the data generated previously in a randomized study investigating the impact of a 2-y exercise intervention on bone mass in young women (5), to explore the relationship of calcium intake on changes in body composition (6). In the parent study, healthy normal weight 18–31 y old women were randomized into an exercise or nonexercise group after baseline testing. Three-day diet records were collected at baseline and 6-mo intervals, and averaged over the 2-y period of the study. Total body bone mineral content was assessed by dual X-ray absorptiometry, allowing an analysis of body composition changes as well. The results of 54 women who completed the 2-y trial were used. Calcium intakes were low (781 ± 212 mg/d), compared to the dietary reference intakes (1000 mg/d for most of this group), and the primary source of dietary calcium was from dairy intake (67%). When dietary calcium was expressed as a nutrient density (calcium/energy, g/kcal), it negatively predicted changes in body weight and body fat, but not lean mass. Dairy calcium predicted the changes as well as did nondairy calcium; however, the range of nondairy calcium intakes was low and may not have been sufficient to demonstrate a relationship.

To further explore why calcium intake predicted the changes only when corrected for calorie intakes, women were categorized into groups either above or below the mean caloric intake of the cohort (1876 kcal/d). Calcium intake did not predict changes in weight or fat mass in the group with calorie intakes above the mean, whereas calories positively predicted these changes; thus the higher the calories, the greater the increase in body fat. On the other hand, calcium, but not calories, negatively predicted changes in weight and fat mass

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in women with caloric intakes below the mean. Between 10 and 13% of the variability in weight and fat mass changes were accounted for by the calcium or dairy calcium intakes. The biological impact can be demonstrated by use of the resulting regression equation to estimate potential changes. In women with calorie intakes of <1876 calories/d, calcium intakes of 1000 mg/d predicted a body fat loss of −2.6 kg over 2 y compared to a gain of ~1.8 kg at calcium intakes of 500 mg/d. These changes are substantial in normal weight young women and thus have important implications for the prevention of obesity.

Clearly, if dairy products are added to a diet without compensation for energy intake, one is likely to gain weight. This is shown in the study by Barr et al. (7), in which 204 men and women, aged 55–85 y, were randomized to either a control group or a dairy intervention group. The dairy intervention group was advised to increase skim or 1% milk intake from <1.5 servings to three servings/d. Although their overall nutrient intakes improved substantially, the dairy intervention group also gained 0.6 kg in the 12-wk trial, significantly more than did the control group. However, this gain was less than would be predicted by the increase in dairy products, suggesting that either the subjects altered their diets to compensate for the additional calories, or potentially that calcium or dairy shifted the energy balance to partially compensate for the additional calories.

Subsequently, other studies have been reanalyzed that, similar to our study, were not originally designed to study body fat mass. The results of these studies have supported the potential impact of calcium intake on body fat across a wide age range in both men and women. For example, dietary intakes of 53 children from the age of 24 to 60 mo were studied, followed by assessment of body fat mass at 70 mo (8). The higher the dietary intake of calcium, the lower the body fat mass at 70 mo. Davies et al. (9) reanalyzed five clinical trials that included ages ranging from the third to the eighth decade, to assess the impact of dietary calcium/protein intake on body weight or body mass index (BMI). These analyses included two cross-sectional, two longitudinal and one randomized, controlled trial totaling 780 women. In every case, the calcium/protein ratio negatively predicted either BMI or change in weight. Zemel et al. (10) used the NHANES III survey to further assess the impact of calcium on body fat. The analysis of women showed that the odds ratio of being in the highest body fat quartile was significantly reduced to 0.16 if they were in the highest calcium intake quartile. These results support the negative impact of calcium intake on body fat and its application to a variety of age groups.

This relationship has been noted in both men and women. One of the first observations of a potential relationship between calcium intake and body fat was noted in men > 10 y ago, similar to the rat studies described above. In this study, obese African-American men (n = 11) were randomized into a 1-y calcium intervention by adding yogurt to their diet to bring their calcium intakes to ~1000 mg/d (10). At the end of the intervention, the men on the yogurt diet had significantly lower body fat compared to those men on the basal diet containing a calcium intake of ~500 mg/d. However, these results remained unpublished until recently. The analysis by Zemel et al. (10) that made use of the NHANES III data demonstrated a similar relationship in men as was noted in women; that is, the odds ratio of being in the highest body fat quartile was significantly reduced if the men were in the highest calcium intake quartile. These results demonstrate that increased calcium intakes were associated with reduced body fat in men who were participating in both a large epidemiological study and a small intervention trial.

In addition to the study in African-American men noted above, a similar relationship between calcium intake and body fat was observed in African-American women (11). In this cross-sectional study, the higher the calcium/kcal ratio, the lower the BMI (R^2 = 0.47) in premenopausal lactose-tolerant women (n = 26). Thus, the associative relationship of calcium and body weight is also apparent in African-Americans.

The growing interest in this field is evidenced by the rapid increase in recent related abstracts or publications, each adding support for the relationship. The Quebec Family Study categorized both men (n = 235) and women (n = 235) aged 20–65 y by their calcium intakes into low (<600 mg/d), medium (600–1000 mg/d) or high (>1000 mg/d) (12). The women in the low calcium intake category were significantly higher than the other two groups in weight, percentage of body fat, fat mass, BMI, waist circumference and total abdominal adipose tissue. Another recent abstract described the results of a double-blind, placebo-controlled 3-y dietary calcium intervention study in young women (n = 52). Results showed that those women in the calcium supplement (1500 mg/d) group had a reduced body fat increase over the intervention period compared to that of placebo controls, further supporting the relationship between calcium and body weight (13). Again, this study was originally designed to assess the impact of calcium intake on bone mass in young women.

The impact of calcium may be particularly beneficial during weight loss, as evidenced by the results presented elsewhere in this symposium (14). However, dairy products, the predominant source of calcium in the U.S. diet, are commonly avoided in weight loss diets. Further evidence for this trend, and the potential importance in reducing this trend, are suggested by another recent abstract describing 181 overweight women aged 24–45 y who were enrolled in a 6-mo behavioral weight loss study (15). Their mean intake of calcium dropped from 833 to 681 mg/d during the weight loss trial. However, being in the highest quartile of calcium intake significantly predicted the change in body weight (R^2 = 0.12). These results as well as those of Zemel (14) suggest that calcium, and perhaps dairy products, should not be removed from weight loss diets, but instead may enhance the effects of the diet.

Finally, recent results generated from the Coronary Artery Risk Development in Young Adults (CARDIA) Study suggest an intriguing and exciting negative relationship between calcium intake or dairy consumption and obesity and insulin resistance syndrome (IRS) in young adults (16). In this prospective study, 3157 black and white adults aged 18 to 30 y were followed for 10 y. Dairy product intake was negatively associated with the cumulative incidence of IRS, including development of obesity, abnormal glucose homeostasis, elevated blood pressure and dyslipidemia, in overweight, but not in leaner participants. Neither lifestyle factors, race nor gender influenced the results. The investigators estimated that each additional serving of dairy products was associated with 21% lower odds of IRS. These interesting results provide support for an impact of dairy consumption, which may or may not be attributed to the calcium content, on reducing the incidence of important risk factors for chronic diseases.

In conclusion, the current and rapidly growing body of evidence is substantial and supports the relationship of dietary calcium intake to reductions in weight and body fat mass. However, it is important to confirm these observations in studies specifically designed to address this issue and in larger trials. It is also important to further understand the underlying mechanism(s) for this effect, and to determine whether the

impact is greater in certain subgroups or while the energy balance is shifting. These results may have a substantial impact of increased calcium intake on reducing the incidence of obesity, as described in the analysis elsewhere in this symposium (17). Finally, studies to confirm the impact of dairy products on the development of IRS, suggested by the recently published study by Pereira et al. (16) are necessary. Although energy balance is the most important factor, if these results are confirmed, increasing the low dairy product and calcium intakes in the United States may greatly contribute to reducing the growing epidemic of obesity and IRS.

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Symposium: Dairy Product Components and Weight Regulation

Normalizing Calcium Intake: Projected Population Effects for Body Weight[1,2]

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ABSTRACT Published data describing the inverse relationship between calcium intake and body weight in 564 women were evaluated for their dispersion around their means, and the fraction above any given weight or rate of weight gain was calculated from the parameters of the normal distribution for the variable concerned. At the 25th percentile of calcium intakes, 15% of young women were overweight, and that fraction fell to only 4% at calcium intakes in the range of currently recommended values. Similarly, obesity prevalence in this cohort fell from 1.4 to 0.2% across the same difference in calcium intakes. At midlife, women at the 25th percentile of intakes gained weight, on average, at a rate of 0.42 kg/y. This gain dropped to −0.011 kg/y at currently recommended calcium intakes. Although calcium intake explains only a small fraction of the variability in weight or weight gain, shifting the mean of the distributions downward by increasing calcium intake can be estimated to reduce the prevalence of overweight and obesity by perhaps as much as 60–80%. J. Nutr. 133: 268S–270S, 2003.

KEY WORDS: • *obesity* • *overweight* • *weight gain* • *calcium intake*

Recent reports have shown an inverse relationship between calcium intake and body fat mass (1–6). Although several of these studies have been observational in nature (and hence unable by themselves to establish definitively that changing calcium intake would change body weight), published reports describe at least three randomized controlled trials, all of which were positive. Hence, although much more needs to be learned, it now seems reasonably well established that high calcium intakes can reduce the risk of being obese and assist in making weight loss regimens more effective.

Obesity is recognized to be multifactorial in character, and calcium intake has been variously estimated to explain from 3% to perhaps as much as 10% of the total variation in adult weight, a relatively small portion of the total variability. Perhaps a more important question, however, is how much difference normalizing calcium intake would make in the prevalence of obesity or overweight in the population. This study presents a preliminary attempt, using published data, to answer this question.

SUBJECTS AND METHODS

The data behind the publication of Davies et al. (3) were reevaluated, focusing on the distribution of values around the regression lines relating calcium intake to body mass index (BMI) in young women and to weight gain at midlife. In both groups of women, calcium intake was obtained from 7-d diet records, and was expressed as the calcium-to-protein ratio (mg:g). This stratagem partially corrects for portion size estimation error; at the same time it adjusts for the countervailing effects of calcium and protein intakes on calcium balance in the range of calcium intakes commonly encountered (7).

The young women constituted two cohorts (total n = 348), one studied in 1984–1985 and the other in 1995–1997, both on entry into studies designed to test skeletal endpoints. For the studies in these women, BMI was taken as the dependent variable. The middle-aged women also constituted two cohorts (total n = 216), each followed prospectively without intervention, one for a mean of 8.5 y and the other for a mean of 21.7 y. In these women rate of change in weight (kg/y) was taken as the dependent variable. Both groups of women have been characterized more fully elsewhere (3).

For both data sets the distribution of values for BMI and weight gain were tested for normality and suitably transformed, as needed. The fraction of the population represented by these samples above any specified value was calculated from the integral of the normal distribution for the respective means and standard deviations. Error terms for these fractions were calculated from the confidence intervals of the slope of the relationship of the dependent variable on calcium intake, in each case at the specifically tested calcium intakes. The ap-

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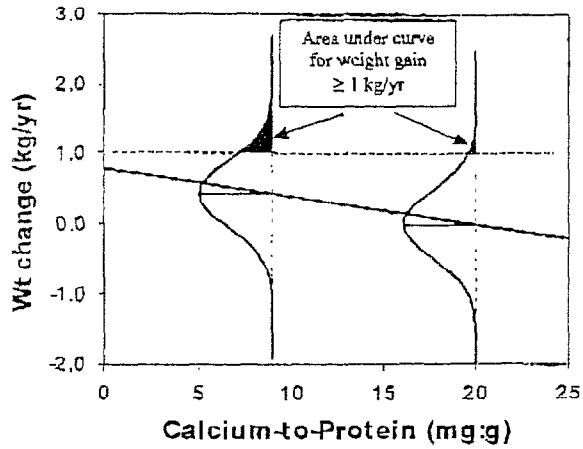

FIGURE 1 Plot of the regression line relating the dietary calcium: protein ratio to weight gain in midlife women, from the data of Davies et al. (3). Superimposed on this regression line are plots of the normal distribution with means at predicted weight gain for calcium:protein ratios of 9 and 20 mg Ca:g protein, and with standard deviations set to the standard error of the estimate for the regression. The areas under the two curves for weight gain ≥ 1 kg/y (the horizontal dashed line) are shaded. (Copyright Robert P. Heaney, 2002. Used with permission.)

proach is illustrated graphically in Figure 1 for the data set from the middle-aged cohort.

RESULTS

Table 1 presents the area under the curve of the distribution of BMI values equal to or >26 kg/m^2 and equal to or >30 kg/m^2, respectively, both at calcium-to-protein ratios of 10 and 20 mg:g. (The former represents the approximate 25th percentile of calcium intakes for this group, and the latter is approximately the currently recommended calcium intake for third-decade women.) Because the distribution of BMI was not normal, the data were log transformed and the foregoing calculations were performed on the transformed data. As can be seen, predicted BMI at 10 mg Ca:g protein was 22.5 and the probability of having a BMI ≥ 26 was 0.146. This probability drops to 0.041 at 20 mg Ca:g protein, and the relative risk of being overweight at the approximate 25th percentile of calcium intakes, relative to the recommended intake, is thus 3.6. Similarly, the probabilities of being obese (BMI ≥ 30) are 0.014 and 0.002 for the two calcium intakes, for a relative risk of 6.9.

Table 2 presents corresponding data for weight gain at midlife, at calcium-to-protein intake ratios of 9 and 20 mg:g, respectively. (The former is the approximate 25th percentile and the latter, approximately the currently recommended intake for middle-aged women.) As can be seen, the predicted weight gain at 9 mg Ca:g protein was 0.425 kg/y, and about one sixth of the women gained at a rate of 1 kg/y or greater. By contrast, the mean predicted change at 20 mg Ca:g protein is actually slightly negative (−0.011 kg/y), and only 3.7% would be predicted to gain at a rate of 1 kg/y or more. The risk of such gain for the lower calcium intake level, relative to recommended intakes, is thus 4.2.

DISCUSSION

The data presented in this analysis suggest that the prevalence of obesity (or weight gain) in women could be reduced by 60–80% by the simple stratagem of ensuring population-wide calcium intakes at the currently recommended levels. However, it must be stressed that there is a great deal of uncertainty around such an estimate, principally because so few women were available in the two cohorts studied to provide weight or weight gain data at the recommended calcium intakes. For this reason any distributional analysis at recommended calcium intakes must involve a certain amount of extrapolation, always a risky enterprise.

Moreover, it must be stressed that, although this analysis adds new insights into the data assembled by Davies et al. (3), it does not add new information. The 564 individuals who contributed data for this analysis are the same subjects reported on by Davies et al. In this instance, however, the data from controlled trials are helpful, given that intakes in these trials are in the desired range, and the observed weight changes are at least directionally consistent with the estimates derived in this study.

Also reassuring in this regard is the analysis of the NHANES-III data earlier reported by Zemel et al. (1) After adjusting for age, sex, race and energy intake, they found a stepwise reduction in risk of obesity for each quartile of calcium intake. At the highest quartile (approximately equal to current recommendations for calcium), the risk of being in the highest BMI quartile was reduced by about 85%. Here the investigators had access to sufficient numbers of individuals at the recommended calcium intakes, and the observed reduction in their prevalence of obesity is quite similar to the estimate developed in this analysis. Consistent with this finding, also, is the recent report of the CARDIA study group (8) that dairy consumption was inversely associated with body weight in a prospective study of over 3000 young adults.

The absolute prevalence of obesity in our sample of young women was relatively low, only nine out of 348 individuals, or slightly <3%. These women were entered into their respective trials 18 and 7 y ago, respectively, and it is known that obesity prevalence in this age range has increased substantially since then (9,10). This means that the distribution of weight has shifted upward and that both the predicted mean values and the population fraction above any given BMI level will probably be higher today than the values presented in Table 1. Whether the slope of BMI on calcium intake will have changed since then cannot be determined from the data analyzed here. However, the response to calcium supplementa-

TABLE 1

BMI and calcium intake in young women

Ca-to-protein ratio (mg:g)	Predicted BMI	Fraction ≥ 26 kg/m^2	Confidence interval	Fraction ≥ 30 kg/m^2	Confidence interval
10	22.5	0.146	0.106–0.152	0.014	0.0087–0.0153
20	19.3	0.041	0.032–0.051	0.002	0.0014–0.0029

TABLE 2

Weight gain and calcium intake at midlife

Ca-to-protein ratio (mg:g)	Predicted weight change (kg/y)	Fraction gaining ≥ 1 kg/y	Confidence interval
9	+0.425	0.154	0.124–0.189
20	−0.011	0.037	0.027–0.049

tion in contemporary trials (2) indicates that the inverse relationship found in these women remains operative today.

The observation, both evident here and previously noted (4), that mean weight gain at midlife is effectively zero if calcium intake is at currently recommended levels is a fortuitous confirmation of the approximate adequacy of those recommendations. It is fortuitous in the sense that the currently recommended intakes were pegged to a skeletal endpoint, and there is no a priori reason to expect that all systems would exhibit the same requirement. It is also interesting to note that, despite the established bone protective benefit of an adequate calcium intake, the data presented here suggest that the effect on obesity prevalence—unrecognized until recently—is likely to be as large as, or larger than, the corresponding effect on osteoporosis prevalence.

Both the skeletal and the weight benefits are manifestations of the pleomorphic effects of dietary calcium, the bases for which are only now becoming clear and which were described in detail elsewhere (11). At the same time, they illustrate, tangentially, a point made by Geoffrey Rose nearly 20 y ago (12) that, when the bulk of the population is exposed to any given, but unrecognized, harmful influence, usual studies of apparent causation are able to identify, not the true etiology, but only predisposing factors (i.e., the reasons why some succumb to the disease and others do not). Low calcium intakes in this case are so widespread in the North American population today that virtually everyone is exposed to that influence. If, as seems increasingly likely, these low intakes are inadequate, then correcting calcium intake at a population level would produce benefits for many body systems. Furthermore, some of the factors currently considered to be causative of the diseases concerned will likely turn out to be only predisposing or triggering factors, operating by exaggerating or uncovering the effects of the real cause, inadequate calcium intake.

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Calcium intake, body composition, and lipoprotein-lipid concentrations in adults[1–3]

Mélanie Jacqmain, Eric Doucet, Jean-Pierre Després, Claude Bouchard, and Angelo Tremblay

ABSTRACT

Background: Recent data suggest that variations in calcium intake may influence lipid metabolism and body composition.

Objective: The association between daily calcium intake and body composition and plasma lipoprotein-lipid concentrations was studied cross-sectionally in adults from phase 2 of the Québec Family Study.

Design: Adults aged 20–65 y (235 men, 235 women) were studied. Subjects who consumed vitamin or mineral supplements were excluded. Subjects were divided into 3 groups on the basis of their daily calcium intake: groups A (<600 mg), B (600–1000 mg), and C (>1000 mg).

Results: Daily calcium intake was negatively correlated with plasma LDL cholesterol, total cholesterol, and total:HDL cholesterol in women and men after adjustment for variations in body fat mass and waist circumference ($P < 0.05$). In women, a significantly greater ratio of total to HDL cholesterol ($P < 0.05$) was observed in group A than in group C after correction for body fat mass and waist circumference. In women, body weight, percentage body fat, fat mass, body mass index, waist circumference, and total abdominal adipose tissue area measured by computed tomography were significantly greater ($P < 0.05$) in group A than in groups B and C, even after adjustments for confounding variables. Comparable trends were observed in men, but not after adjustment for the same covariates.

Conclusion: A low daily calcium intake is associated with greater adiposity, particularly in women. In both sexes, a high calcium intake is associated with a plasma lipoprotein-lipid profile predictive of a lower risk of coronary heart disease risk compared with a low calcium intake. *Am J Clin Nutr* 2003;77:1448–52.

KEY WORDS Calcium, body weight, adiposity

INTRODUCTION

Human studies have shown negative relations between high calcium intake and obesity-related metabolic disorders such as hypertension (1–4) and diabetes and insulin resistance (3–7). Other data show an inverse association between calcium intake and body weight (8–10) and the risk of becoming obese (11). Furthermore, some research groups have reported an inverse association between calcium consumption and body fat, particularly in women (10–12) and in children (13–15). Finally, animal models have provided mechanistic insight as to how low calcium intakes could influence body fat stores (11, 16, 17).

The plausible relation between the amount of calcium ingested in the diet and adipocyte intracellular calcium $[Ca^{2+}]_i$ was examined by Zemel et al (4, 11, 18, 19). In brief, an inverse relation between dietary calcium and $[Ca^{2+}]_i$ was found. It appears that an increase in dietary calcium intake results in a decrease in $[Ca^{2+}]_i$, which in turn increases lipolysis (11). In contrast, low calcium consumption induces high blood parathyroid hormone and 1,25-dihydroxyvitamin D concentrations, which could increase $[Ca^{2+}]_i$ in human adipocytes, switching their metabolism from lipolysis to lipogenesis (4, 11). Thus, an increase in $[Ca^{2+}]_i$ appears to promote triacylglycerol accumulation in adipocytes by exerting a coordinated control over lipogenesis and lipolysis (18). The increase in $[Ca^{2+}]_i$ would suppress the latter, resulting in lipid storage and adipocyte hypertrophy.

Most of the data on calcium intake and body composition in humans are observational (9, 11, 15, 20), although the results remain useful for the formulation of new hypotheses. Furthermore, the potential relation between calcium intake and plasma lipoprotein-lipid concentrations has not been investigated. Thus, the present study was performed to further investigate the relation between daily calcium intake and direct measures of body composition as well as to test the hypothesis of an association between dietary calcium intake and plasma lipoprotein-lipid concentrations.

SUBJECTS AND METHODS

Subjects

This study is based on data obtained from 235 men and 235 women aged 20–65 y, who were recruited in phase 2 (1991–1998) of the Québec Family Study. Subjects who regularly consumed vitamin or mineral supplements were excluded from the study. However, the questionnaire on food habits that was used in this study did not permit us to specifically identify calcium supplement consumers among the subjects who reported consumption of nutrient supplements. Therefore, consumers of all types of dietary and nutrient supplements were excluded. For some analyses, participants were divided into 3 groups on the basis of their daily calcium consumption: group A (<600 mg), group B (600–1000 mg), and group C (>1000 mg). The classification of subjects was a priori decided in accordance with our intent to compare subjects with either a calcium intake markedly below nutrient reference intakes or above adequate calcium intakes. The cutoffs of 600 and 1000 mg Ca/d

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TABLE 1
Descriptive characteristics of women and men divided into 3 groups by daily calcium intake[1]

Variable	Women[2]			Men[2]		
	Group A ($n = 52$)	Group B ($n = 113$)	Group C ($n = 70$)	Group A ($n = 36$)	Group B ($n = 94$)	Group C ($n = 105$)
Age (y)	43.5 ± 1.6	38.8 ± 1.2	36.7 ± 1.5	45.3 ± 2.0	43.2 ± 1.4	37.9 ± 1.3
Body weight (kg)	82.3 ± 3.3[a]	69.8 ± 1.9[b]	65.0 ± 2.7[b]	86.8 ± 3.5	83.0 ± 2.0	82.5 ± 2.0
BMI (kg/m^2)	31.8 ± 1.2[a]	27.0 ± 0.7[b]	25.2 ± 1.0[b]	28.8 ± 1.1	27.7 ± 0.6	27.6 ± 0.7
Percentage body fat (%)	37.3 ± 1.6[a]	31.3 ± 0.9[b]	28.9 ± 1.2[b]	24.6 ± 1.5	23.5 ± 0.9	23.7 ± 0.9
FM (kg)[3]	32.4 ± 2.5[a]	23.6 ± 1.4[b]	19.8 ± 1.9[b]	21.2 ± 2.4	20.5 ± 1.3	21.2 ± 1.3
FFM (kg)[3]	48.9 ± 1.2	45.7 ± 0.7	44.4 ± 0.9	62.1 ± 1.5	60.8 ± 0.8	61.7 ± 0.8
Waist circumference (cm)	93.6 ± 2.6[a]	82.0 ± 1.6[b]	78.4 ± 2.2[b]	98.0 ± 2.7	94.0 ± 1.6	94.2 ± 1.6
Abdominal AT (cm^2)[4]	552.2 ± 40.8[a]	405.7 ± 24.4[b]	373.3 ± 33.7[b]	356.5 ± 39.5	340.2 ± 21.9	362.3 ± 22.4
Mean calcium intake (mg/d)	448.0 ± 12.3[a]	789.0 ± 9.9[b]	1286.8 ± 31.3[c]	455.0 ± 23.4[a]	773.5 ± 11.0[b]	1426.4 ± 36.1[c]

[1] $\bar{x}$ ± SEM. Variables of body composition were adjusted for age, daily energy intake, percentage dietary fat, dietary protein, and socioeconomic status by analysis of covariance. FM, fat mass; FFM, fat-free mass, AT, adipose tissue. Within a sex group, values in the same row with different superscript letters are significantly different, $P < 0.05$.

[2] Group A, <600 mg Ca/d; group B, 600–1000 mg Ca/d; and group C, >1000 mg Ca/d.

[3] $n = 214$ women and 216 men

[4] Cross-sectional area measured by computed tomography.

appeared justified because they also allowed a sufficient statistical power within each group. The Québec Family Study received approval from the Laval University Medical Ethics Committee, and written informed consent was obtained from each participant.

Anthropometric measurements

Waist circumference was measured according to Lohman et al (21), whereas body weight was measured with a standard beam scale. The closed-circuit helium dilution method (22) was used to assess residual lung volume. Body density was determined by hydrodensitometry (23), and the Siri formula (24) was used to estimate the percentage body fat from body density. Fat mass (FM) was calculated from the derived percentage body fat and total body weight. Fat-free mass (FFM) was calculated by subtracting FM from body weight.

Computed tomography measurements

Computed tomography (CT) was performed with a Siemens Somaton DRH scanner (Siemens, Erlangen, Germany) according to the method described by Sjöström et al (25). Briefly, subjects were examined in the supine position with both arms stretched above their head. CT scans were performed at the abdominal level (between the L4 and L5 vertebrae). Abdominal adipose tissue (AT) was calculated by delineating the area with a graph pen and then computing the total AT surface with an attenuation range of −190 to −30 Hounsfield units (25), as previously described (26)

Dietary record

Daily energy, macronutrient, and micronutrient intakes were determined by using a 3-d dietary record, as previously described (27). Information was subsequently coded, and the energy, macronutrient, and micronutrient contents of the diet were calculated with the Canadian Nutrient File (28). The dietary journal was completed on 2 weekdays days and 1 weekend day.

Plasma lipids and lipoproteins

Serum blood lipids were determined from blood samples collected at 0800 after the subjects had fasted overnight for 12 h. Total cholesterol and triacylglycerol concentrations were determined enzymatically with the use of commercial kits, as described elsewhere (29). HDL-cholesterol and LDL-cholesterol concentrations were analyzed after precipitation of LDL in the infranatant fluid with heparin and magnesium chloride (30). The ratio of total cholesterol to HDL cholesterol was also derived as a lipid index of ischemic heart disease risk (31).

Statistical analysis

JMP software 3.1.6.2. (SAS Institute, Inc, Cary, NC) was used for all analyses. The values for men and women were analyzed separately. Pearson's correlations were calculated between daily calcium intake and all body-composition variables [body weight, body mass index (BMI), FM, FFM, percentage body fat, waist circumference, and abdominal AT] and plasma lipoprotein-lipid variables (HDL cholesterol, LDL cholesterol, triacylglycerol, total cholesterol, and total:HDL cholesterol). Correlations were subsequently calculated with the residual scores between daily calcium intake and body-composition variables after taking into account the effects of age, daily energy intake, percentage dietary fat, dietary protein, and socioeconomic status (total income and highest academic level). Moreover, correlations were performed with the residual scores between daily calcium intake and plasma lipoprotein-lipid concentrations after control for FM and waist circumference.

A one-way analysis of variance was used to test for differences in body weight, BMI, FM, FFM, percentage body fat, waist circumference, and abdominal AT between the groups with different calcium intakes. A one-way analysis of covariance was used to control for a series of covariates (age, daily energy intake, percentage dietary fat, dietary protein, and socioeconomic status), which can potentially affect energy balance and body weight control. The one-way analysis of variance and analysis of covariance were also used to compare the plasma lipoprotein-lipid profile (HDL cholesterol, LDL cholesterol, triacylglycerol, total cholesterol, and total:HDL cholesterol) across the subgroups of daily calcium intake with FM and waist circumference as covariates. When a statistical difference was detected, a Tukey's test was then performed to assess specific differences between groups. All values are expressed as means ± SEMs.

RESULTS

The descriptive characteristics of the 3 calcium intake subgroups, by sex, are shown in **Table 1**. After adjustment for age,

TABLE 2
Plasma lipid-lipoprotein concentrations in women and men divided into 3 groups by daily calcium intake[1]

Variable	Women[2]			Men[2]		
	Group A	Group B	Group C	Group A	Group B	Group C
HDL cholesterol (mmol/L)	1.29 ± 0.05	1.36 ± 0.03	1.37 ± 0.04	1.06 ± 0.05	1.09 ± 0.03	1.11 ± 0.03
LDL cholesterol (mmol/L)	3.27 ± 0.13	3.03 ± 0.08	2.88 ± 0.10	3.43 ± 0.15	3.36 ± 0.09	3.08 ± 0.08
Triacylglycerol (mmol/L)	1.43 ± 0.09	1.25 ± 0.06	1.24 ± 0.07	1.86 ± 0.17	1.70 ± 0.10	1.61 ± 0.09
Total cholesterol (mmol/L)	5.19 ± 0.16	4.94 ± 0.10	4.80 ± 0.12	5.32 ± 0.17	5.20 ± 0.10	4.88 ± 0.09
Total:HDL cholesterol	4.16 ± 0.14[a]	3.81 ± 0.09[a,b]	3.69 ± 0.11[b]	5.23 ± 0.23	5.01 ± 0.14	4.64 ± 0.13

[1] $\bar{x}$ ± SEM. Variables were adjusted for fat mass and waist circumference by analysis of covariance. Within a sex group, values in the same row with different superscript letters are significantly different, $P < 0.05$.
[2] Group A, <600 mg Ca/d; group B, 600–1000 mg Ca/d; and group C, >1000 mg Ca/d

daily energy intake, percentage dietary fat, dietary protein, and markers of socioeconomic status, the women who consumed <600 mg dietary Ca/d had greater values of body weight, BMI, percentage body fat, FM, waist circumference, and abdominal AT than did those with daily calcium intakes >600 mg ($P < 0.05$). No significant differences were found across subgroups of men. Women and men with the lower calcium intake were 6–7 y older than the group with the highest calcium intake. This finding agrees with the significant correlation that was observed between age and adiposity in both women ($r = 0.40$, $P < 0.01$) and men ($r = 0.42$, $P < 0.01$) and justifies the statistical adjustment for age in the present study.

A comparison of the plasma lipoprotein-lipid profile among the subgroups of men and women, classified by daily calcium intake, is shown in **Table 2**. In women, group A had a significantly greater ratio of total to HDL cholesterol ($P < 0.05$) than did group C, whereas no significant differences were observed for HDL cholesterol, LDL cholesterol, triacylglycerol, or total cholesterol between groups. No significant differences in plasma lipoprotein-lipid concentrations were found between subgroups of men.

Calcium intakes in women and men were 861.8 ± 22.8 and 1016.4 ± 30.3 mg/d, respectively ($P < 0.01$). As expected, most of the dietary calcium was derived from dairy products. In women, 61.8% of the daily calcium intake was from milk, cheese, yogurt, ice cream, pudding, desserts with milk, and soups prepared with milk. In men, 59.5% of the daily calcium intake was provided by the same dairy products. In both sexes, bread and cereals contributed 11% and 12% of daily calcium intake, respectively. Other foods contributed smaller amounts of calcium.

Simple correlations and adjusted correlations between daily calcium intake and body-composition variables in women and men are provided in **Table 3**. After correction for confounding variables such as age, daily energy intake, percentage dietary fat, dietary protein, and markers of socioeconomic status, significant correlations persisted only in women. Thus, adjusted correlations were significant for percentage body fat ($P < 0.01$), FM ($P < 0.05$), BMI ($P < 0.05$), and waist circumference ($P < 0.05$). Trends were also observed for FFM ($P = 0.08$). For men, after control for the same covariates, no significant association with daily calcium intake was observed.

Simple correlations and adjusted correlations between daily calcium intake and plasma lipoprotein-lipid concentrations in women and men are shown in **Table 4**. In women, after adjustment for FM and waist circumference, LDL cholesterol, total cholesterol, and the ratio of total to HDL cholesterol were all inversely correlated with daily calcium intake ($P < 0.05$). In men, after control for the same covariates, LDL cholesterol, total cholesterol, and the ratio of total to HDL cholesterol were also negatively correlated with calcium intake ($P < 0.01$).

DISCUSSION

This study was performed to examine the association between daily calcium intake and body composition and plasma lipid-lipoprotein concentrations in both women and men. Our results are generally consistent with recent data, which show a potential effect of calcium intake on body weight and FM in humans (8, 9, 11–15). One of the intriguing observations in the present study is

TABLE 3
Correlations and adjusted correlations between daily calcium intake and body-composition variables in women and men[1]

	Percentage body fat	FM	FFM	BMI	Waist circumference	Abdominal AT[2]
Correlations						
Women, calcium intake	−0.17[3]	−0.11	0.01	−0.07	−0.07	−0.17[3]
Men, calcium intake	−0.20[4]	−0.10	0.25[4]	0.00	−0.05	−0.02
Adjusted correlations[5]						
Women, calcium intake	−0.19[4]	−0.17[3]	−0.12[6]	−0.14[3]	−0.15[3]	−0.10
Men, calcium intake	−0.10	−0.09	0.02	−0.09	−0.10	0.04

[1] FM, fat mass; FFM, fat-free mass; AT, adipose tissue.
[2] Cross-sectional area measured by computed tomography.
[3] $P < 0.05$.
[4] $P < 0.01$.
[5] After correction for age, daily energy intake, percentage dietary fat, protein intake, and socioeconomic status.
[6] $P = 0.08$.

TABLE 4
Correlations and adjusted correlations between daily calcium intake and plasma lipid-lipoprotein concentrations in women and men

	HDL cholesterol	LDL cholesterol	Triacylglycerol	Total cholesterol	Total.HDL cholesterol
Correlations					
Women, calcium intake	0.06	−0.20[1]	−0.13[2]	−0.19[1]	−0.19[1]
Men, calcium intake	0.10	−0.24[1]	−0.11	−0.24[1]	−0.22[1]
Adjusted correlations[3]					
Women, calcium intake	0.03	−0.18[1]	−0.08	−0.16[2]	−0.15[2]
Men, calcium intake	0.09	−0.26[1]	−0.11	−0.26[1]	−0.24[1]

[1] $P < 0.01$
[2] $P < 0.05$.
[3] After correction for the effects of fat mass and waist circumference.

that the significant relations with dietary calcium were observed mainly in women. These observations, however, agree with those of Teegarden et al (12) and Zemel et al (11). As shown in Table 1, body weight, BMI, percentage body fat, FM, waist circumference, and abdominal AT were all significantly greater in women reporting a low calcium intake (<600mg/d). This was observed despite adjustments for a series of potentially confounding variables.

As proposed by Zemel et al (4, 11), a low calcium intake could also influence calcitrofic hormones. In humans, a rise in parathyroid hormone and 1,25-dihydroxyvitamin D favors an increase in $[Ca^{2+}]_i$-promoting lipogenesis (4, 11). Conversely, a high calcium intake results in lower blood parathyroid hormone and 1,25-dihydroxyvitamin concentrations and an increase in lipolysis (4, 11).

Our study is the first to show a difference in the lipoprotein-lipid profile by daily dietary calcium intake, independently of adiposity. Thus, in women and in men, LDL cholesterol, total cholesterol, and the ratio of total to HDL cholesterol were inversely correlated with daily calcium intake. The ratio of total to HDL cholesterol was significantly greater in women who consumed lower amounts of calcium (groups A and B) than in group C (Table 2). Accordingly, a recent study of postmenopausal women showed a beneficial effect of calcium citrate on blood lipids (32). These data strongly suggest that the effects of calcium on the lipolysis-lipogenesis balance as well as on plasma lipid and lipoprotein concentrations warrant further investigation.

Zemel et al (11, 16) studied the implication of the agouti protein on the regulation of $[Ca^{2+}]_i$. Agouti stimulates Ca^{++} influx and promotes energy storage in adipocytes by stimulating the expression and activity of fatty acid synthase, an enzyme involved in lipogenesis, and by inhibiting lipolysis in a Ca^{++}-dependent rat model (11). This model is useful for the assessment of calcium regulation in adipocytes of rodents, but human studies are also needed to test these pathways.

The relation between dietary calcium intake, adiposity, and lipoprotein-lipid metabolism may also be affected by sex hormones. Indeed, variations in plasma estrogen concentrations were recently found to be associated with those in intestinal calcium absorption (33, 34). This could affect dietary calcium availability and result in significant metabolic changes long term.

As we reported previously, men who consumed micronutrient supplements had a lower mean body weight (8.5 kg) than did nonconsumers of supplements (35). Thus, we could expect a potential role of other micronutrients on variations in energy balance and body composition. Nevertheless, the influence of calcium on daily and resting energy expenditure and on feeding behavior (eg, level of satiety, level of hunger, desire to eat, and prospective food consumption) should be considered in future research.

As expected, dietary calcium was mainly provided by dairy products in both men and women. Because these foods are good sources of fat and protein, which are known to affect both energy balance and adiposity (36, 37), analyses were performed by correcting for variations in these 2 nutrients. However, as indicated above, this statistical adjustment did not alter the calcium-adiposity relation, suggesting that the potential effect of calcium on body fatness and lipid metabolism is independent of the macronutrient content of dairy products.

In summary, dietary calcium intake is associated with body composition, particularly in women who report a low calcium intake. Moreover, the plasma lipoprotein-lipid profile in both women and men is apparently affected by a low calcium intake, independently of the concomitant variation in body fatness. We conclude that dietary calcium should be considered in the study of the regulation of energy balance if a more complete picture of the factors predisposing to obesity is to be achieved. More research is needed to establish whether there is a causal association between calcium intake, body composition, and plasma lipoprotein-lipid concentrations.

MJ reviewed the relevant literature, performed the statistical analyses, interpreted the data, and drafted the manuscript. AT, J-PD, and CB were involved in the study design and data collection and revised the manuscript. ED contributed to the statistical analyses and to the interpretation of the global issue of micronutrient supplementation and revised the manuscript. None of the authors had a personal interest or a potential personal conflict.

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PAPER

Relation between calcium intake and fat oxidation in adult humans

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OBJECTIVE: To determine if total calcium (Ca^{2+}) intake and intake of Ca^{2+} from dairy sources are related to whole-body fat oxidation.
DESIGN: Cross-sectional study.
SUBJECTS: A total of 35 (21 m, 14 f) non-obese, healthy adults (mean ± s.d., age: 31 ± 6 y; weight: 71.2 ± 12.3 kg; BMI: 23.7 ± 2.9 kg m^{-2}; body fat: 21.4 ± 5.4%).
MEASUREMENTS: Daily (24 h) energy expenditure (EE) and macronutrient oxidation using whole-room indirect calorimetry; habitual Ca^{2+} intake estimated from analysis of 4-day food records; acute Ca^{2+} intake estimated from measured food intake during a 24-h stay in a room calorimeter.
RESULTS: Acute Ca^{2+} intake (mg · $kcal^{-1}$) was positively correlated with fat oxidation over 24 h ($r = 0.38$, $P = 0.03$), during sleep ($r = 0.36$, $P = 0.04$), and during light physical activity ($r = 0.32$, $P = 0.07$). Acute Ca^{2+} intake was inversely correlated with 24-h respiratory quotient (RQ) ($r = -0.36$, $P = 0.04$) and RQ during sleep ($r = -0.31$, $P = 0.07$). After adjustment for fat mass, fat-free mass, energy balance, acute fat intake, and habitual fat intake, acute Ca^{2+} intake explained ~10% of the variance in 24-h fat oxidation. Habitual Ca^{2+} intake was not significantly correlated to fat oxidation or RQ. Total Ca^{2+} intake and Ca^{2+} intake from dairy sources were similarly correlated with fat oxidation. In backwards stepwise models, total Ca^{2+} intake was a stronger predictor of 24 h fat oxidation than dairy Ca^{2+} intake.
CONCLUSION: Higher acute Ca^{2+} intake is associated with higher rates of whole-body fat oxidation. These effects were apparent over 24 h, during sleep and, to a lesser extent, during light physical activity. Calcium intake from dairy sources was not a more important predictor of fat oxidation than total Ca^{2+} intake.
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Keywords: body weight regulation; lipolysis; room calorimeter; energy expenditure

Introduction

There is little understanding of the optimal dietary composition necessary to promote weight loss and prevent weight gain. While much attention has been focused on macronutrient intake and body weight regulation, particularly dietary fat,[1] an emerging body of literature suggests that dietary calcium (Ca^{2+}) may play a role in the regulation of body weight and body fat. In an analysis of the first National Health and Nutrition Examination Survey (NHANES I), McCarron *et al*[2] reported that body weight was inversely related to self-reported Ca^{2+} intake. In subsequent cross-sectional studies, self-reported Ca^{2+} intake was shown to be inversely related with body fat mass,[3] body weight,[4] and the relative risk of obesity in NHANES III.[5] Low self-reported Ca^{2+} intake was later shown to predict gains in body fat in children[6] and young women.[7] In humans, greater weight loss was observed with Ca^{2+} supplementation in placebo-control trials of the effect of dietary Ca^{2+} on osteoporotic risk,[4] and an unexpected 4.9 kg weight loss was observed in a clinical trial investigating the anti-hypertensive effects of increasing dietary Ca^{2+}.[5] It has been estimated that a 1000 mg Ca^{2+} intake difference is associated with an 8 kg difference in mean body weight, and that Ca^{2+} intake explains approximately 3% of the variance in body weight.[4] Thus, there is an increasing interest in understanding the mechanism by which dietary Ca^{2+} potentially regulates body weight and fat mass.

It has been hypothesized that high Ca^{2+} diets protect against fat gain by creating a balance of lipolysis over

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lipogenesis in adipocytes.[5] Ca^{2+} homeostasis is maintained by the concerted actions of parathyroid hormone (PTH), calcitonin, and the vitamin D metabolite 1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$). When serum Ca^{2+} levels fall below normal (8.5–10.5 mg dl^{-1}), counter-regulatory increases in PTH promote increased bone resorption, decreased Ca^{2+} excretion in the kidneys, and increased formation of 1α,25-$(OH)_2D_3$. Both 1α,25-$(OH)_2D_3$ and PTH stimulate increases in intracellular concentrations of Ca^{2+} ($[Ca^{2+}]_i$) in human and murine adipocytes.[5] *In vitro* data demonstrate that lipolysis and lipogenesis in adipocytes are regulated by Ca^{2+}-dependent mechanisms. Agouti protein exerts a potent inhibition of forskolin-induced lipolysis, which is blocked by treatment with the calcium channel blocker nitrendipine.[8] Treatment of 3T3-L1 adipocytes with recombinant agouti protein increases fatty acid synthase (FAS, a key enzyme in *de novo* fatty acid synthesis) mRNA levels 1.5-fold, and this effect is attenuated by treatment with nitrendipine.[9] Thus, it is hypothesized that low dietary Ca^{2+} leads to increased $[Ca^{2+}]_i$, mediated by changes in circulating 1α,25-$(OH)_2D_3$ and PTH, thereby reducing lipolysis and enhancing lipogenesis in adipocytes.[5]

A mechanistic link between calcium intake and adiposity is supported by observations in both mice and humans. In mice on an obesity-promoting diet, those animals on high-Ca^{2+} diets compared to animals on a low-Ca^{2+} diet had lower $[Ca^{2+}]_i$ in adipocytes,[10] and gained less weight[5,10] and fat pad mass,[5,10] despite no differences in food intake. In obese humans, basal adipocyte concentrations of $[Ca^{2+}]_i$[11] and circulating 1α,25-$(OH)_2D_3$[12–14] are elevated. A mechanistic link between Ca^{2+} intake and lipolysis is supported by the observation that mice on the high-Ca^{2+} diets have higher levels of forskolin-stimulated glycerol release.[5,10] Although FAS expression and activity are lower in adipocytes from mice on high-Ca^{2+} diets,[5,10] a contribution from *de novo* lipogenesis in the development of human obesity remains doubtful.[15]

Implicit in the hypothesis that high Ca^{2+} intake promotes maintenance of lower body fat mass in humans by enhancing lipolysis is the assumption that high-Ca^{2+} diets promote greater rates of whole-body fat oxidation. In Pima Indians, a low rate of fat oxidation has been shown to be predictive of future weight gain.[16] To our knowledge, the association between Ca^{2+} intake and whole-body fat oxidation in humans has not been previously examined. Using data from subjects previously studied in our whole-room calorimeter, we examined the association between 24-h fat oxidation and (a) self-reported (habitual) Ca^{2+} intake, and (b) measured (acute) Ca^{2+} intake to test the hypothesis that a higher intake of dietary Ca^{2+} is associated with higher levels of fat oxidation. As data in humans[17] and animals[6,10] suggest that dairy sources of calcium exert a stronger effect than non-dairy sources, we also considered the effects of total and dairy calcium separately. To eliminate the effect of variations in fat oxidation during the day because of the timing of meals and activity, the association between Ca^{2+} intake and sleeping fat oxidation was also examined. Finally, we examined fat oxidation during light physical activity to determine if fat oxidation during exercise is related to Ca^{2+} intake.

Methods

Subjects

Data from healthy, normal-weight subjects, who previously completed a 24-h stay in the whole-room calorimeter, were used in the current analyses. Subjects were moderately active (3–5 h per week of exercise, as determined from self-report), and between 20 and 45 y of age. Smokers or individuals reporting a history of diabetes, cardiovascular disease, or metabolic disorders known to affect intermediary metabolism were excluded. A health history and physical examination was performed to confirm that there were no medical reasons for exclusion. Subjects provided informed written consent. The study protocols were approved by the Colorado Multiple Institutional Review Board and the Scientific Advisory Board of the General Clinical Research Center (GCRC) at the University of Colorado Health Sciences Center.

All subjects had completed one 24-h stay in the whole-room calorimeter under similar conditions. A standardized walking and stepping protocol was performed each day between 14.20 and 16.30 to account for activity level outside the calorimeter. This protocol consisted of 10-min periods alternating between either walking or stepping and sitting quietly. Subjects were free to move about the calorimeter during other times of the day, but primarily this time was spent in sedentary behavior (reading, writing, or watching television). Subjects were instructed to remain awake and not to nap or perform any exercise other than that prescribed by the protocol. During each stay in the calorimeter, subjects consumed a diet designed to achieve energy balance, estimated from fat-free mass. The composition of the diet was 30% energy fat, 15% energy protein, and 55% energy carbohydrate. Subjects were permitted to select their food preferences (eg some subjects avoided dairy products), so there was a wide range of Ca^{2+} intake.

Measurements

Body composition. Body composition was determined by hydrodensitometry, with residual volume measured simultaneously using the open-circuit nitrogen-dilution technique.[18] Nitrogen was measured using a Med-Science 505-D Nitralizer (St Louis, MO, USA). Percent body fat was estimated from body density (average of 7–10 repeat measurements) using the revised equation of Brozek *et al.*[19]

Daily (24 h) energy expenditure and substrate oxidation. Total daily energy expenditure (EE) and substrate oxidation were determined from oxygen consumption and carbon

dioxide production measured in a whole-room calorimeter. Gas concentrations were determined from the flow rate and the differences in CO_2 and O_2 concentrations between entering and exiting air using Hartman and Braun (Frankfurt, Germany) oxygen (Magnos 4 G) and carbon dioxide (Uras 3 G) analyzers. Values were corrected for temperature, barometric pressure, and humidity. Urine was collected for the duration of the calorimeter stay and analyzed for total nitrogen concentration, which was then used to determine 24-h protein oxidation.[20] EE and substrate oxidation were calculated from oxygen consumption and the respiratory quotient (RQ) based on the equations of Jequier *et al*.[21] Values for all indices were averaged over 1 min intervals and recorded to a data file. The operation of the calorimeter was controlled and data collected minute by minute using a customized program operating on a personal computer. An advantage of the room calorimeter is that it permits the determination of EE and substrate oxidation during different segments of the day (eg during sleep and the walking/stepping protocol). The accuracy and precision of the calorimeter is evaluated regularly by burning propane at a variable rate. Calibration tests consistently demonstrate a 97–98% recovery of the predicted values for oxygen consumption and carbon dioxide production. Quick response rates (in the order of 1–2 min) are observed, allowing for an accurate determination of EE and substrate oxidation over short intervals (eg $\geq$30 min).

Energy and macronutrient intake. Habitual Ca^{2+} intake was determined from 4-day food diaries completed over four consecutive days that included a weekend day. Each subject was individually trained by a dietitian to weigh and record all food and beverage intakes. The diaries were completed 2–4 weeks before subjects were studied in the room calorimeter. Subjects were instructed to consume their usual diets during the measurement period. In the presence of the subject, the dietitian reviewed the completed food intake records for clarity and completeness. Caloric and macronutrient contents of the 4-day food were determined using Food Intake Analysis Software (FIAS, Version 3.98, University of Texas

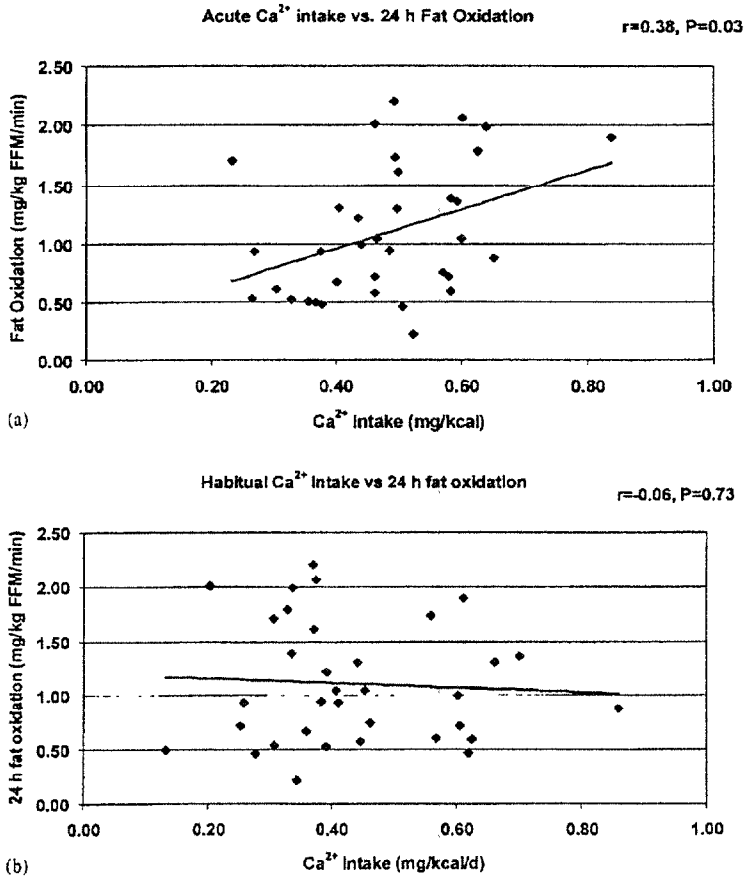

Figure 1 Relation between 24-h fat oxidation and acute (a) and habitual (b) Ca^{2+} intake.

Health Sciences Center, Houston, TX, USA). Acute Ca^{2+} intake was determined from measured food intake during the calorimeter stay. Diets consumed in the calorimeter were developed using Diet Planner (Version 2.12, San Francisco, CA, USA), which provides macro- and selected micronutrient data, based on the gram weight of food consumed. Calcium intake from dairy sources was calculated from the estimated intake of milk, cheese, ice cream, and yogurt. To minimize estimation errors, only whole-food sources of dairy Ca^{2+} were used in the estimation of dairy Ca^{2+}; dairy Ca^{2+} from mixed or prepared foods (eg lasagna) was not used.

Statistical analysis. Statistical analyses were done using SAS (SAS Institute Inc., Cary, NC, USA, 2000). To minimize the effects of body weight, calcium intake is expressed relative to total calories consumed ($mg\,kcal^{-1}$), and fat oxidation relative to fat-free mass ($gm\,kg\,FFM^{-1}\,min^{-1}$). Unadjusted correlation coefficients between Ca^{2+} intake and measures of fat oxidation were determined using Pearson correlations. To further examine the relationship between Ca^{2+} intake and fat oxidation, multiple regression models were developed adjusted for factors known to affect 24 h fat oxidation (fat mass, fat-free mass, energy balance, and acute and habitual fat intake), and partial correlations adjusting for these factors were used to estimate variance in outcomes that is explained by Ca^{2+}. Finally, to consider the effects of dairy *vs* total Ca^{2+} intake on fat oxidation, backwards stepwise multiple regression was performed using total (acute and habitual) and dairy (acute and habitual) Ca^{2+} as predictors.

Results

A total of 35 subjects (21 m, 14 f) were studied (mean ± s. d., age: 31 ± 6 y; weight: 71.2 ± 12.3 kg; BMI: 23.7 ± 2.9 $kg\,m^{-2}$; body fat: 21.4 ± 5.4%). Mean self-reported Ca^{2+}

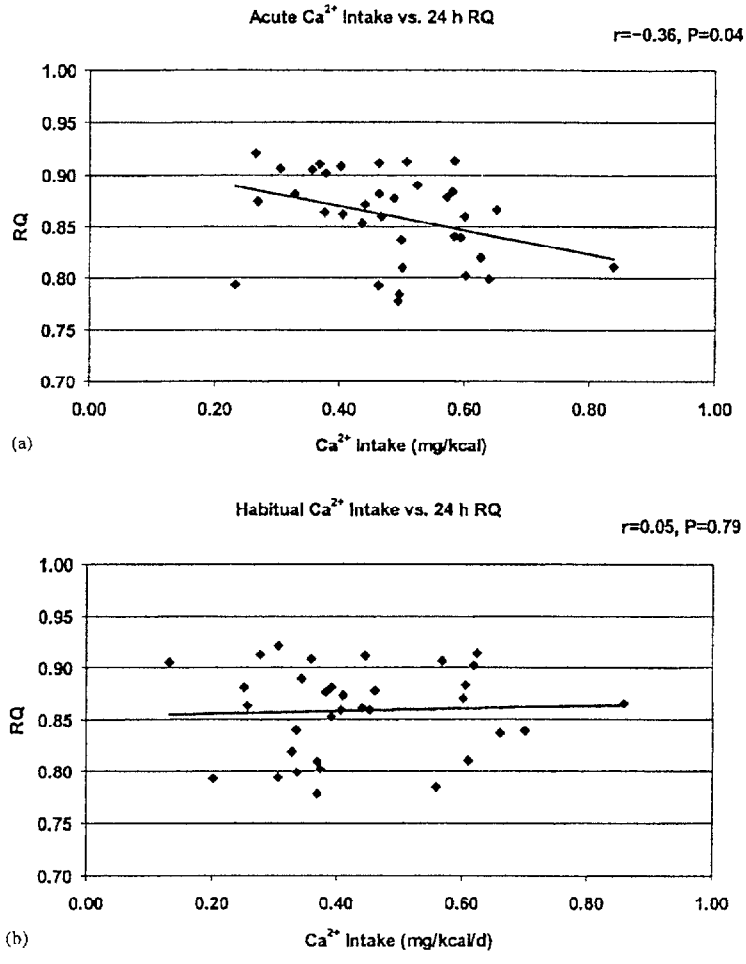

Figure 2 Relation between 24-h RQ and acute (a) and habitual (b) Ca^{2+} intake.

intake (mean ± s.e.m.) was 1222 ± 116 mg day^{-1} (range, 485–4109 mg day^{-1}). Self-reported dairy calcium intake was 664 ± 94 mg day^{-1}, corresponding to 50.3 ± 3.6% of total calcium intake. During the 24 h stay in the whole-room calorimeter, acute Ca^{2+} intake was 1046 ± 55 mg day^{-1} (range, 477–1768 mg day^{-1}). Acute dairy Ca^{2+} intake was 640 ± 44 mg day^{-1}, which was 59.5 ± 1.7% of total Ca^{2+} intake. Self-reported Ca^{2+} intake was greater than Ca^{2+} intake measured in the calorimeter ($P=0.09$), but were significantly correlated whether expressed in absolute (mg day^{-1}, $r=0.46$, $P<0.01$) or relative (mg kcal^{-1}, $r=0.35$, $P=0.04$) terms. Neither habitual ($r=0.04$) nor acute ($r=0.26$) Ca^{2+} intake were significantly correlated with 24-h EE.

The association between Ca^{2+} intake and 24-h fat oxidation and RQ is illustrated in Figures 1 and 2. Acute Ca^{2+} intake (mg kcal^{-1}) was positively correlated with 24-h fat oxidation (Figure 1a, $r=0.38$, $P=0.03$) and inversely correlated with 24-h RQ (Figure 2a, $r=-0.36$, $P=0.04$). Acute dairy Ca^{2+} intake was also significantly correlated with 24-h fat oxidation ($r=0.35$, $P=0.04$). In the adjusted multiple regression model, total acute Ca^{2+} (partial $r=0.33$, $P=0.08$) and acute dairy Ca^{2+} (partial $r=0.31$, $P=0.11$) were positively correlated with 24-h fat oxidation. Neither habitual total Ca^{2+} intake (mg·kcal^{-1}, Figures 1b and 2b) nor habitual dairy Ca^{2+} intake (data not shown) were significantly related to 24-h fat oxidation or RQ. After adjustment, total habitual (partial $r=-0.23$, $P=0.22$) and habitual dairy Ca^{2+} (partial $r=-0.20$, $P=0.30$) were not significant predictors of 24-h fat oxidation.

Similar to the 24-h data, acute Ca^{2+} intake was positively correlated with sleeping fat oxidation (Figure 3a, $r=0.36$, $P=0.04$) and inversely correlated with sleeping RQ (Figure 4a, $r=-0.31$, $P=0.07$). Acute dairy Ca^{2+} intake was also significantly correlated with sleeping fat oxidation ($r=0.45$, $P<0.01$). After adjustment, total acute Ca^{2+} (partial $r=0.29$, $P=0.13$) and acute dairy Ca^{2+} (partial $r=0.38$, $P=0.04$)

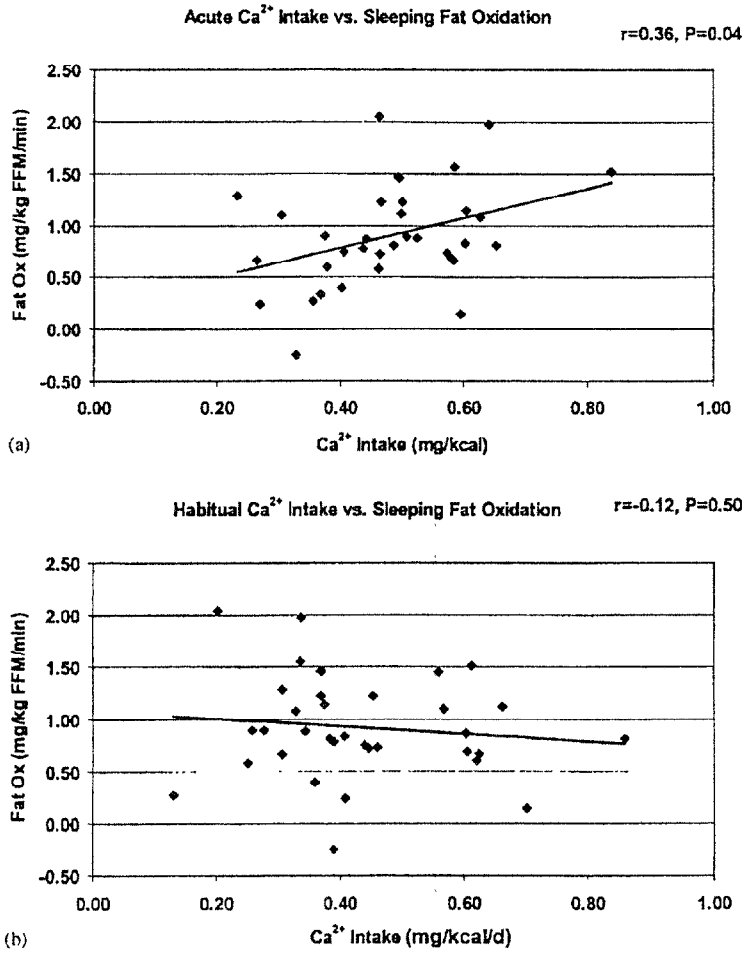

Figure 3 Relation between sleeping fat oxidation and acute (a) and habitual (b) Ca^{2+} intake.

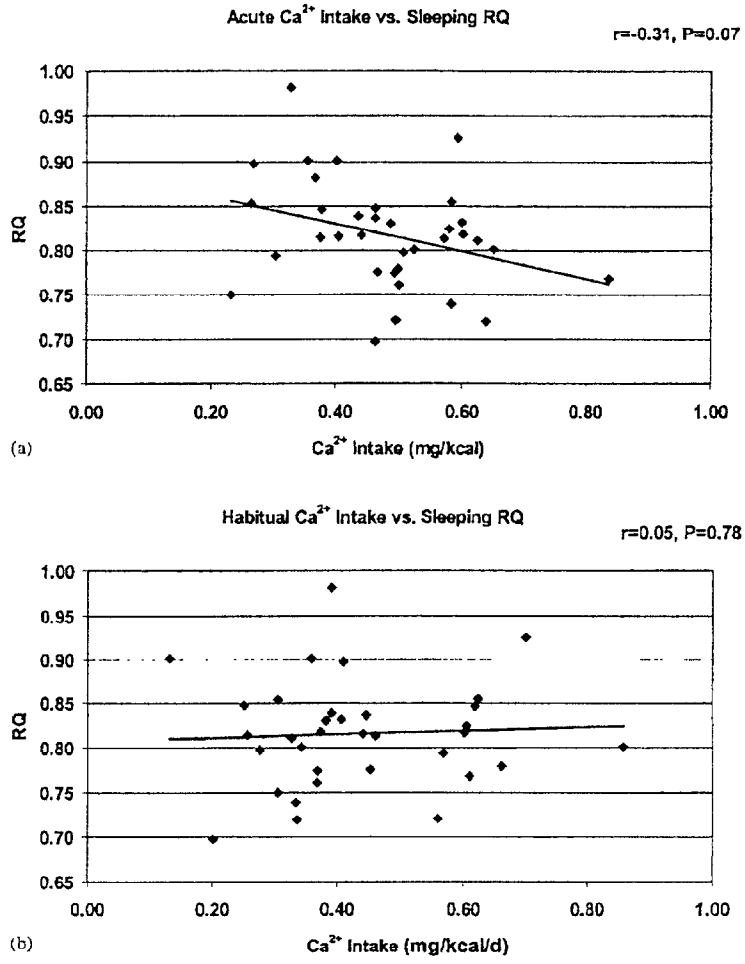

Figure 4 Relation between sleeping RQ and acute (a) and habitual (b) Ca^{2+} intake.

intakes were weakly but positively correlated with sleeping fat oxidation. Habitual Ca^{2+} intake was not related to sleeping fat oxidation (Figure 3b) or RQ (Figure 4b). After adjustment, total habitual Ca^{2+} (partial $r=-0.23$, $P=0.07$) and habitual dairy Ca^{2+} (partial $r=-0.31$, $P=0.11$) were not significant predictors of sleeping fat oxidation.

Acute Ca^{2+} intake was positively correlated with walk/step fat oxidation ($r=0.32$, $P=0.07$) and inversely correlated with walk/step RQ ($r=-0.25$, $P=0.16$, Figure 5a), although these correlations were not significant. After adjustment, acute total Ca^{2+} (partial $r=0.25$, $P=0.20$) and acute dairy Ca^{2+} (partial $r=0.18$, $P=0.35$) explained less than 5% of the variance in walk/step fat oxidation. Habitual Ca^{2+} intake (Figure 5b) was not related to walk/step RQ. Similarly, neither acute nor habitual dairy Ca^{2+} intake were significantly correlated with walk/step RQ or fat oxidation, even in the adjusted regression models (data not shown).

In the backwards stepwise multiple regression models, acute ($P=0.02$) and habitual ($P=0.04$) total Ca^{2+} intake remained significant predictors of 24-h fat oxidation, whereas acute and habitual dairy Ca^{2+} were not. However, sleeping fat oxidation was best predicted by total habitual ($P=0.01$) and acute dairy ($P<0.01$) Ca^{2+} intake.

Discussion

In healthy, young, non-obese humans, acute Ca^{2+} intake is significantly and positively related to fat oxidation measured using whole-room, indirect calorimetry. Thus, the findings of the current study are consistent with the hypothesis that high dietary Ca^{2+} diets protect against fat mass gain by promoting lipolysis,[5] which may in turn promote increased fat oxidation. To our knowledge, this is the first study in humans to report an association between Ca^{2+} intake and fat oxidation.

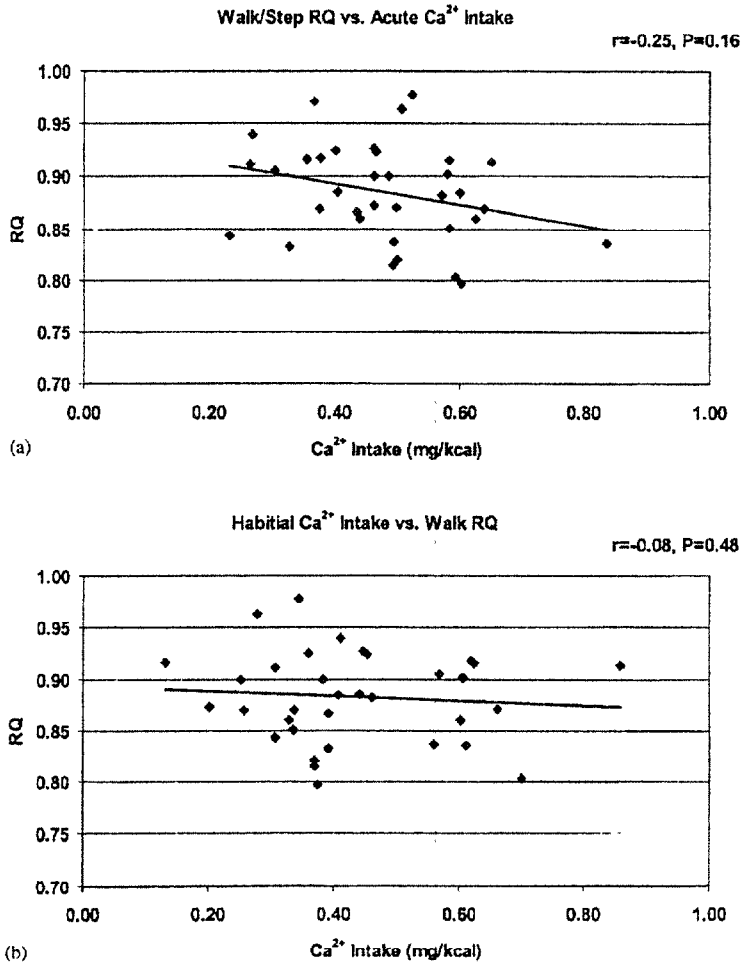

Figure 5 Relation between RQ during the standardized walking and stepping routine and acute (a) and habitual (b) Ca^{2+} intake.

Interestingly, habitual self-reported Ca^{2+} intake was not related to fat oxidation. This could be because of problems with self-report, or with imprecision for estimating Ca^{2+} intake from food recall methods. Self-report errors were not a factor in our estimate of acute Ca^{2+} intake, although estimating Ca^{2+} from measured intake would still introduce errors. Alternatively, the significant association of fat oxidation with acute but not habitual Ca^{2+} intake may suggest that the effects of dietary Ca^{2+} on adipocyte metabolism occur rather quickly. $1\alpha,25\text{-}(OH)_2D_3$ exerts physiological effects through post-nuclear transcription,[22] and also generates rapid non-genomic signal transductions, including stimulation of $[Ca^{2+}]_i$, via a putative vitamin D receptor expressed in a wide variety of cells.[24–27] Glycerol release from human adipocytes is suppressed only after 2 h treatment with $1\alpha,25\text{-}(OH)_2D_3$. Pretreatment with a specific antagonist (1β-dihydroxyvitamin D) of the putative membrane vitamin D receptor blocks this effect, whereas treatment with an agonist ($1\alpha,25$-dihydroxylumisterol$_3$) specific for this receptor makes the effect more pronounced.[28] Thus, it is possible that the association between acute Ca^{2+} intake and 24-h fat oxidation in the current study can be attributed to non-genomic effects of $1\alpha,25\text{-}(OH)_2D_3$.

Because the current study was a cross-sectional analysis, we were not able to determine if Ca^{2+} intake exerts a direct effect on fat oxidation. Indeed, it is possible that dietary Ca^{2+} only serves as a marker for other nutrients that directly modulate lipolysis and fat oxidation. Direct *in vivo* effects of alterations in dietary Ca^{2+} on circulating levels of PTH and $1\alpha,25\text{-}(OH)_2D_3$, changes in intracellular Ca^{2+} concentrations, and the subsequent effects on lipolysis and fat oxidation remain to be demonstrated.

It has been suggested that the beneficial role for dietary Ca^{2+} in weight management is markedly greater from dairy *vs* non-dairy sources of Ca^{2+}.[5,17] However, in the current analysis, the correlations between fat oxidation and Ca^{2+}

intake were nearly identical whether total or dairy Ca^{2+} was used. Moreover, total, but not dairy, Ca^{2+} remained as significant predictors of 24-h fat oxidation in backwards stepwise regression models. However, acute dairy Ca^{2+} intake was a significant predictor of sleeping fat oxidation in the backwards models. Thus, although our data do not support an additive effect of dairy Ca^{2+} on fat oxidation, we cannot exclude the possibility that the effects of dairy Ca^{2+} on fat oxidation are only apparent at rest.

In summary, in this cross-sectional analysis we found that subjects with higher intakes of dietary calcium during a 24-h period also had higher rates of fat oxidation during that period. After adjustment for factors known to affect fat oxidation measured with room calorimetry, intake of this single micronutrient explained ~10% of the variance in fat oxidation between individuals. Although these results do not show directly that calcium promotes fat oxidation, the findings are consistent with the hypothesis that high intakes of calcium are associated with lower levels of fat mass.

Acknowledgements

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Dietary patterns and changes in body mass index and waist circumference in adults[1–3]

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ABSTRACT

Background: Obesity has increased > 20% in the past decade in the United States, and more than one-half of US adults are overweight or obese.

Objective: Our objective was to further elucidate the nutritional etiology of changes in body mass index (BMI; in kg/m^2) and waist circumference by dietary intake pattern. We hypothesized that a healthy dietary pattern would lead to smaller changes in BMI and waist circumference than would other dietary patterns.

Design: Subjects were 459 healthy men and women participating in the ongoing Baltimore Longitudinal Study of Aging. Diet was assessed with the use of 7-d dietary records, from which 41 food groups were created and entered into a cluster analysis.

Results: Five dietary patterns were derived (healthy, white bread, alcohol, sweets, and meat and potatoes). The mean annual change in BMI was 0.30 ± 0.06 for subjects in the meat-and-potatoes cluster and 0.05 ± 0.06 for those in the healthy cluster ($P < 0.01$). The mean annual change in waist circumference was more than 3 times as great for subjects in the white-bread cluster (1.32 ± 0.29 cm) as for those in the healthy cluster (0.43 ± 0.27 cm) ($P < 0.05$).

Conclusions: Consuming a diet high in fruit, vegetables, reduced-fat dairy, and whole grains and low in red and processed meat, fast food, and soda was associated with smaller gains in BMI and waist circumference. Because foods are not consumed in isolation, dietary pattern research based on natural eating behavior may be useful in understanding dietary causes of obesity and in helping individuals trying to control their weight. *Am J Clin Nutr* 2003;77:1417–25.

KEY WORDS Dietary patterns, cluster analysis, obesity, body composition, diet assessment, BMI, waist circumference

INTRODUCTION

Obesity has increased > 20% in the past decade in the United States (1), and more than one-half of US adults are overweight or obese (2). Obese adults are at increased risk of the major diseases that afflict Western populations, notably cardiovascular disease, type 2 diabetes, and certain cancers (3). Body fat distribution may also be a factor significantly associated with morbidity and mortality. The ratio of waist-to-hip circumference and waist circumference alone have been associated with cardiovascular disease, premature death, stroke, type 2 diabetes, some cancers, and hypertension (4–10).

Despite considerable research effort, the nutritional etiology of obesity remains unclear and controversial, especially with regard to the roles of dietary fat (3, 11) and carbohydrate (12). Inconsistent findings may be due in part to the traditional single-nutrient approach commonly used in nutritional epidemiologic research, which is limited by colinearity among nutrients (13) and by an inability to detect small effects from single nutrients (14). A recent review of intervention trials found that mean weight loss was greater in studies in which subjects consumed a high-fiber diet compared witha low-fiber diet than in studies in which subjects consumed a low-fat diet compared with a high-fat diet (15). However, weight loss was more than 3 times as great in those studies in which subjects consumed a combination low-fat, high-fiber diet (3.4 kg over 6 mo) compared with a low-fat diet alone (1.0 kg over 6 mo), which suggests additive effects of fat and fiber in weight loss (15).

In response to the challenges of the traditional approach to understanding diet-disease relations, the measurement of dietary or eating patterns, in which various foods or nutrients (or both) are combined into a composite variable (16), has been suggested as an alternative method in nutritional epidemiologic research. A recent review of eating patterns and body mass index (BMI; in kg/m^2) found that patterns defined with the use of either a diet index, factor, or cluster analysis were inconsistently related to BMI (17). All articles reviewed were cross-sectional in design. We are not aware of any prospective studies that have measured dietary patterns by using factor or cluster analysis in relation to changes in either BMI or waist circumference.

This study was designed to use cluster analysis to examine prospectively the relation between dietary patterns and body composition among women and men participating in the Baltimore Longitudinal Study of Aging (BLSA). Our objective was to further elucidate the nutritional etiology of changes in adiposity over time as measured by BMI and waist circumference by using dietary patterning methods—specifically, cluster analysis. Many studies of dietary patterns have empirically derived a "healthy" type of dietary pattern that is relatively high in fruit, vegetables, fiber, and

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other "healthy" foods (18–23). We hypothesized that a healthy dietary pattern would lead to smaller changes in BMI and waist circumference than would other dietary patterns.

SUBJECTS AND METHODS

Study population

The BLSA was initiated in 1963 to study the physical, mental, and emotional effects of aging among healthy, active persons; the original study design and data collection were described in detail elsewhere (24). The initial study participants were white men aged 27–88 y who were living in the Baltimore area. The study protocol was expanded in 1978 to include women and minorities. All subjects were volunteers who were recruited from Baltimore City Hospitals. Enrollment in the BLSA is open, and subjects may have entered the study as early as 1963 or as recently as today. Once enrolled, participants return approximately every 12–24 mo for repeated evaluation, eg, height and weight measurements, body-composition analysis, and dietary assessment.

Our study population was limited to those entering the study in or after 1980 ($n = 921$) to incorporate both men and women and to avoid biasing our study because of changing dietary trends over time. Of these candidates, we excluded those aged < 30 y ($n = 46$) and > 80 y ($n = 92$), because persons in the former group are less likely to have developed stable dietary patterns and those in the latter group are more likely to be ill or to have limited dietary intake. All subjects gave written informed consent for their participation in the study, and the institutional review boards of the Johns Hopkins Bayview Medical Research Center and the Gerontology Center approved the BLSA protocol.

Of the remaining 783 subjects who met the year of entry and age range inclusion criteria for the study, those who had not completed ≥ 4 d of dietary records were excluded ($n = 9$), as were those whose food group intake appeared implausible (> 6 SDs from the mean for each food group; $n = 63$). An additional 134 subjects were excluded because they did not have measures of height or weight at the time the dietary record was collected and at follow-up, which meant that they could not be used in a prospective study. Finally, to create a disease-free cohort, all subjects who were diagnosed with cancer, diabetes, stroke, or heart disease either before or at baseline were excluded ($n = 190$), which left 459 subjects available for the analysis. Of those 459 subjects, 10 were excluded from the waist circumference analysis because they did not have baseline or follow-up information on waist circumference.

Dietary assessment

Dietary intake was assessed by 7-d dietary records; reports detailing dietary collection methods and dietary intake in the BLSA population were published previously (25–27). In summary, trained dietitians instructed study participants in the procedure for completing 7-d food records. Food records were completed at home by the participant and sent back to the study center. Before 1993, subjects were given food models and a booklet of food pictures to help them assess portion size. From 1993 on, subjects were given a portable scale for weighing food portions. Participants were contacted by telephone if there were any questions about their diet records.

Dietary records from 1984–1991 were originally coded and entered into a nutrient database maintained by the BLSA, whereas diet records completed since 1994 were coded and entered into the Minnesota Nutrient Database (NDS) at Tufts University, no dietary data were collected in 1992 and 1993. Dietary data from 1984–1991 were then reentered in the NDS, and nutrient intakes were back-adjusted to correct for changes in the food supply (eg, nutrient content due to fortification of cereals) with the use of data from the USDA to correspond to appropriate intervals (28, 29).

To perform a dietary pattern analysis with the use of cluster analysis, individual foods and food ingredients from the dietary records must first be aggregated into groups. We formed 41 food groups, mainly according to macronutrient composition (eg, fat or fiber content) and culinary use; several foods (eg, pizza, eggs) composed their own groups (**Appendix A**). Where possible, foods were separated into full- and reduced-fat groups (eg, high-fat dairy and low-fat dairy). Cereals were divided into those that were a good source of fiber (≥ 2.5 g/serving) and those that were low in fiber (< 2.5 g/serving) (30).

For entry into the cluster analysis, food groups may be measured by absolute weight in grams, the number of servings, or the percentage of energy intake from each food group (31). We chose to consider the food group variables in terms of the percentage of energy contributed by each of the 41 groups, and we calculated those values for each subject from the average of diet records. The percentage contribution from each food group for each subject was then entered into the cluster analysis.

Cluster analysis requires a priori selection of the number of clusters to be used in the analysis. To decide which number of clusters we would specify, we ran 4–8 cluster solutions and examined each solution to see which set of clusters most meaningfully described distinct eating patterns while they also maintained adequate statistical power in each group to detect effects. From these analyses, a 5-cluster solution was selected. With the use of the PROC FASTCLUS procedure in SAS software (32), the K-means method was then used to classify subjects into 5 nonoverlapping groups in a process that iteratively compares Euclidean distances between each subject for each solution.

Anthropometric and covariate assessments

Anthropometric measurements were made by following standardized procedures (33) as fully described elsewhere (34). In summary, weight and height were measured for each subject at each visit, and BMI was calculated from these values. At each visit, waist circumference was measured with an inelastic tape used at the narrowest part of the torso at the end of expiration (34).

Demographic data were collected from each study participant at the first visit and were used to adjust for potential confounding in regression analyses. Race-ethnicity, physical activity, smoking status, education, and vitamin supplement use were determined by questionnaire when the dietary records were collected. Physical activity was measured by an adapted version of the Harvard Alumni questionnaire, which asked participants about all daily activities (eg, activities at home, at work, and during recreation or sports). The amount of time spent for each activity was summed across all activities to determine the daily energy output per kilogram of body weight (kJ/kg) and has been described previously (25, 35).

Statistical analysis

Sample means and frequencies were calculated separately for the women and the men at the baseline visit at which dietary data

TABLE 1
Characteristics of 459 adults participating in the Baltimore Longitudinal Study of Aging

Characteristics	Women	Men
Number of subjects [*n* (%)]	219 (47.7)	240 (52.3)
Age (y)	57.3 ± 14.0[1]	60.8 ± 13.3
BMI (kg/m^2)	24.7 ± 4.0	25.2 ± 3.0
Overweight, BMI 25–29.99 [*n* (%)]	68 (31.1)	102 (42.5)
Obese, BMI ≥ 30 [*n* (%)]	23 (10.5)	12 (5.0)
Waist circumference (cm)	77.2 ± 9.4	90.4 ± 8.9
Length of follow-up (mo)	25.5 ± 6.9	25.4 ± 7.4
Physical activity (kJ/kg)	63.6 ± 16.3	61.5 ± 15.9
Vitamin user [*n* (%)]	124 (56.6)	94 (39.2)
Smoking status [*n* (%)]		
Never smoker	168 (77.0)	181 (75.4)
Current smoker	23 (10.6)	22 (9.2)
Former smoker	7 (12.4)	37 (15.4)
Education [*n* (%)]		
No high school	10 (4.6)	27 (11.3)
High school or vocational degree	31 (14.2)	16 (6.7)
Some college	32 (14.6)	17 (7.1)
College degree	66 (30.1)	68 (28.3)
Graduate degree	80 (36.5)	112 (46.6)
Race-ethnicity [*n* (%)]		
White	206 (94.1)	231 (96.2)
African American	13 (5.9)	9 (3.8)

[1] $\bar{x}$ ± SD.

were collected. The percentage of subjects who were overweight (BMI: 25–29.99) and obese (BMI: ≥ 30) was calculated with the use of recommended international cutoffs (36).

To describe food and nutrient intake across the 5 clusters (dietary patterns), we calculated separately for each pattern the mean energy contribution from each food group, the mean nutrient intake from selected macronutrients and alcohol, and sample characteristic means and frequencies. We tested for differences across patterns by using PROC GLM software with Tukey-Kramer's adjustment for multiple comparisons across groups (32). The mean annual changes in BMI and waist circumference were calculated for each dietary pattern.

We performed 2 separate regression analyses to test whether dietary patterns were associated with changes in adiposity as measured by the annual change in BMI and the annual change in waist circumference, respectively. The annual change in BMI was calculated by subtracting the BMI at visit 1 from that at visit 2, dividing that figure by the interval between visits (no. of mo), and then multiplying by 12 (representing mo). The annual change in waist circumference was calculated with the use of the same algorithm. We tested the a priori hypotheses that the healthy dietary pattern would be associated with smaller changes in BMI and waist circumference than would other dietary patterns in models adjusted for baseline anthropometry (baseline BMI and baseline waist circumference, respectively), age, sex, and sociodemographic covariates. Because total energy intake is the mechanism through which nutrients and foods may affect BMI, it is arguable whether energy should be included in the model. We fitted a final model for each analysis in which we added total energy to the multivariate model to ascertain whether adjustment for baseline energy changed our estimates. We also added terms to adjust for development of disease (stroke, cancer, diabetes, and heart disease) during the follow-up period. Because age may not be linearly related to BMI, we fitted regression models by using quadratic terms for age. We checked for effect modification by creating interaction terms for age and dietary pattern, sex and dietary pattern, and age and sex, and we then tested the interaction terms in the final models to see if the model fit improved. All analyses were performed with SAS for WINDOWS software, version 8.2 (32).

RESULTS

Sample characteristics at baseline are shown in **Table 1**. Most of the subjects were white. Fifty-two percent of the study participants were men, aged 60.8 y on average; the mean age of the women was 57.3 y. More of the men (42.5%) than of the women (31.1%) were overweight at baseline, whereas twice as many of the women (10.5%) than of the men (5.0%) were obese. The men had a greater mean waist circumference (90.4 cm) than did the women (77.2 cm).

Five clusters were derived, which we labeled healthy, white bread, alcohol, sweets, and meat and potatoes, on the basis of the food that contributed relatively greater proportions of energy to each cluster. The energy contributions from selected food groups for the 5 clusters (dietary patterns) are shown in **Table 2**. Differences in energy intake from food groups were seen across patterns. Energy intakes in the white-bread, alcohol, and sweets patterns were greatest from these respective food sources. The healthy pattern contained relatively greater contributions from "healthy" foods, including fruit, high-fiber cereal, and reduced-fat dairy, and relatively smaller contributions from fast food, nondiet soda, and salty snacks.

A significantly higher percentage of energy from carbohydrate (61.9 ± 1.5%; $P < 0.0001$) and a higher intake of fiber (26.6 ± 1.1 g; $P < 0.001$) was seen in the healthy pattern than in all other clusters (**Table 3**); no significant differences were seen in protein or total energy intake across patterns. The alcohol pattern contained the highest percentage of current smokers (29.3%, $P < 0.01$), and 62.2% of subjects in the healthy pattern used vitamins compared with 39.0% of subjects in the meat-and-potatoes pattern and 37.6% of subjects in the sweets pattern ($P < 0.001$). Subjects in the healthy pattern had the smallest waist circumference ($P < 0.05$) at baseline, but there were no significant differences in baseline BMI ($P > 0.05$).

Regression results associating change in BMI and change in waist circumference for all clusters compared with the healthy pattern are shown in **Table 4**. There was a significantly greater annual increase in BMI among subjects in the meat-and-potatoes pattern ($\beta = 0.25$; 95% CI: 0.07, 0.43; $P < 0.05$) and in waist circumference among subjects in the white-bread pattern ($\beta = 0.90$ cm; 95% CI: 0.12, 1.68; $P < 0.05$) than among subjects in the healthy cluster. The annual change in waist circumference for subjects in the meat-and-potatoes pattern was 0.75 cm, which was nearly significant (95% CI: −0.03, 1.53; $P < 0.10$) compared with changes among subjects in the healthy pattern. Estimates were similar when total energy was included in the model and when models were adjusted for development of disease during the follow-up period. Including quadratic terms for age in the model did not improve model fit. Because no significant interaction was observed between age and dietary pattern, sex and dietary pattern, or age and sex, these interaction terms were removed from the final model. For all study participants, the overall annual rate of change in BMI was 0.11, and the overall annual rate of change in waist circumference was 0.84 cm (data not shown).

TABLE 2
Percentage energy contribution from selected food groups across the 5 dietary patterns identified at baseline among 459 adults participating in the Baltimore Longitudinal Study of Aging[1]

	Energy contribution[2]				
Food or food group	Cluster 1: healthy pattern (n = 98)	Cluster 2 white-bread pattern (n = 79)	Cluster 3: alcohol pattern (n = 60)	Cluster 4: sweets pattern (n = 140)	Cluster 5: meat-and-potatoes pattern (n = 82)
White bread or refined grains	3.2 ± 3.7	15 8 ± 4.8	6.3 ± 3 6	7.1 ± 3.2	5.3 ± 2.9
Nonwhite bread	4.5 ± 3.6	1.7 ± 1.9	2.5 ± 2.1	3 5 ± 3.3	4.2 ± 3.4
Whole grains	1.1 ± 2.0	0.3 ± 0.6	0.4 ± 0.9	0.5 ± 1.3	0.6 ± 1.2
Low-fiber cereal	0.6 ± 1.1	0 8 ± 1.8	0.9 ± 1.4	0.7 ± 1.2	0.4 ± 0.9
High-fiber cereal	7.0 ± 5.5	3.0 ± 2.9	2.9 ± 3.1	3.3 ± 3.4	2.8 ± 3.0
Rice or pasta	3.9 ± 4.0	3.7 ± 3.7	4.0 ± 3.4	2.9 ± 2.6	3 3 ± 3.3
Beans and legumes	1.3 ± 1.8	0.9 ± 1.4	0.8 ± 1.2	0.8 ± 1.3	1.0 ± 1.3
Potatoes	2.2 ± 1.8	2.2 ± 1.9	2.1 ± 1.8	2.8 ± 2.2	3.2 ± 2.3
Fruit	9.9 ± 4.5	4.5 ± 3 4	4.1 ± 2.5	5.1 ± 3.2	5.7 ± 3.6
Vegetables	3 5 ± 2.0	2.6 ± 1 4	3.0 ± 1.7	2.8 ± 1.4	3.3 ± 1.8
Salty snacks	0.8 ± 1.7	1.6 ± 2.2	2.1 ± 2.6	1.3 ± 2 2	1.9 ± 2.8
High-fat baked goods	4.7 ± 3.6	5.2 ± 3 9	4.0 ± 4.1	11.2 ± 4 9	3.4 ± 2.6
High-fat dairy desserts	2.1 ± 2.5	3.0 ± 2 9	1.5 ± 2.1	3.6 ± 3 6	2.8 ± 3 7
Nuts and seeds	2.7 ± 3.2	2.0 ± 2 7	1.8 ± 2.6	2.2 ± 2 8	3 6 ± 4 3
Poultry	4.2 ± 3.8	5 2 ± 4.4	4 4 ± 3 7	2.7 ± 2.8	4.8 ± 3.5
Meat	4.1 ± 3.5	6 4 ± 4 2	7 3 ± 4.9	6 6 ± 4.0	8.0 ± 5 0
Processed meat	1.2 ± 1.4	2.6 ± 2 9	3 0 ± 3 0	3 2 ± 3.0	3.7 ± 3.6
Seafood	3.9 ± 3.4	2.6 ± 2 4	3.6 ± 3.0	2.3 ± 2.5	3.8 ± 3.7
Fast food	1.2 ± 2.4	1 8 ± 2.7	1 5 ± 2 5	1.9 ± 2.6	2.7 ± 3 7
Pizza	1.0 ± 2.6	1.2 ± 2.5	1.1 ± 2 3	1.4 ± 3.1	1.5 ± 3 0
Alcohol	1.8 ± 2.7	2.7 ± 2.7	14.8 ± 5.6	2.2 ± 2 8	3 0 ± 2 9
Nondiet soda	0.9 ± 2.1	1.3 ± 2.0	1 2 ± 1 7	1.6 ± 2.5	1 8 ± 2.8
High-fat dairy	4.3 ± 3.7	7.3 ± 4.5	7.3 ± 4.2	10.1 ± 6.1	6.3 ± 3.9
Reduced-fat dairy	7.8 ± 5.0	2.0 ± 2.9	1.5 ± 1.7	2.3 ± 2.9	1 7 ± 1.8

[1] $\bar{x}$ ± SD.

[2] Energy contribution from selected foods or food groups in each cluster does not total 100% for each column because energy contribution is not presented for all 41 food groups. Data are not shown for the following food groups: chocolate, chowders, nonchowder soups, eggs, fruit juices, low-fat dairy desserts, margarine, miscellaneous fats, miscellaneous sugars, miscellaneous foods, fruit drinks, starchy vegetables, liver and organ meats, low-fat baked goods, meal replacements, and seasonings.

The mean annual change in BMI was 0.30 ± 0.06 for subjects in the meat-and-potatoes pattern and 0.05 ± 0.06 for those in the healthy pattern ($P < 0.01$) (**Figure 1**). The mean annual change in waist circumference among subjects in the white-bread pattern (1.32 ± 0.29 cm) was more than 3 times that among subjects in the healthy pattern (0.43 ± 0.27 cm; $P < 0.05$) (**Figure 2**).

DISCUSSION

We derived 5 nonoverlapping dietary patterns by using cluster analysis, and the smallest gains in BMI and waist circumference were seen for subjects consuming a healthy diet. Our healthy dietary pattern is similar to the Dietary Approaches to Stop Hypertension diet, which has been shown to decrease blood pressure (37). We are not aware of any studies that have examined the relation between the Dietary Approaches to Stop Hypertension diet and changes in BMI or body composition or both. The overall annual rate of change in BMI that we observed in this study (0.11) is similar to that reported for control subjects in the Normative Aging Study with a similar baseline BMI (0.09; 38) and to that in a study among representative Canadians (0.11 for women and 0.09 for men; 39).

Our findings were strengthened by the prospective study design. Most studies of dietary patterns and weight have employed a cross-sectional design (18, 19, 40), and such studies are susceptible to reverse causation. In a cross-sectional study of 16 621 women participating in the National Health Screening Service in Oslo, Jacobsen and Thelle (41) found that high BMI was most strongly associated with lower consumption of low-fat milk and higher consumption of fish, and they concluded that this pattern may have resulted from the subjects' attempts to lose weight, rather than reflecting the reason for weight gain.

Our study used patterning methods to assess diet, an approach that has only recently been used in observational obesity research. A recent review on the topic considered 30 cross-sectional studies that observed the relation between diet patterns defined by diet index, factor, or cluster analysis and BMI (17). The results were inconclusive, possibly because of variability in dietary assessment methods (including food-frequency questionnaires, diet diaries, single 24-h diet recalls) and inadequate control of confounding factors. However, there was limited evidence suggesting that a diet high in fruit and vegetables and low in meat and fat was associated with a lower BMI (17), as also seen in the present study. We assessed diet with the use of 7-d diet diaries, which are considered the gold standard of dietary assessment, and we were able to adjust for age, sex, physical activity, education, and smoking in our models. We were not able, however, to adjust for the role of genetics, which may modify the relation between diet and adiposity (42).

We know of no prospective studies that have considered the relation between empirically derived dietary patterns and body fat distribution. In 2 cross-sectional studies, persons with type 1 diabetes

TABLE 3
Daily nutrient intakes and sample characteristics across the 5 dietary patterns identified at baseline among 459 adults participating in the Baltimore Longitudinal Study of Aging[1]

Nutrient or sample characteristic	Cluster 1: healthy pattern (n = 98)	Cluster 2: white-bread pattern (n = 79)	Cluster 3: alcohol pattern (n = 60)	Cluster 4: sweets pattern (n = 140)	Cluster 5: meat-and-potatoes pattern (n = 82)	P
Nutrient						
Energy (kJ)[2]	8079 ± 189.5[3]	8150 ± 208.8	8096 ± 239.7	8749 ± 155.6	8272 ± 204.6	—
Carbohydrate (% of energy)[4]	61.9 ± 1.5[b]	53.6 ± 2.1[a]	48.0 ± 2.9[a]	53.1 ± 1.7[a]	51.8 ± 2.4[a]	< 0.0001
Protein (% of energy)[4]	16.6 ± 0.6	14.6 ± 0.9	16.3 ± 1.2	15.2 ± 0.7	16.9 ± 1.0	—
Fat (% of energy)[4]	24.8 ± 1.4[a]	31.4 ± 2.0[a,b]	24.4 ± 2.8[a,b]	33.5 ± 1.7[b]	32.1 ± 2.3[a,b]	< 0.001
Saturated fat (% of energy)[4]	7.6 ± 0.6[a]	9.8 ± 0.8[a,b]	9.0 ± 1.1[a,b]	11.6 ± 0.7[b]	9.5 ± 0.9[a,b]	<0.01
Fiber (g)[4]	26.6 ± 1.1[b]	18.1 ± 1.2[a]	18.1 ± 1.4[a]	21.7 ± 0.9[a]	20.4 ± 1.2[a]	<0.001
Sucrose (g)[4]	44.0 ± 1.9[a]	42.8 ± 2.1[a]	31.2 ± 2.4[b]	53.3 ± 1.6[c]	38.8 ± 2.0[a,b]	<0.001
Starch (g)[4]	94.8 ± 2.7[a]	102.5 ± 2.9[a]	81.0 ± 3.3[b]	94.3 ± 2.2[a]	83.3 ± 2.8[b]	<0.001
Alcohol (g)[4]	4.5 ± 1.0[a]	6.0 ± 1.1[a]	35.9 ± 1.3[b]	5.3 ± 0.8[a]	7.6 ± 1.1[a]	<0.001
Sample characteristic						
Age (y)	63.4 ± 1.4[b]	56.1 ± 1.5[a]	58.2 ± 1.8[a,b]	58.8 ± 1.1[a,b]	58.3 ± 1.5[a,b]	<0.01
BMI (kg/m^2)[3]	24.1 ± 0.4	25.4 ± 0.4	25.2 ± 0.5	25.1 ± 0.3	25.0 ± 0.4	—
Waist circumference (cm)[3]	81.6 ± 0.9[b]	85.0 ± 1.0[a,b]	85.6 ± 1.1[a]	84.5 ± 0.7[a,b]	84.5 ± 1.0[a,b]	< 0.05
Physical activity (kJ/kg)[3]	61.9 ± 1.7	62.3 ± 1.7	62.3 ± 2.1	63.2 ± 1.3	61.5 ± 1.7	—
Length of follow-up (mo)	25.0 ± 0.7	25.4 ± 0.8	25.1 ± 0.9	25.6 ± 0.6	26.0 ± 0.8	—
Female [n (%)]	57 (58.2)[b]	34 (43.0)[a,b]	20 (33.3)[a]	62 (44.0)[a,b]	46 (56.1)[a,b]	< 0.05
White [n (%)]	91 (92.9)	77 (97.5)	59 (98.3)	136 (96.5)	75 (91.5)	—
Vitamin user [n (%)]	61 (62.2)[a]	38 (48.1)[a,b]	34 (56.7)[a,b]	53 (37.6)[b]	32 (39.0)[b]	< 0.001
Smoking status [n (%)]						
Current smoker	4 (4.1)[b]	8 (10.1)[a,b]	14 (29.3)[a]	11 (7.8)[b]	8 (9.8)[b]	<0.01
Never smoker	81 (82.6)[a]	60 (76.0)[a]	33 (55.0)[b]	111 (78.7)[a]	65 (80.3)[a]	<0.01
Former smoker	13 (13.3)	11 (13.9)	13 (21.7)	19 (13.5)	8 (9.9)	—
Education [n (%)]						
No high school	5 (5.1)	4 (5.1)	5 (8.3)	11 (7.8)	12 (14.6)	—
High school or vocational degree	7 (7.2)	10 (12.7)	5 (8.3)	16 (11.4)	9 (10.9)	—
Some college	12 (12.2)	7 (8.9)	6 (10.0)	13 (9.3)	11 (13.5)	—
College degree	26 (26.5)	23 (29.0)	20 (33.4)	39 (27.9)	26 (31.7)	—
Graduate degree	48 (49.0)	35 (44.3)	24 (40.0)	61 (43.6)	24 (29.3)	—

[1] Values in the same row with different superscript letters are significantly different, $P < 0.05$ (Tukey-Kramer's adjustment for multiple comparisons).
[2] Adjusted for age and sex.
[3] $\bar{x} \pm$ SD.
[4] Adjusted for age, sex, and total energy intake.

(n = 2868) who consumed a diet high in carbohydrates, cereal fiber, and low-glycemic-index foods had a significantly smaller waist circumference and BMI (43), and healthy adults (n = 2110) participating in the original and offspring US Framingham Heart Study and European SENECA studies who were in the meat and fat cluster had a larger waist circumference and BMI than did those who were in the fish and grain cluster. A recent crossover study among 11 healthy men who consumed a high-glycemic-index diet and a low-glycemic-index diet for 5 wk each found that the low-glycemic-index diet led to a decrease in trunk mass and an increase in lean body mass with no change in body weight (44). The authors suggest several possible mechanisms, including shifts in substrate utilization, decreases in the proteolytic counterregulatory hormones, and a reduction in lipoprotein lipase activity, which could explain the change in fat mass but not body weight (44). In our study, those in the white-bread pattern had significantly greater gains in waist circumference, but not in BMI, than did those in the healthy pattern. These studies and ours suggest that a diet high in fiber-rich, low-glycemic-index foods may result in lesser amounts of central adiposity.

Our study has several limitations. Dietary exposure measurements that use empirical methods such as cluster or factor analysis are data-driven and involve subjective decisions by investigators, and there is no gold standard for determining the number of clusters (17). Patterns may therefore be difficult to reproduce and compare across studies, especially between populations in whom diet differs. However, there does seem to be homogeneity of dietary patterns across populations. With the use of cluster or factor analysis, several studies have identified healthy and meat-based patterns (18–23, 45–49), a white-bread pattern (13, 50–52), a sweets pattern (18, 22, 50, 52–55), and an alcohol pattern (18, 22, 23, 48, 51, 53, 54, 56) in which food intakes were similar to those of the same-named patterns observed in this study. The consistency of patterns across studies derived from either cluster or factor analysis suggests that dietary patterns are reasonably reproducible.

A limitation of the dietary pattern approach is the inability to isolate nutrient-specific biologic effects, because dietary patterns include many foods and nutrients that act differently to affect hunger, satiety, energy metabolism, and food intake. In our study, subjects consuming the healthy dietary pattern had the highest intake of foods such as high-fiber cereal, reduced-fat dairy, fruit, nonwhite bread, whole grains, beans and legumes, and vegetables and the smallest gains in BMI and waist circumference. Several

TABLE 4
Regression coefficients (β) and SE for dietary patterns for predicting relative change in BMI and change in waist circumference, comparing each pattern with the healthy pattern, among adults participating in the Baltimore Longitudinal Study of Aging[1]

Dietary pattern	Change in BMI[2] (n = 459) β	SE	Change in waist circumference (cm)[3] (n = 449) β	SE
Cluster 2: white bread				
Adjusted for age and sex	0 06	0.09	0.84	0.40[4]
Multivariate model, adjusted[5]	0.05	0 08	0 90	0.40[4]
Multivariate model, adjusted + energy	0.05	0 09	0.90	0.40[4]
Cluster 3 alcohol				
Adjusted for age and sex	0.07	0.09	0.56	0.43
Multivariate model, adjusted[5]	0 06	0.10	0.72	0.44
Multivariate model, adjusted + energy	0 06	0.10	0 72	0.44
Cluster 4: sweets				
Adjusted for age and sex	0.01	0.09	0.03	0.35
Multivariate model, adjusted[5]	0.02	0.08	0.10	0.36
Multivariate model, adjusted + energy	0.04	0.08	0.17	0.36
Cluster 5: meat and potatoes				
Adjusted for age and sex	0.23	0.09[4]	0.68	0.39[6]
Multivariate model, adjusted[5]	0.25	0.09[4]	0.71	0.39[6]
Multivariate model, adjusted + energy	0.26	0.09[4]	0.74	0.40[6]

[1] Absolute mean changes in BMI and waist circumference are given in Figures 1 and 2, respectively.
[2] Adjusted for baseline BMI.
[3] Adjusted for baseline waist circumference.
[4] $P < 0.05$
[5] Multivariate models are further adjusted for ethnicity, physical activity, smoking status, education, and vitamin supplement use.
[6] $P < 0.10$.

mechanisms may be responsible for this effect. These foods are high in fiber, which may affect weight by increasing satiety and satiation through decreased gastric emptying, increased colonic transit, and decreased insulin response (57). This hypothesis with regard to the role of fiber may be extended to the role of the

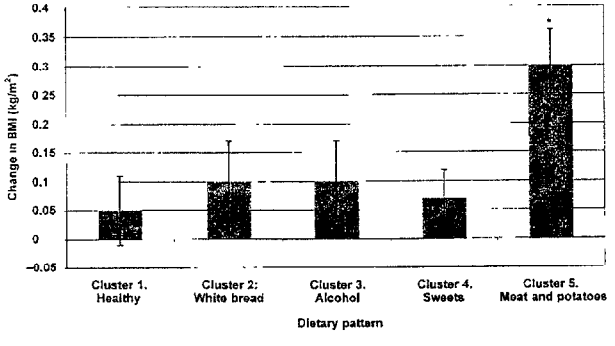

FIGURE 1. Mean (±SE) annual change in BMI across the 5 dietary patterns identified at baseline among adults participating in the Baltimore Longitudinal Study of Aging. Healthy pattern, n = 98; white-bread pattern, n = 79; alcohol pattern, n = 60; sweets pattern, n = 140; meat-and-potatoes pattern, n = 82. *Significantly different from the healthy pattern, $P < 0.05$.

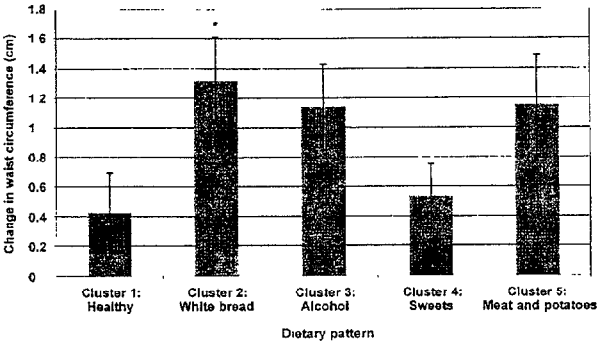

FIGURE 2. Mean (±SE) annual change in waist circumference across the 5 dietary patterns identified at baseline among adults participating in the Baltimore Longitudinal Study of Aging. Healthy pattern, n = 98, white-bread pattern, n = 79, alcohol pattern, n = 60; sweets pattern, n = 140, meat-and-potatoes pattern, n = 82. *Significantly different from the healthy pattern, $P < 0.05$.

glycemic index (58). Many of the foods in the healthy pattern are low in glycemic load, which evokes a decreased insulin response and therefore decreases hunger and energy intake (59). The healthy dietary pattern also contained foods that are low in energy density (eg, fruit and vegetables), which may be responsible for mediating energy intake rather than dietary composition per se (60–62). Compared with subjects in the healthy pattern, those in the meat-and-potatoes pattern had relatively higher intakes of meat, potatoes, fast food, pizza, and nondiet soda—foods that are low in fiber and high in energy density and glycemic load—whereas those in the white-bread pattern received almost 16% of their daily energy intake from white bread—the food with the highest glycemic index value (63).

This study cannot conclude whether one of these mechanisms or another, as yet unknown mechanism was responsible for the gains in BMI and waist circumference, but it is likely, rather, that there are multiple pathways through which food intake, including both dietary composition and total food volume, affects energy balance. Because foods are consumed not in isolation but as part of an overall dietary pattern, research based on natural eating behavior may be useful in understanding the effect of diet on weight. Such research is also more easily translated to specific dietary recommendations or advice (31), which could be helpful to persons trying to control their weight.

Our study did not address social or demographic issues that may affect dietary intake. There may be sex differences in dietary intake (13, 51, 64), and dietary patterns may also have different effects on BMI in men and women (53, 65). In our study, preliminary analysis revealed similar patterns in the men and the women, and we therefore did not stratify our analysis by sex. In addition, this study population was mainly white and highly educated, whereas dietary patterns may differ among ethnic (13) and educational (19) groups. Our population was also relatively lean (5% of the women and 10% of the men were obese) compared with a nationally representative sample of US adults, of whom 31% were obese (66). For these reasons, our findings may not be generalizeable to the overall US population.

In conclusion, our results suggest that consuming a diet high in fruit, vegetables, reduced-fat dairy, and whole grains and low in red and processed meat, fast food, and soda was

associated with smaller gains in BMI and waist circumference. Additional prospective research studies are needed to further assess the relations among dietary patterns, body weight, and central fat deposition.

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RA and JH contributed to the original design and to data collection for the Baltimore Longitudinal Study of Aging. PKN was responsible for the design and analysis for this report and drafted the manuscript. NQ provided statistical programming support. KT contributed to the analysis and design and oversaw Tufts' collaboration with the BLSA. All authors made critical comments during the preparation of the manuscript, and they fully accept responsibility for the work. None of the authors had a personal or professional conflict of interest.

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APPENDIX A

Food groups (n = 41) used in the cluster analysis

Food group	Foods in the group
High-fat dairy	Whole or 2%-fat milk (white or chocolate), cream (heavy, light, or half-and-half), hard cheese, yogurt, butter, sour cream, and cream cheese
Reduced-fat dairy	Skim or 1%-fat milk (white or chocolate), reduced-fat dairy products (butter, cheese, yogurt, sour cream, cream cheese, and cottage cheese)
High-fat dairy desserts	Pudding, cheesecake, custard, and ice cream
Low-fat dairy desserts	Pudding, ice milk, frozen yogurt, and sherbet
Margarine	All full- and reduced-fat hard margarine, and shortening
Vegetable oils	Salad dressing, mayonnaise, vegetable and nut oils, spray oil or margarine, and liquid margarine
Miscellaneous fats	Gravy, lard, salt pork, nondairy creamer, fats from meat, and nondairy "dairy" items (eg, nondairy sour cream)
Fruit	Oranges, grapefruit, lemons, limes, bananas, mangoes, dried fruit, apples, pears, melons, berries, tropical fruit, kiwi fruit, and fruit salad
Fruit juices	Orange, grapefruit, apple, other 100% fruit juices, and nectars
Fruit drinks	Sweetened fruit drinks (not 100% fruit)
Vegetables	Winter squash, carrots, broccoli, Brussels sprouts, leafy greens (mustard, turnip, spinach, other), cauliflower, tomatoes, tomato or vegetable juice, tomato products (salsa, sauce), all lettuces (leafy green, romaine, iceberg), okra, sweet peas, green or red peppers, onions, shallots, leeks, string beans, green beans, avocadoes, coleslaw, radishes, mixed vegetables, and mixed vegetable dishes
Potatoes	Potatoes (all preparations)
Starchy vegetables	Green bananas, plantains, sweet potatoes, corn, and other root crops
Beans and legumes	Beans, hard peas, legumes, baked beans and pork, cowpeas, mixed dishes with beans or legumes, soybeans, tofu, meat substitutes made from soy products, soy nuts, soymilk, and vegetable protein products
Eggs	Eggs (all preparations, including egg salad and egg substitutes)
Poultry	Chicken or turkey, with or without skin (all preparations)
Meat	Steak, ground beef, mixed dishes with beef, lamb, veal, game, whole or ground pork, venison, mixed dishes with these meats, pork turnovers, dumplings, egg rolls, and neckbones
Processed meat	Processed lunch meats (including lean and fat-free), sausage, hot dogs, bacon, and breakfast sausage
Seafood	Whole fish (all kinds), sardines, tuna fish, shellfish, tuna fish salad sandwich, other fish, and mixed dishes with fish
Liver and organ meats	Liver (all preparations) and organ meats
Sweet baked goods, full-fat	Cake, cookies, quick bread, doughnuts, sweet rolls, granola bar, muffins, sweet potato pie, crisps, and cobblers
Sweet baked goods, reduced-fat	All of the above, reduced-fat versions
Low-fiber cereals[1]	Low-fiber cereals (fortified and nonfortified, hot or cold)
High-fiber cereals[1]	High-fiber cereals (fortified and nonfortified, hot or cold)
White bread and refined grains	White bread (including light), rolls, stuffing, crackers, biscuits, bagels, pancakes, waffles, white flour, cornmeal, cornbread, hush puppies, grits, cracked wheat bread, croutons, and pretzels
Rice, pasta, and mixed dishes with rice or pasta	White rice (steamed or fried), mixed dishes with rice, rice and beans, pasta with vegetables, macaroni and cheese, mixed pasta dishes without beef, and wheat pasta
Nonwhite breads	Whole-wheat bread, rye bread, other multigrain and whole-grain breads (including light), wheat crackers, and whole-wheat flour
Whole grains	Barley, quinoa, bulgur, kasha, couscous, wheat germ, processed bran, oats and oatbran, other grains, brown rice, and popcorn
Salty snacks	Potato chips, corn chips, cereal mix, corn nuts, tortilla chips, pretzels, and other salty snacks
Nondiet soda	Cola or noncola, with or without caffeine
Alcohol	Red or white wine, beer (regular or lite), and liquor
Nuts and seeds	Almonds, peanuts, walnuts, seeds, other nuts and seeds, peanut butter, tahini (sesame butter), and coconut
Meal-replacement food products	Ensure,[2] breakfast bars, and Slim Fast[3]
Pizza	Pizza (plain or with toppings)
Miscellaneous sugary foods	Syrup, jam, table sugar, hard candy (including light and fat-free versions), popsicles, gelatin, and sorbet
Chocolate	Chocolate and candy bars with chocolate
Soups (broth or bouillon)	Noncream, broth-based soups
Chowders, cream-based soups	Cream soups and chowders
Fast food	Any food from a fast-food restaurant (hamburgers, chicken, fish, French fries, breakfast sandwiches, milkshakes, pancakes, eggs, etc)
Seasonings	All condiments (eg, ketchup) and spices (fresh or dried)
Miscellaneous foods	Yeast, Hamburger Helper dry mix,[4] macaroni and cheese dry mix, and seasoning mix from mixed dishes

[1] Foods that contain ≥10% of the daily value for fiber, or ≥2.5 g/serving, are considered a good source of fiber and are in the high-fiber cereal group. Cereals containing <2.5 g fiber/serving are in the low-fiber cereal group (30).

[2] Ross Products Division of Abbott Laboratories Inc, Abbott Park, IL.

[3] Slim-Fast Foods Company (Unilever United States Inc), West Palm Beach, FL.

[4] General Mills Inc, Minneapolis.

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Dietary Calcium and Dairy Products Accelerate Weight and Fat Loss During Energy Restriction in Obese Adults. Michael B. Zemel, University of Tennessee, Knoxville, TN; Warren Thompson, Mayo Clinic, Rochester, MN; Paula Zemel, Ann-Marie Nocton, Anita Milstead, Kristin Morris, Peter Campbell, University of Tennessee, Knoxville, TN

We have previously demonstrated that increasing intracellular Ca^{2+} stimulates adipocyte lipogenic gene expression and lipogenesis and suppresses lipolysis, resulting in increased lipid accumulation. Moreover, we have recently demonstrated that 1,25-dihydroxyvitamin D stimulates Ca^{2+} influx in human adipocytes and thereby promote adiposity, while suppressing 1,25-dihydroxyvitamin D levels by increasing dietary calcium markedly inhibits lipogenesis, accelerates lipolysis, increases thermogenesis and suppresses fat accretion and weight gain in animals maintained at identical caloric intakes and markedly accelerates fat loss in mice subjected to caloric restriction. To extend these findings to humans, 32 obese adults were maintained for 24 weeks on balanced deficit diets (500 kcal/day deficit) and randomized to control (0-1 serving/day and 400-500 mg Ca/day supplemented with placebo), high calcium (control diet supplemented with 800 mg Ca/day), or high dairy (3-4 servings of low-fat dairy products/day, total Ca intake of 1200-1300 mg/day). Control patients lost 6.4+2.5% of their body weight, which was increased by 26% on the high calcium diet and 70% (to 10.9+1.6%) on the high dairy diet ($p<0.01$). Fat loss (via DEXA) followed a similar trend, with the high calcium and high dairy diets augmenting the fat loss found on the low calcium diet by 38 and 64%, respectively ($p<0.01$). Moreover, fat loss from the trunk region represented 19.0+7.9% of total fat loss on the low calcium diet, and this fraction was increased to 50.1+6.4 and 66.2+3.0% on the high calcium and high dairy diets, respectively ($p<0.001$). Thus, increasing dietary calcium significantly augments weight and fat loss secondary to caloric restriction and increases the percentage of fat lost from the trunk region. Moreover, dairy products exert a substantially greater effect on both fat loss and fat distribution compared to an equivalent amount of supplemental calcium.

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Dairy Consumption, Obesity, and the Insulin Resistance Syndrome in Young Adults

The CARDIA Study

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ORIGINAL CONTRIBUTION

Dairy Consumption, Obesity, and the Insulin Resistance Syndrome in Young Adults
The CARDIA Study

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Context Components of the insulin resistance syndrome (IRS), including obesity, glucose intolerance, hypertension, and dyslipidemia, are major risk factors for type 2 diabetes and heart disease. Although diet has been postulated to influence IRS, the independent effects of dairy consumption on development of this syndrome have not been investigated.

Objective To examine associations between dairy intake and incidence of IRS, adjusting for confounding lifestyle and dietary factors.

Design The Coronary Artery Risk Development in Young Adults (CARDIA) study, a population-based prospective study.

Setting and Participants General community sample from 4 US metropolitan areas of 3157 black and white adults aged 18 to 30 years who were followed up from 1985-1986 to 1995-1996.

Main Outcome Measure Ten-year cumulative incidence of IRS and its association with dairy consumption, measured by diet history interview.

Results Dairy consumption was inversely associated with the incidence of all IRS components among individuals who were overweight (body mass index ≥25 kg/m^2) at baseline but not among leaner individuals (body mass index <25 kg/m^2). The adjusted odds of developing IRS (2 or more components) were 72% lower (odds ratio, 0.28; 95% confidence interval, 0.14-0.58) among overweight individuals in the highest (≥35 times per week, 24/102 individuals) compared with the lowest (<10 times per week, 85/190 individuals) category of dairy consumption. Each daily occasion of dairy consumption was associated with a 21% lower odds of IRS (odds ratio, 0.79; 95% confidence interval, 0.70-0.88). These associations were similar for blacks and whites and for men and women. Other dietary factors, including macronutrients and micronutrients, did not explain the association between dairy intake and IRS.

Conclusions Dietary patterns characterized by increased dairy consumption have a strong inverse association with IRS among overweight adults and may reduce risk of type 2 diabetes and cardiovascular disease.

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RISK OF TYPE 2 DIABETES AND cardiovascular disease is affected by a number of medical and lifestyle factors. In recent years, increasing attention has been focused on a constellation of risk factors termed the insulin resistance syndrome (IRS), also known as the metabolic syndrome or syndrome X.[1,2] In this syndrome, obesity, insulin resistance, and hyperinsulinemia are thought to cause glucose intolerance, dyslipidemia (low serum high-density lipoprotein cholesterol (HDL-C), and high serum triglyceride concentrations), hypertension, and impaired fibrinolytic capacity.[3] An increasing incidence of IRS in all racial, ethnic, and social class groups in the United States can be inferred from the increasing prevalence of obesity[4,5] and type 2 diabetes[6-8] over the last 3 decades. Recently, this syndrome has been observed in youth,[9-11] and age-adjusted prevalence among adults has been estimated at 24%.[12] An increase in the prevalence of IRS may partly explain the recent plateau or increase in cardiovascular disease rates, after several decades of decline.[13]

Although various environmental influences, including smoking and physical inactivity, are known to promote insulin resistance, the effect of dietary composition on IRS is poorly understood. For most of the past 3 decades, the US Department of Agriculture and the American Heart Association have

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recommended low-fat diets in the prevention and treatment of cardiovascular disease. Recently, however, some have questioned these recommendations out of concern that high-carbohydrate consumption might promote IRS.[14-17] Other dietary factors that have been linked to components of IRS include the ratios of monounsaturated or polyunsaturated to saturated fatty acids,[15,18,19] dietary fiber,[20,21] and glycemic index.[22-24]

Dairy consumption is another dietary factor that might affect IRS. Milk intake has decreased significantly over the past 3 decades[25-27] as the prevalence of obesity and type 2 diabetes has increased. Epidemiologic and experimental studies suggest that dairy products may have favorable effects on body weight in children[28] and adults.[29-31] In addition, dairy and/or calcium may decrease the risk for hypertension,[32,33] coagulopathy,[34] coronary artery disease,[35,36] and stroke.[37,38] An inverse cross-sectional association between dairy intake and IRS was observed in men but not in women although the influence of physical activity, fruit and vegetable intake, and other lifestyle factors was not considered.[39] The purpose of this study was to examine, in a prospective fashion, the independent association between dairy consumption and IRS, after taking into account physical activity level, macronutrient and fiber intake, and other potentially confounding variables.

METHODS

The Coronary Artery Risk Development in Young Adults (CARDIA) Study is a multicenter population-based prospective study of cardiovascular disease risk factor evolution in a US cohort of black and white young adults. The 4 study centers are Birmingham, Ala; Chicago, Ill; Minneapolis, Minn; and Oakland, Calif. Stratification was used to obtain nearly equal numbers of individuals in each race, age group (age ranges, 18-24 and 25-30 years), and educational level (high school diploma and <high school diploma). Participants have been followed up for 15 years, with the present analyses including the first 10 years and 5 clinic examinations beginning with the baseline in 1985 and including 1987, 1990, 1992, and 1995. Fifty-one percent of 5115 eligible participants underwent the baseline examination. Participation has been excellent at approximately 80% through 1995. More details of the CARDIA Study design and its participants have been reported.[40]

Study Participants

From a total sample of 5115, we excluded from our analysis those who had no year 0 or year 7 dietary data (n=1175); had unusually high or low dietary intake values (<800 and >8000 cal/d for men; <600 and >6000 cal/d for women), consistent with CARDIA procedures (n=707); were pregnant at baseline or within 180 days of year 10 clinic examination (n=184); or were taking medications that affect blood lipid levels (n=87). Many participants belonged to more than 1 of these categories, leaving 3563 study participants. Two hundred sixty-five of these individuals had 2 or more components of the IRS at baseline, and 141 had missing IRS data, resulting in a final sample size of 3157. For stratified analyses, 923 of these individuals were overweight (body mass index [BMI] ≥25 kg/m^2).

Standard questionnaires were used to maintain consistency in the assessment of demographic (age, sex, race, educational level) and behavioral (physical activity and cigarette smoking) information across CARDIA examination visits. The CARDIA Physical Activity History questionnaire[41] queries the amount of time per week spent in leisure, occupational, and household physical activities over the past 12 months. Physical activity level is summarized as units of total activity averaged from the baseline and year 7 examination. Educational level was quantified as the number of years of school completed by the year 10 examination, and cigarette smoking status as current vs other smoker at the baseline and year 7 examination.

Dietary Assessment

The CARDIA Diet History[42] queries usual dietary practices and obtains a quantitative food frequency of the past 28 days. Starting with the Western Electric dietary history as a model, the list of foods was expanded from 150 to approximately 700 items in the hope of developing a dietary assessment tool that would be suitable across various populations and ethnic groups. Liu et al[43] reported on the reliability and validity of the CARDIA Diet History in 128 young adults. The validity correlations between mean daily nutrient intakes from the CARDIA Diet History and means from 7 randomly scheduled 24-hour recalls were generally above 0.50.[43] The correlations of calorie-adjusted calcium intake ranged from 0.56 to 0.69 across race and sex groups. After correction for within-person variability, they ranged from 0.66 to 0.80.[43]

The University of Minnesota Nutrition Coordinating Center (NCC) tape 10 nutrient database was used at baseline[44] and tape 20 at year 7.[45] Foods containing dairy were identified by matching all CARDIA food codes to the entire NCC code listings for dairy products. We identified dairy products as any items reported during the diet history interview that were either 100% dairy (eg, milk) or included dairy as one of the main ingredients (eg, dips made with sour cream). We did not include mixed dishes or recipes when the contribution of dairy to the weight or caloric content of the item was unclear or likely to be minimal. The most frequently consumed dairy product at the baseline examination was milk and milk drinks, followed by butter, cream, and cheeses. Together these items comprised approximately 90% of dairy intake. Most of the remaining products were yogurts, dips, ice cream, and puddings and other dairy-based desserts. Weekly frequency of consumption for each food (times per week) was used to estimate relative intake per week for each food for each individual. In addition to using specific commonly consumed dairy foods, such as milk, as independent variables in our analyses, we

also performed analyses for various dairy food groups based on type of product and amount of fat. Milk was considered to be reduced fat if it consisted of 2% milk fat whereas cheeses and desserts were considered to be reduced fat if they had less than 15% milk fat (eg, reduced fat sour cream). The summation of dairy intake across all foods in the respective food groups was computed for each individual. To improve the accuracy of estimating habitual intake, we averaged the intake reported during the interviews of the baseline and year 7 examinations. Total dairy intake was classified into 5 categories. To ensure sufficient numbers in each race per dairy category, approximate quintile cut points from the dairy distribution of the total cohort were used. Therefore, when stratified by race or baseline overweight status, we did not have equal numbers of observations per category.

We also considered intake of other food groups that may confound associations between dairy intake and IRS. These food groups included fruits, nonstarchy and starchy vegetables, fruit juices, soft drinks and sugar-sweetened beverages, whole and refined grains, meat, and fish. In attempt to maximize our adjustment for lifestyle factors that may confound associations between dairy intake and IRS, we created a healthy propensity score based on the following lifestyle factors, coded as 0 for unhealthy, and 1 for healthy: cigarette smoking (nonsmoker, 1), physical activity (above median total activity score, 1), fruit and vegetable intake (≥5 servings per day, 1), whole grain intake (above median intake level, 1), and soft drink consumption (below median intake level, 1). Thus, this healthy propensity score had a range of 0 (least healthy) to 5 (most healthy). We also created 2 groups among overweight individuals—those with a healthy propensity score below 3 (490/923) and those with a healthy propensity score of 3 or higher (433/923). Other dietary and nutrient measures from the CARDIA Diet History used in our analyses as potential confounders or mediators of our hypotheses included caloric intake; alcohol; fiber (grams per 1000 cal/d); caffeine (mg/d); percentage of calories from carbohydrates, protein, total fat, saturated and unsaturated fatty acids; and the micronutrients from supplements and foods including calcium, magnesium, sodium, potassium, and vitamin D.

Clinic Measurements

All clinic procedures were conducted in accordance with the CARDIA Study Manual of Operations. Participants were standing and dressed in light clothing without shoes for anthropometric measures. Body weight was measured to the nearest 0.2 kg with a calibrated balance beam scale. Height was measured with a vertical ruler to the nearest 0.5 cm. Body mass index was computed as weight in kilograms divided by height in meters squared. Waist and hips were measured with a tape in duplicate to the nearest 0.5 cm around the minimal abdominal girth and the maximal protrusion of the hips at the level of the symphysis pubica, respectively. Waist-hip ratio (WHR) was computed from the average of the 2 values for each respective measure.

Prior to each CARDIA examination participants were asked to fast and to avoid smoking and heavy physical activity for the final 2 hours. For the patients who did not fast for at least 8 hours prior to clinic examinations, data on triglycerides, insulin, and glucose were considered missing. Blood pressure was measured at each examination on the right arm using a Hawksley random 0 sphygmomanometer (WA Baum Co, Copaigue, NY) with the participant seated and following a 5-minute rest. Three measurements were taken at 1-minute intervals. Systolic and diastolic blood pressures were recorded as phase I and phase V Korotkov sounds.[46] The second and third measurements were averaged. Vacuum tubes containing no preservative were used to draw blood for insulin and glucose. Serum was separated by centrifugation at 4°C within 60 minutes, stored in cryovials and frozen at −70°C within 90 minutes until laboratory analysis. The radioimmunoassay for insulin required an overnight, equilibrium incubation and used a unique antibody that has less than 0.2% cross-reactivity to human proinsulin and its primary circulating split form Des 31,32 proinsulin (Linco Research, St Louis, Mo). Blind analysis of split serum samples resulted in a technical error of 16.6% of the mean, and $r=0.98$. Northwest Lipid Research Clinic Laboratory (Seattle, Wash), which is a participant in the Centers for Disease Control and Prevention standardization program, was used to measure all lipids. Triglyceride levels were estimated using enzymatic procedures, and HDL-C levels were measured according to the method of Warnick et al.[47] Although not a component of IRS, we also included low-density lipoprotein cholesterol as a separate independent variable to include a balanced view of risk factors for cardiovascular disease.

Insulin Resistance Syndrome

Abnormal glucose homeostasis was defined as a fasting plasma insulin concentration of at least 20 μU/mL (approximately the 90th percentile of the fasting insulin distribution), fasting glucose concentration of at least 110 mg/dL (6.1 mmol/L), or use of medications to control blood glucose. Obesity was defined as a BMI of at least 30 kg/m^2 or a WHR of at least 0.85 for women or 0.90 for men. Elevated blood pressure was defined as blood pressure of at least 130/85 mm Hg or use of antihypertensive medications.[48] Dyslipidemia was defined as low HDL-C (≤35 mg/dL [≤0.90 mmol/L]) or high triglyceride (≥200 mg/dL [≥2.26 mmol/L]) concentrations. Insulin resistance syndrome was defined as the presence of 2 or more of the 4 components: abnormal glucose homeostasis, obesity, elevated blood pressure, and dyslipidemia. If 2 components of IRS were positive, the individual was considered to have IRS, even if other components were missing. If 3 components were negative, the individual was not considered to have IRS even if the fourth

Table 1. Dairy Food Intake Medians by Race and Baseline Overweight Status in the Coronary Artery Risk Development in Young Adults (CARDIA) Study*

	Median Intake Frequency, Times per Week			
	BMI <25 kg/m²		BMI ≥25 kg/m²	
Variables	Blacks (n = 906)	Whites (n = 1328)	Blacks (n = 503)	Whites (n = 420)
All dairy products	16.6	23.2	13 2	21.3
Reduced fat	2.4	9.3	2.3	9.3
High fat	12.2	11.2	8.8	10.4
Milk and milk drinks	5.7	8.2	4.0	7.8
Reduced fat	0.3	5.9	0 5	5.7
High fat	2.4	0.1	1.0	0.0
Cheese and sour cream	2.8	4.7	2.7	4.7
Reduced fat	0.2	1.2	0.1	1.2
High fat	2.3	3.0	2.2	3.0
Butter and cream	3 7	3.9	2.0	2.9
Desserts	1.0	1.0	0.7	0.8
Yogurt	0.0	0.5	0.0	0.3

*BMI indicates body mass index

was missing. In all other cases of missing components, the IRS status was considered missing and the individual was not included in our analyses. To ensure true incident cases, baseline (year 0) cases of IRS were excluded from all analyses. When the outcome variable was an individual component of IRS (eg, obesity), we excluded from the analysis the baseline cases of this particular component.

Statistical Analysis

All analyses were performed using SAS statistical software version 8 (SAS Institute, Cary, NC). General linear regression models were used to compare the incidence of components of IRS and of IRS itself across categories of dairy intake. We used multiple logistic regression to evaluate associations between dairy consumption and the odds of developing IRS during the 10-year study after excluding all individuals who had IRS at baseline. Odds ratios (ORs) and their 95% confidence intervals (CIs) were computed for the second through fifth category of the respective dairy food group with the first category (lowest intake) as the referent group. A linear trend across categories was tested with contrast statements using orthogonal polynomial coefficients.[49] In addition to assessing the influence of potential confounders, models were constructed to evaluate micronutrients and macronutrients as possible physiologic mediators of the association between dairy intake and IRS. We evaluated whether adding these variables to the final regression models attenuated the strength of the association between dairy intake and risk factors and disease outcomes. We tested 2-way interactions based on a priori hypotheses about potential differences in the association between dairy intake and IRS by race and sex, and by baseline overweight (BMI <25 kg/m² vs ≥25 kg/m²) status. Statistical significance was set at $P=.05$.

RESULTS

TABLE 1 presents total dairy intake and specific dairy food groups by race. Dairy intake was higher in whites than in blacks ($P<.001$), and this difference was generally consistent across the dairy subgroups. One exception was when dairy was classified according to amount of fat; whites tended to consume more reduced fat dairy products than blacks, whereas the reverse was true for higher fat dairy products. We also observed differential dairy intake according to baseline BMI, with overweight individuals consuming dairy products at a lower frequency than their normal-weight counterparts ($P<.001$). These differences were larger for blacks than for whites. We observed a small decline in dairy intake of approximately 13% from the year 0 to the year 7 examination (−3.1 times per week, 95% CI, −3.7 to −2.4]), but change in dairy intake was not associated with baseline BMI (Pearson correlation coefficient, 0.02). Thus, any potential misclassification related to dairy consumption over time should not affect lean and obese individuals differently.

Demographic, lifestyle, and dietary correlates of dairy intake are shown in TABLE 2, with adjustment for age, sex, race, caloric intake, and study center. In comparison, the baseline characteristics of the 1552 participants excluded from the present analyses were generally similar although 64% were black and 46% were white. Higher dairy consumers were much less likely to be black and somewhat more likely to be women. Notably, dairy consumption was positively associated with whole grain, fruit, vegetable, saturated fat intake, and inversely associated with sugar-sweetened soft drink intake.

Ten-year cumulative incidence rates of each IRS component, as well as the IRS itself, are shown in TABLE 3 stratified by race and overweight status (BMI ≥25 kg/m²). Incidence rates for all components were higher for individuals who were overweight at baseline. Blacks have higher rates of each component with the exception of dyslipidemia. The incidence of IRS (developing 2 or more components over 10 years) was nearly 4-fold higher in overweight blacks and nearly 5-fold higher in overweight whites compared with their normal-weight counterparts.

FIGURE 1 shows 10-year cumulative incidence of the 4 components of IRS by dairy categories stratified by baseline overweight status and adjusted for age, sex, race, caloric intake, study center, and baseline BMI as a continuous variable within each BMI category. There was a consistent reduction in incidence for each of the 4 components with increasing categories of dairy intake for overweight individu-

als only. Associations between dairy intake and these IRS components were much weaker and less consistent in normal-weight individuals. Although not part of IRS, we found no association between dairy intake and incidence of high LDL-C (≥140 mg/dL [3.6 mmol/L], data not shown).

With IRS as the dependent variable, there was an interaction between dairy intake and baseline overweight status ($P=.03$). No association was observed between dairy intake and IRS incidence in those who were not overweight at baseline. Among overweight individuals of either race, incidence of IRS decreased by more than 50% from lowest to highest categories of dairy consumption. This association behaved in a dose-response manner and was monotonic for blacks. Associations between dairy intake and IRS were similar between races and sexes (P value for interaction terms >.10).

TABLE 4 includes ORs for IRS according to categories of total dairy intake. Because of the interaction between dairy intake and overweight status described above, we only present models for individuals who were overweight or obese at baseline. Model 1 includes basic demographic factors and BMI. We observed a substantial reduction in the odds of IRS over the 10-year period with increasing category of dairy intake. The reduction in odds was 71% (OR, 0.29, 95% CI, 0.14-0.58) for the highest category of dairy intake relative to the lowest category (P value for linear trend across all quintiles <.001). Among those who were not overweight or obese at baseline, the OR of IRS for those in the highest category of dairy intake was 0.72 (95% CI, 0.39-1.34, P for trend=.22).

In the adjusted models 2 and 3A involving overweight or obese individuals, we observed little evidence of confounding by other lifestyle and dietary factors. Confidence intervals became wider in model 3A due to the inclusion of many dietary variables, most of which are themselves nonsignificant predictors of IRS. Adjustment for the healthy propensity score revealed very similar findings (OR for highest vs lowest category of dairy intake, 0.37; 95% CI, 0.18-0.79). In model 3B, we evaluated several macronutrients and micronutrients as possible mediators of the association between dairy intake and IRS. Results for models 2 and 3B are very similar, suggesting that these factors do not explain the inverse association between dairy intake and IRS incidence. Finally, in model 3C, we performed stepwise logistic regression for all dietary variables included in models 3A and 3B while forcing all demographic and nondietary lifestyle factors into the model. Other than dairy, fiber and protein were the only dietary variables with significant associations with IRS. Of particular note is the strong inverse association between dietary fiber intake and IRS (OR for each 3 g/1000-cal increment in fiber [ap-

Table 2. Adjusted Mean Values of Demographic and Dietary Factors by Level of Dairy Intake and Baseline Overweight Status*

	Median Intake Frequency, Times per Week					
	BMI <25 kg/m²			BMI ≥25 kg/m²		
Variables	0 to <10 (n = 366)	16 to <24 (n = 527)	≥35 (n = 463)	0 to <10 (n = 223)	16 to <24 (n = 214)	≥35 (n = 116)
Demographics						
Age, y	25.2	24 7	24.9	25 8	25 1	25.6
Black, %	67.9	41.2	16.4	85.8	41.7	16 3
Women, %	54 9	52.3	60.5	51.5	55.5	65.8
Education, y	14.9	15.2	15.1	14 4	14.8	15.6
Lifestyle						
Physical activity, units	354	402	409	324	381	392
Current smoker, %	27.9	21.8	26.3	26.8	24.7	25.9
Alcohol intake, mL/d	12 7	9.6	9.6	11.5	10.6	11 1
Dietary factors						
Saturated fat, % of daily calories	11.7	13 2	14.7	12.0	13.5	14.6
Carbohydrates, % of daily calories	49.0	48.5	47.3	47.0	46.5	47.0
Protein, % of daily calories	13.8	14.5	14.9	14.4	15.1	15.5
Calcium intake, mg/d	884	1172	1480	877	1133	1500
Soda intake, times/wk	6.5	5.0	3.6	6.4	4.5	3.3
Whole grain intake, times/wk	6.2	8.6	9 5	5.4	7.5	9.9
Fruits and vegetable intake, times/wk	21.4	23.1	23.7	20.4	22.5	23.4

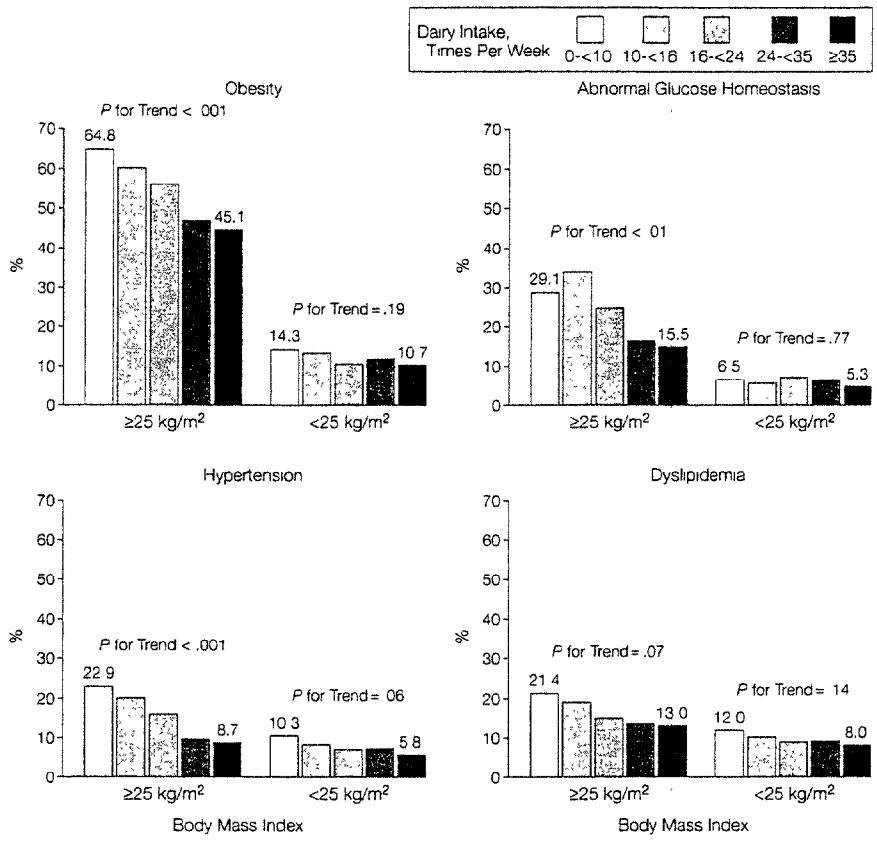
*Means are adjusted for age, caloric intake, race, sex, and study center. The second and fourth dairy categories are omitted to conserve space. BMI indicates body mass index.

Table 3. Ten-Year Cumulative Incidence of the Insulin Resistance Syndrome and Its Components by Race and Baseline Overweight Status in the Coronary Artery Risk Development in Young Adults (CARDIA) Study

	No./Total (%)			
	BMI <25 kg/m²		BMI≥25 kg/m²	
Variables	Black	White	Black	White
Obesity	116/818 (14.2)	129/1237 (10.4)	228/346 (65 9)	146/328 (44.5)
Body mass index ≥30 kg/m²	76/819 (9.3)	39/1237 (3.2)	207/347 (59.7)	121/328 (36.9)
Waist-hip ratio[a]	60/819 (7.3)	101/1239 (8.2)	69/348 (19.8)	76/328 (23 2)
Abnormal glucose homeostasis	74/646 (11.5)	35/1099 (3.2)	124/393 (31.6)	70/384 (18.2)
Elevated blood pressure	101/779 (13 0)	47/1201 (3.9)	113/545 (20 7)	50/431 (11.6)
Dyslipidemia	65/709 (9.2)	108/1114 (9.7)	58/486 (11.9)	91/385 (23.6)
Insulin resistance syndrome (≥2 components)	85/715 (11.9)	89/1160 (7.7)	170/412 (41 3)	123/367 (33.6)

[a] Men, ≥0 90, Women, ≥0 85. BMI indicates body mass index.

Figure 1. Ten-Year Cumulative Incidence of Insulin Resistance Syndrome Components by Categories of Total Dairy Intake With Stratification by Baseline Overweight Status



Rates are adjusted for age, study center, caloric intake, race, sex, and baseline body mass index

proximate interquartile range], 0.66; 95% CI, 0.53-0.80). However, fiber was not a confounder of the association between diary and IRS. The strong and independent joint association of dairy and fiber intake with odds of IRS is shown in FIGURE 2. Odds of IRS for individuals in the lowest tertiles of both fiber and dairy were nearly 7-fold higher than those in the highest tertiles of both fiber and dairy intake. Dietary protein demonstrated a positive association with IRS incidence (OR for each 1% caloric increment of protein, 1.12; 95% CI, 1.04-1.21), and this association was due to animal rather than vegetable protein (data not shown). Although dietary calcium appeared to be inversely associated with IRS incidence in a model without dairy intake (OR for each 600-mg increment [approximate interquartile range], 0.79: 95% CI, 0.61-1.03), the association between calcium intake and IRS was entirely explained by adding dairy intake to the model (OR, 0.99; 95% CI, 0.76-1.29).

To examine the extent to which weight gain explains the association between dairy and IRS, we added 10-year weight gain to the final model as a continuous variable. In this model, the OR for the highest category of dairy in-

Table 4. Odds Ratios for Insulin Resistance Syndrome According to Dairy Intake Categories Among Overweight Individuals at Baseline*

Independent Variables	Weekly Dairy Intake, Odds Ratio (95% Confidence Interval)					
	0 to <10 (n = 219)	10 to <16 (n = 201)	16 to <24 (n = 212)	24 to <35 (n = 161)	≥35 (n = 116)	P for Trend
Model 1						
Demographics†	1.00	1.15 (0 73-1 80)	0.58 (0.35-0.94)	0.41 (0.24-0.71)	0.29 (0 14-0.58)	< 001
Model 2						
Demographics and nondietary lifestyle factors‡	1.00	1.19 (0.76-1.88)	0.62 (0.38-1.01)	0.45 (0 26-0.79)	0.32 (0.16-0.66)	<.001
Model 3A						
Demographics, nondietary lifestyle factors and dietary factors§	1.00	1.20 (0.75-1.93)	0.64 (0.38-1.08)	0 51 (0.28-0.94)	0.38 (0.17-0 83)	.002
Model 3B						
Demographics, nondietary lifestyle factors and components of dairy‖	1.00	1 19 (0.75-1.90)	0.59 (0.35-1.00)	0.43 (0.23-0.78)	0.31 (0.14-0.70)	<.001
Model 3C						
Demographics, nondietary lifestyle factors, and dietary fiber and protein¶	1 00	1.12 (0.71-1.78)	0.56 (0.34-0.92)	0.42 (0.24-0 75)	0.28 (0.14-0.58)	<.001

*Overweight is defined by body mass index of 25 kg/m² or more
†Demographics include age, sex, race, calorie intake per day, study center, and baseline body mass index.
‡Nondietary lifestyle factors include educational level in years, daily alcohol intake by milliliters per day, current smoking status, units of daily physical activity, and use of vitamin supplement.
§Dietary factors include caloric percentage of daily polyunsaturated fat consumption, milligrams of daily caffeine intake, grams of fiber per 1000 calories, intake frequency of whole and refined grains, meat, fruit, vegetables, soda, and dietary intake of milligrams of magnesium and calcium and micrograms of vitamin D.
‖Components of dairy include caloric percentage of protein and saturated fat and dietary intake of milligrams of magnesium, calcium, and potassium and micrograms of vitamin D
¶Based on a stepwise procedure with a $P \leq .10$ for inclusion (demographic and lifestyle factors were forced into the model), fiber and protein were the only variables included.

take compared with the lowest category was 0.33 (95% CI, 0.16-0.72). This finding was similar when adjusting for weight gain as quintiles or deciles. We also stratified the sample by 10-year weight gain, based on a median split. In both weight gain strata, the odds of IRS were lowest for those with highest dairy intakes although CIs became wide because of the imbalance in the number of cases between these 2 groups.

As shown in FIGURE 3, the association between dairy intake and IRS incidence was very similar for both races and sexes. With the same covariates as in model 3C of Table 4, the odds of IRS associated with an increment of 1 daily eating occasion of dairy was 0.96 (95% CI, 0.73-1.28) for black men, 0.70 (95% CI, 0.54-0.91) for black women, 0.74 (95% CI, 0.59-0.93) for white men, and 0.62 (95% CI, 0.46-0.84) for white women.

In TABLE 5, ORs of the components of IRS and of IRS itself are shown for 1 daily increment (7/wk) of total dairy intake and of specific types of dairy. Odds were generally lower, and in most cases considerably reduced, with increasing intake of all types of dairy products. Inverse associations were observed for both reduced-fat and high-fat dairy products. Odds of obesity, abnormal glucose homeostasis, and elevated blood pressure were lower by nearly 20% for each daily eating occasion of total dairy products, and odds of IRS were lower by 21%. When a BMI of 30 kg/m^2 and a WHR of 0.90 for men and 0.85 for women were evaluated separately, odds of both were lower (OR, 0.81 for BMI; OR, 0.89 for WHR) with each daily increment of total dairy. The association between dairy intake and dyslipidemia was somewhat weaker. However, dairy intake appeared to be inversely associated with the odds of elevated triglyceride levels (OR, 0.79 for 1 daily increment of total dairy; 95% CI, 0.67-0.94) but not with low HDL-C (OR, 0.99; 95% CI, 0.87-1.12).

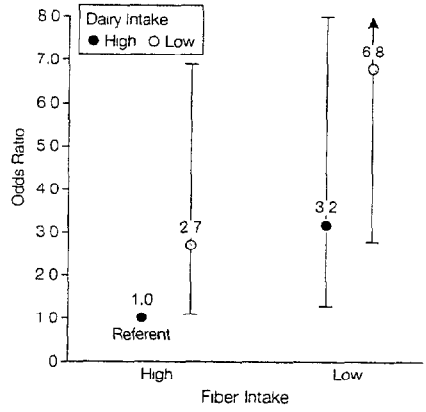
COMMENT

We observed inverse associations between frequency of dairy intake and the development of obesity, abnormal glucose homeostasis, elevated blood pressure, and dyslipidemia in young overweight black and white men and women. The 10-year incidence of the IRS was lower by more than two thirds among overweight individuals in the highest category of dairy consumption (≥5/d) compared with those in the lowest category (<1.5/d). These associations were not confounded by other lifestyle factors or dietary variables that are correlated with dairy intake and did not differ materially by race or sex

The main limitation of our study is its observational nature. Therefore, we cannot rule out residual confounding, and we cannot conclude that increased dairy intake reduced the incidence of IRS in a causal manner. The strengths of the study include its longitudinal design, allowing us to exclude participants with existing IRS at baseline and to compare the 10-year cumulative incidence of IRS across dairy categories from the average of 2 comprehensive diet history interviews. Self-reported diet averaged over time should be a better estimate of habitual intake than a single measure.[50] Remaining errors in the measure of diet are likely to bias associations toward the null hypothesis (no association), resulting in an underestimation of the true magnitude of the association. Indeed, we observed somewhat stronger associations between dairy intake and IRS incidence when modeling the average dairy intake compared with the year 0 and year 7 dairy intake separately, although these differences were not large and do not materially affect the results or conclusions (data not shown). The diet history method was chosen for use in the CARDIA study because of its comprehensiveness, interviewer-administered format, suitable timeframe for capturing habitual diet without exacerbating recall error, and applicability to populations differing in social and cultural characteristics.

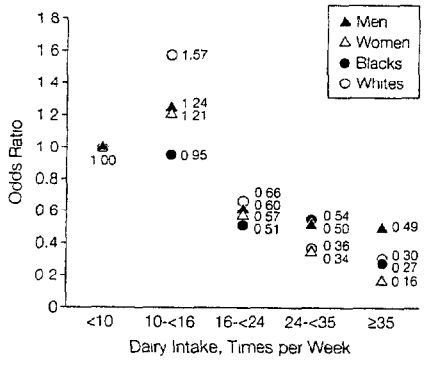
Although saturated fat contained in dairy products may raise LDL-C levels, there are several mechanisms by which dairy intake may protect against insulin resistance, obesity, and cardiovascular disease. Many single-nutrient studies, but not all,[51] suggest that calcium, potassium, and magnesium may lower the risk of hypertension,[32,33] coronary heart disease,[35,36] stroke,[37,38] or type 2 diabetes.[52] Other studies have suggested an intracellu-

Figure 2. Joint Associations of Dairy Intake and Dietary Fiber With Insulin Resistance Syndrome (IRS) Incidence



Odds ratios for IRS are shown for high and low tertiles of the dairy and fiber distributions, after adjustment for age, sex, race, caloric intake, study center, baseline body mass index, educational level (years), alcohol intake, current smoker (yes/no), physical activity, vitamin supplement use (yes/no), and protein intake (% of daily calories). Error bars indicate 95% confidence intervals

Figure 3. Race- and Sex-Stratified Models of Odds Ratios for Insulin Resistance Syndrome Incidence



Odds ratios are shown after adjustment for age, sex, race, caloric intake (cal/d), study center, baseline body mass index (kg/m^2), educational level (years), alcohol intake (mL/d), current smoker (yes/no), physical activity (units/d), vitamin supplement use (yes/no), fiber (g/1000 cal), and protein intake (% of daily calories).

Table 5. Odds Ratios for Components of Insulin Resistance Syndrome (IRS) per 1 Daily Eating Occasion of Specific Types of Dairy Products Among Individuals Who Were Overweight at Baseline*

	Odds Ratio (95% Confidence Interval)				
Variables	Obesity	Abnormal Glucose Homeostasis	Elevated Blood Pressure	Dyslipidemia	IRS
All dairy products	0.82 (0.72-0.93)	0.83 (0.73-0.95)	0.81 (0.71-0.93)	0.92 (0.82-1.04)	0.79 (0.72-0.88)
Reduced fat	0.84 (0.70-1.02)	0.95 (0.79-1.14)	0.79 (0.64-0.98)	0.95 (0.80-1.13)	0.78 (0 65-0.93)
High fat	0.84 (0.73-0.97)	0.77 (0.65-0.91)	0.84 (0 71-0.99)	0.91 (0.79-1.05)	0.82 (0.71-0.94)
Milk and milk drinks	0.83 (0.68-1.00)	0.84 (0 70-1.03)	0.80 (0.64-0.99)	0.91 (0.76-1.08)	0.74 (0.62-0.89)
Cheese and sour cream	0.82 (0.55-1.22)	0.96 (0.65-1.42)	0.67 (0.43-1.06)	1.23 (0.85-1.77)	0.64 (0.43-0 94)
Butter and cream	0.85 (0.72-1.02)	0 84 (0.69-1.03)	0.86 (0.70-1.05)	0.91 (0.76-1.08)	0.90 (0.76-1.05)
Dairy-based desserts	0 63 (0.24-1.64)	0.26 (0.09-0.80)	0.37 (0.12-1.13)	0.68 (0.26-1.79)	0.32 (0.13-0.83)
Yogurt	0.47 (0.16-1.43)	0.44 (0.12-1.62)	0.78 (0.22-2 72)	0.51 (0.15-1.72)	0.58 (0.20-1.66)

*Adjusted for all variables listed in Table 4

lar role of calcium or other components of dairy products in body weight regulation,[30] a hypothesis supported by several,[28-31] but not all,[53] observational and experimental studies. In our study, the inverse association between calcium intake and IRS was entirely explained by dairy intake whereas the association between dairy consumption and IRS was not materially affected by adjustment for the intake of calcium or any other nutrients. It is also possible that the lactose, protein, and fat in dairy foods may enhance satiety and reduce the risk of overweight and obesity relative to other high-carbohydrate foods and beverages. However, adjustment for these nutrients also had no meaningful effect on the associations between dairy intake and the risk factors of the present study.

Alternative explanations for a possible effect of dairy on the development of IRS include alterations in dietary patterns associated with dairy intake (eg, low glycemic index[22-24]), presence in dairy of unrecognized biologically active components, or residual confounding by recognized dietary or lifestyle factors. Further observational and experimental work is needed to examine these possibilities.

The association between dairy intake and IRS was not observed in individuals who were not overweight (BMI <25 kg/m^2) at baseline of this 10-year study, perhaps because these individuals were protected from insulin resistance and obesity by other lifestyle or genetic factors. Other epidemiologic studies of coronary disease or lipid levels have reported similar interactions between overweight status and dietary patterns related to insulin sensitivity.[22]

Changing dietary patterns may play an important role in the epidemics of obesity[4,5] and type 2 diabetes,[6,7] as well as the plateauing or increase in heart disease rates[13] in the United States in recent years. Trends in dietary intake behaviors over the past few decades have revealed decreasing intake of dairy products, especially milk, and increasing amounts of soda consumption and snacking among children and adolescents.[25-27,54] In summary, our study suggests that dietary patterns characterized by increased dairy consumption may protect overweight individuals from the development of obesity and the IRS, which are key risk factors[1,2] for type 2 diabetes and cardiovascular disease. Indeed, other major clinical trials and official nutritional recommendations would appear to be supportive of this dietary pattern.[55,56]

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Calcium Intake and Body Weight*

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ABSTRACT

Five clinical studies of calcium intake, designed with a primary skeletal end point, were reevaluated to explore associations between calcium intake and body weight. All subjects were women, clustered in three main age groups: 3rd, 5th, and 8th decades. Total sample size was 780. Four of the studies were observational; two were cross-sectional, in which body mass index was regressed against entry level calcium intake; and two were longitudinal, in which change in weight over time was regressed against calcium intake. One study was a double-blind, placebo-controlled, randomized trial of calcium supplementation, in which change in weight during the course of study was evaluated as a function of treatment status. Significant negative associations between calcium intake and weight were found for all three age groups, and the odds ratio for being overweight (body mass index, >26) was 2.25 for young women in the lower half of the calcium intakes of their respective study groups ($P < 0.02$). Relative to placebo, the calcium-treated subjects in the controlled trial exhibited a significant weight loss across nearly 4 yr of observation. Estimates of the relationship indicate that a 1000-mg calcium intake difference is associated with an 8-kg difference in mean body weight and that calcium intake explains ~3% of the variance in body weight. (*J Clin Endocrinol Metab* **85:** 4635–4638, 2000)

McCARRON (1), IN HIS analysis of NHANES-I data, noted an inverse association between calcium intake and body weight. The lack of any plausible basis for connecting these two variables effectively relegated this observation to the status of a curiosity or a chance association. But recently, Zemel *et al.* (2), in an analysis of the NHANES-III database, found a very strong inverse association between relative risk of obesity and calcium intake. Moreover, this observation was not itself an isolated one. Teegarden *et al.* (3), Carruth *et al.* (4), and Skinner *et al.* (5) have recently reported a similar inverse association between body fat gain and calcium intake in children and young women. Now that Zemel *et al.* (2, 6–8) have established a plausible physiological basis for the association, it seemed useful to examine other databases and particularly randomized controlled trials in which calcium supplementation was used for a skeletal end point, to see whether, in a different context, calcium intake was also associated with a weight effect.

Accordingly, we examined the data accumulated in several studies conducted out of our Osteoporosis Research Center over the past 12 yr. Four of these, for their primary skeletal end points, have been published elsewhere (9–12). One is an ongoing randomized trial in which the blind has not been broken, but the entry data were available for cross-sectional analysis.

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Materials and Methods

Subjects

The studies from which our data come are: "YWS" denotes a cohort of 184 healthy women in their early 20s followed for 4 yr (9); "TCD" denotes a similar cohort of young women participants in a randomized controlled trial of calcium supplementation; "Nuns" denotes a prospective study of calcium metabolism and bone health at 5-yr intervals in a cohort of 191 nuns as they passed from premenopause to postmenopause (12); "MBx" denotes a study of bone dynamics and biochemical markers in a cohort of 75 healthy perimenopausal women observed at 6-month intervals over 5 yr (11); and "Van" denotes a randomized controlled trial of calcium supplementation in 216 elderly women (10). The subjects have all been described in greater detail in the respective publications. Table 1 presents the several studies involved, providing relevant information with respect to type of analysis, age group of the subjects concerned, duration of observation, pertinent intake variables, and method of assessing dietary intake. Table 1 also contains the numbers of subjects in each study on whom suitable data were available for this analysis. (For the longitudinal studies we included only women in whom we had at least three observations over time, and we excluded women who, while under study, developed illnesses that might influence weight.) All these projects had been reviewed and approved by Creighton University's Institutional Review Board, and all subjects gave written consent.

Dietary intake assessment

For the nonintervention studies, 7-day food diaries were assessed by registered dietitians using a succession of methods over time. For the Nuns study, beginning in 1967, intakes were assessed using hand calculation, referring to *USDA Handbook 8* and later Bowes and Church (13), computer software was used exclusively in the other four studies and in the Nuns study as it became available. The YWS and MBx studies used NutriPractor (Practorcare, San Diego, CA). Finally, TCD began in 1995 and has used Food Processor (ESHA Research, Salem, OR). For YWS and MBx, both of which had 6-month visit intervals, only the initial diet analysis was used. But for the Nuns study, which had 5-yr visit intervals, the average intake values over the period of observation was used.

Calcium intake was expressed as the calcium to protein ratio, both because this stratagem explicitly factors in the countervailing effects of the two nutrients (13) and because the ratio eliminates most of the portion size estimation error. As we have shown previously (9, 14, 15), the ratio better correlates with an outcome variable known to be asso-

The size of the presumed effect can be estimated best by taking apart the calcium to protein ratio and BMI. In the two studies in young women, each 1.0-mg increment in this ratio was associated with a 0.186-kg/m^2 decrement in BMI. For the mean protein intake in these two studies (62.4 g/day), and the mean height (1.66 m), these numbers translate to a predicted 0.82-kg weight decrement for each 100-mg calcium intake increment. And in the middle-aged women, the best estimate of weight change is −0.038 kg/yr/100 mg calcium intake. At a 55% compliance level in the calcium-supplemented group in the Van study (10), the observed difference in weight change translates to −0.052 kg/yr/100 mg calcium intake. This rate of change is of approximately the same magnitude as in the middle-aged women and the difference between them is probably not biologically meaningful.

It may be of interest to note that the predicted weight change in the Nuns and MBx combined cohort (Fig. 2) crosses zero at a calcium to protein ratio of almost exactly 20 mg/g, a figure very close to that derived from current dietary recommendations for both nutrients. Very few women in this age range achieve calcium to protein ratios even close to 20 (see Table 1), and what our data suggest is that the general tendency to gain weight observed in mid life may be due to effectively very low calcium intakes.

Perhaps the largest barrier to prior recognition of a role for calcium intake in body weight has been the lack of a conceptual framework in which to situate the effect or explain its operation, even when it might have been observed. M. B. Zemel (personal communication) has commented that, in his 1990 study of hypertensive blacks (17), he observed substantial weight loss with calcium supplementation but did not report it because it did not seem to fit with what was known either about calcium metabolism or about obesity. However, the same investigator has recently shown that high blood PTH and 1,25(OH)$_2$ vitamin D levels, as would be evoked by a low calcium diet, increase cytosolic [Ca^{2+}] in human adipocytes in culture, switching their metabolism from lipolysis to lipogenesis (2, 6–8). Furthermore, in mice expressing the agouti gene, high calcium diets raised core body temperature and reduced the body fat accumulation that accompanies a baryogenic diet (2, 6). Conversely, low calcium diets resulted in lowered core body temperature and increased fat accumulation.

A plausible background to these phenomena may be found in reflection on the fact that the primitive human diet would have been calcium rich, with calcium to energy ratios two to four times what modern humans ingest (18). High circulating PTH [and correspondingly elevated levels of 1,25(OH)$_2$ vitamin D] would have been experienced only intermittently (*i.e.* at times of food shortage). Because a low calcium intake would have been tantamount to a low food intake, it may be that human physiology used the PTH and 1,25(OH)$_2$ vitamin D response evoked by low calcium intake to regulate its energy metabolism and thereby adapt to imminent food shortage. Today, with calcium intake disconnected from energy intake, the primitive energy-conserving response predisposes to weight gain.

Zemel's mouse model also presents a useful way of thinking about the calcium effect. Briefly, full expression of obesity in the mouse requires a combination of the obesity gene, a baryogenic diet, and low calcium intake. It is likely that some analogous combination is involved in the weight effects observed in humans (*i.e.* ready access to excess energy intake, low calcium intake, a genetic predisposition that impairs adipocyte regulation of cytosolic [Ca^{2+}], and perhaps other factors as well).

It should be noted that, with the exception of the controlled trial, in which calcium carbonate was the calcium source, it cannot be unequivocally determined whether the effect noted in our studies was due to calcium *per se* or to other nutrients for which calcium was a fortuitous marker. The bulk of the calcium in the diets of those with higher intakes was from dairy sources, as would have been expected, and other coingested nutrients may well have been partly responsible for the observed association, as in the DASH study (19). However, calcium itself, presumably through its effect on circulating PTH and 1,25(OH)$_2$ vitamin D, would clearly seem to be involved, as both our controlled human trial and the animal data show. What cannot be excluded at this point is some additional effect produced by other unrecognized dietary elements.

Finally, it may be worth noting the importance of maintaining a high calcium intake during attempts to lose or control weight. The tendency to eliminate milk from many reducing diets may be a partial reason for their frequent failure.

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Increasing fluid milk favorably affects bone mineral density responses to resistance training in adolescent boys

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ABSTRACT

This study examined the effects of increasing milk on bone and body composition responses to resistance training in adolescents. Twenty-eight boys (13 to 17 years of age) were randomly assigned to consume, in addition to their habitual diet, 3 servings/day of 1% fluid milk (n=14) or juice not fortified with calcium (n=14) while engaged in a 12-week resistance-training program. For all subjects combined, there were significant ($P \leq .05$) changes in height (+0.5%), Σseven skin folds (−7.7%), body mass (+2.6%), lean body mass (+5.1%), fat mass (−9.3%), whole-body bone mineral content (+3.6%), bone mineral density (+1.8%), and maximal strength in the squat (+43%) and bench press (+23%). Compared with juice, the milk group had a significantly greater increase in bone mineral density (0.014 vs 0.028 g/cm^2). Increasing intake of milk in physically active adolescent boys may enhance bone health. *J Am Diet Assoc. 2003;103:1353-1356.*

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Changing beverage consumption patterns in children has contributed to poor calcium intake in addition to inadequate intake of several other nutrients (1-3) Over half of young adults consume <1 serving of milk/day, well below the recommended 3 servings/day (4) with no trends of improved intake (5). The optimal intake of dietary calcium needed for optimal bone mineral accrual during periods of rapid growth and physical exercise is a debatable issue (6-8) A large body of literature has shown that both calcium and physical exercise have a favorable effect on bone In cross-sectional and placebo-controlled intervention studies, increasing calcium (either through fluid milk or supplementation) in adolescents favorably affects the rate of bone mineral acquisition (9-15). Resistance training may also have a favorable effect on bone because young male power lifters and Olympic lifters have significantly greater bone mineral density (BMD) compared with age-matched controls (16-18), and resistance training is effective in improving muscle strength and muscle size in youth (19-23). The importance of weight-bearing exercise in optimizing bone mineralization is also becoming more apparent in adolescents (24). The primary purpose of this study was to examine the effects of increasing milk consumption on bone health in response to resistance training in adolescent boys.

METHODS

A two-group prospective study design involving 12 weeks of resistance training was used to examine the effects of increasing milk consumption on bone responses in adolescent boys. Anthropometric measures, body composition, bone density, dietary intakes, and performance measures were assessed at zero, six, and 12 weeks. All subjects and a parent were informed of the purpose and possible risks of this investigation prior to signing an informed consent document approved by the Institutional Review Board. Subjects were excluded who consumed three servings (≥236 mL) of fluid milk or ≥1,500 mg calcium/day or met any of the following criteria: extreme dietary practices, history of lactose intolerance, goals of weight loss/gain, use of nutritional supplements, and smoking. Maturity status was self reported (Tanner stage) by subjects with the help of their parent(s). We selected 28 healthy boys (13 to 17 years of age) who, after baseline testing, were matched on physical characteristics and perfor-

mance measures and then randomly assigned to consume three servings (708 mL or 24 oz) of 1% fluid milk (n=14) or unfortified apple juice alternated with grape juice (n=14) group. Milk and juice were provided to subjects weekly at their exercise training sessions and consumed in addition to their habitual diet. On exercise days, two servings were consumed after the workout in the presence of a member of the investigative team, and the remaining serving was consumed at another time, which was recorded on log sheets. Subjects (and parents) were provided with specific instructions for recording all foods/beverages consumed for a seven-day period at weeks one, six, and 12. All milk and juice intake was recorded daily. Dietary information was analyzed using nutritional software (Nutritionist V, Version 2.3; N-Squared Computing, First Databank Division, The Hearst Corporation, San Bruno, CA).

The resistance exercise program consisted of supervised one-hour exercise sessions three days/week (Monday, Wednesday, Friday) for 12 weeks. The program consisted of varying training loads within each week of training as well as increasing intensity with concomitant decreasing volume over the 12 weeks to optimize strength and power performance gains (25,26). Both groups exercised in the same facility at the same time(s) of day and utilized identical equipment, which consisted of a combination of free weights and Nautilus (Nautilus International, Independence, VA) exercise machines.

Body mass was measured on an electronic scale, and height was determined with a wall-mounted stadiometer. Seven skin folds (triceps, subscapular, midaxillary, chest, suprailiac, abdomen, and thigh) and three circumference (upper arm, thigh, and chest) measurements were serially obtained in duplicate on the right side by the same investigator. A whole-body scan was performed using dual-energy x-ray absorptiometry (DXA) with a total body scanner (Prodigy; Lunar Corporation, Madison, WI; Software version 2.17.008) to determine body composition BMD and bone mineral content (BMC).

After familiarization sessions, maximal lower and upper body strength were assessed using a squat and bench press exercise. The squat was performed on a modified Smith machine as described previously (27) and the bench press using a free-weight Olympic-style barbell. After two to three submaximal warm-up trials, the load was increased to a point at which the subject had three to four max-

Table 1
Subject characteristics and daily intake of dietary nutrients in adolescents who increased either milk or juice intake

	Milk group		Juice group		DRI[a]
	Mean±SD	%	Mean±SD	%	
Age (y)	14.7±1.7		14 0±0.7		
Height (cm)	166.9±15.0		171 8±8.2		
Weight (kg)	59.5±14.8		65.6±14.2		
Tanner stage	3.6±0.9		4.0±0.4		
Energy (kcal)	2,274±491	13	2,521±515	14	
Protein (g)	100.6±18.5	24	87.0±33.2	0	
Protein (% energy)	17.8±2.0*		13.4±2 6		
Carbohydrate (g)	287.3±63.6*	12	365.3±58 9	24	
Carbohydrate (% energy)	49.9±4.5*		58.1±7.4		
Total fat (g)	84 0±25.2	10	82.5±28.4	0	
Total fat (% energy)	32.3±3.8*		28.5±5 1		
Cholesterol (mg)	238±60	12	256±109	0	
Fiber (g)	8.7±2 4		10.0±4.1		
Vitamin A (RE)	1,113±228*	40	853±338	0	900
Vitamin D (μg)	10.4±1 8*	74	3 5±2 1	0	5
Vitamin E (mg)	5 6±8 5		2 9±1 5		15
Thiamin (mg)	1 8±0.4		1.8±0.5		1.2
Riboflavin (mg)	3 0±0.5*		2.2±0.6		1 3
Niacin (mg)	22.6±6.2		24.2±8 3		16
Pyridoxine (mg)	1.8±0.6		1.8±0 7		1.3
Vitamin B-12 (μg)	6 2±1.0*		4.2±1.6		2.4
Folate (μg)	226±109		288±198		400
Vitamin C (mg)	68±28*		235±108		75
Calcium (mg)	1,723±274*	49	979±286	0	1,300
Phosphorus (mg)	1,885±367*		1,336±421		1,250
Iron (mg)	13 9±3.3		16.5±4.3		11
Magnesium (mg)	288±58*		233±82		410
Potassium (mg)	3,191±604	36	2,774±783	30	

[a]Dietary Reference Intakes for calcium, phosphorus, magnesium, vitamin D, and flouride (1997); Dietary Reference Intakes for thiamine, riboflavin, niacin, vitamin B-6, folate, Vitamin B-12, panothenic acid, biotin, and choline (1998); Dietary Reference Intakes for vitamin C, vitamin E, selenium, and carotenoids (2000); and Dietary Reference Intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, manganese, nickel, silicon, vanadium, and zinc (2001). Percentages refer to the amount of nutrient supplied by the additional 3 servings of milk or juice. Dietary values are mean of three, seven-day diet records obtained during weeks one, six, and 12 (21 days total).
*$P \leq .05$ vs corresponding value for the juice group.

Table 2
Total and regional body composition responses in adolescents who increased either milk or juice intake

	Milk group			Juice group			*P* value		
	wk 0	wk 6	wk 12	wk 0	wk 6	wk 12	Time	Group	Int.
Body composition	←		*Mean±SD*			→			
BM (kg)	59.5±14.8	60.7±14.5	60 6±14.1	65.6±14.2	66.7±13 8	67.4±13.9	.000	.252	.460
% Fat	17.1±10 2	15.9±10.3	14.9±9.8	18.0±8.9	16.5±8.1	16.6±8.5	.000	761	.121
LBM (kg)	47.2±14.1	49.1±14.2	49.6±13.8	50.7±7 3	52.6±7.3	53.1±7 5	.000	271	168
FM (kg)	9.8±5 3	9.3±5.3	8.7±5.1	12.8±8.5	11.8±7.9	12.0±8.2	000	.412	.970
BMC (g)									
Arms	322±135	336±138	344±139	340±93	345±88	360±93	.000	.738	.142
Legs	1,022±356	1,037±349	1,051±350	1,077±215	1,100±225	1,107±225	.000	.605	.537
Trunk	813±324	832±310	846±309	793±211	815±194	818±193	002	.830	.794
Ribs	259±98	256±85	263±88	247±73	257±68	255±70	.230	.836	192
Pelvis	348±154	360±149	365±147	345±92	352±88	356±86	003	.885	.697
Spine	206±75	216±79	218±77	201±49	206±43	207±41	.001	.725	543
Whole body	567±883	2,615±869	2,657±874	2,591±540	2,639±583	2,667±525	.000	.945	.729
BMD (g/cm^2)									
Arms	0.852±0.12	0.866±0.138	0.877±0.138	0.853±0.077	0.859±0.080	0.871±0.077	.000	.930	.365
Legs	1.289±0.237	1.315±0 242	1.323±0.242	1.283±0.110	1.298±0.114	1.297±0.109	.000	.818	.104
Trunk	0.917±0.157	0.932±0 157	0.948±0.156	0.917±0.089	0.920±0.092	0.933±0.080	.000	.849	.096
Ribs	0.698±0.084	0.703±0.085	0.715±0.086	0.690±0.065	0.698±0.061	0.696±0.054	007	.618	.352
Pelvis	1.165±0.227	1.185±0.218	1.215±0.224	1.178±0.134	1.197±1.138	1.207±0.129	.000	938	157
Spine	0.961±0.202	0.971±0.190	0.984±0.189	0.939±0.103	0.944±0.116	0 960±0.103	003	.684	.899
Whole body[a]	1.126±1.167	1.142±0.171	1.154±0.172	1.111±0.089	1.117±0.095	1.125±0 087	.000	.656	017

BM=body mass, LBM=soft-tissue lean body mass, FM=fat mass, BMC=bone mineral content, BMD=bone mineral density, Int =interaction (group×time).
[a]Significant interaction (group × time) effect for whole-body BMD

imal efforts to determine the one repetition maximum. Adequate rest was allowed between trials (3 to 5 minutes).

Dependent variables were analyzed using a two-way analysis of variance (ANOVA) with group as a between and time (pre, mid, and post) as a within factor. Significant main effects or interactions were further analyzed using a Fisher LSD post hoc test. Differences in dietary nutrients between milk and juice groups were analyzed using independent *t* tests. Relationships among dietary nutrients and the changes in bone measures were examined using Pearson's product-moment correlation coefficients. Statistical power ranged from 0.80 to 0.85 at the *P* value selected to establish significance in this study (.05).

RESULTS

Compared with the juice group, the milk group had significantly higher intakes of protein, fat, vitamins A and D, riboflavin, calcium, phosphorus, and magnesium and lower intakes of carbohydrate and vitamin C (Table 1). There were significant main time effects for height (+0.8 cm), biceps circumference (0.8 cm), and Σskin folds (−9 mm). There were significant main time effects but no group effects for all measures of body composition, bone, and maximal strength. The only variable that changed differently between the milk and juice groups was whole-body BMD as indicated by a significant group × time interaction effect (Table 2). The milk group had a two-fold greater increase than the juice group (0.028 vs 0.014 g/cm^2, respectively). As a group, there was a significant relationship between vitamin D intake and the absolute change in BMC (r=0.38). No significant relations were observed among nutrient intakes and changes in bone (data not shown).

Maximal strength significantly increased in both groups after six weeks, and there was a further increase at week 12. Squat strength at weeks zero, six, and 12 was 69.4±26.6, 76.7±24.6, and 91.6±25.2, respectively, in the milk group and 61.6±10.8, 71.5±11.8, and 94.7±14.6, respectively, in the juice group. Bench press strength was 48.5±20.4, 53.6±19.8, 59.6±19.0, respectively, in the milk group and 48.5±9.3, 52.8±9.3, and 59.9±9.5, respectively, in the juice group.

DISCUSSION

This was the first study to examine the effect of increasing milk and juice consumption during resistance training in adolescent boys. A primary finding was that 12 weeks of resistance training increased whole-body BMD to a greater extent in boys who added to their diet three additional servings of milk/day compared with a juice group. The increase in BMD was two-fold greater in the milk vs the juice group (0.028 and 0.014 g/cm^2, respectively). Because a control "non-training" group was not included, we cannot comment on the importance of resistance training in contributing to this favorable effect of milk on bone.

A primary finding was that 12 weeks of resistance training increased whole-body BMD to a greater extent in boys who added to their diet three additional servings of milk/day compared with a juice group

Cross-sectional data indicate that competitive adolescent weightlifters have increased BMC and BMD well above age-matched controls (16-18). Blimkie and colleagues (28) examined the effects of a 26-week resistance-training program on bone in postmenarcheal adolescent girls. Although there were no statistically significant increases in BMC and BMD after 26 weeks of training, the

changes tended to be greater in the girls who trained compared with a control group who did not train. In the present study, we observed significant increases in both BMC and BMD in response to a much shorter resistance-training program (12 vs 26 weeks, respectively).

The most likely reason for the greater whole-body BMD response is the additional calcium and/or vitamin D consumed by the milk group. In cross-sectional studies, calcium intake is positively related to BMD in both adolescent boys (29) and girls (30,31). Randomized, placebo-controlled, intervention studies in adolescents also indicate a positive effect of calcium or milk supplementation on the rate of bone mineral acquisition (9-15). Although vitamin D has an important role in bone mineralization and calcium homeostasis, there are limited data on how vitamin D intakes affect bone status in adolescents. A recent study reported that vitamin D intakes in females during adolescence and young adulthood showed the most consistent positive associations with BMD measured at young adulthood (32).

The most likely reason for the greater whole-body BMD response is the additional calcium and/or vitamin D consumed by the milk group

These significant increases in maximal strength were similar to gains observed in prior studies (19-23). These data indicate that additional milk does not provide an advantage over juice in terms of augmenting maximal force production.

APPLICATIONS

■ Increasing milk vs juice intake resulted in significantly greater increases in whole-body BMD, emphasizing the potential importance of calcium and perhaps other nutrients in milk in optimizing bone development in physically active adolescent boys. The physiologic importance of these changes later in life cannot be determined from this study but could protect against risk of osteoporosis and fracture in susceptible individuals (33). These data indicate that adequate milk intake should be encouraged and be part of nutritional education messages targeted to young persons and their parents.

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Translating the Genome
Friday, April 11 - Tuesday, April 15 • San Diego,CA

Abstract # 2277

Higher dairy intake is associated with lower body fat during adolescence.

R. Novotny, S. Acharya, J.S. Grove, Y.G. Daida and T.M. Vogt.
Univ. of Hawaii at Manoa and Kaiser Permanente Clin. Res. Ctr., Honolulu.

Due to the increasing prevalence of overweight adolescents in the US, it is imperative to identify life style factors that could help maintain body fat and weight in this group. The purpose of this study was to examine factors influencing body fat and weight. Three hundred and twenty three girls, nine to 14 years, were selected from all age eligible girls at Kaiser Permanente Oahu. Girls' age, ethnicity and levels of physical activity were obtained by questionnaire. Anthropometry was obtained by measurement. The mean age, weight and iliac skinfold thickness of girls was 11.5 ± 1.4 years, 98.1 ± 28.5 lbs and 12.4 ± 6.1 mm, respectively. In multiple regression analysis adjusted for ethnicity, 17.2% of the variation in iliac skinfold thickness was negatively explained by dairy intake, age, and physical activity; height, breast Tanner staging and calorie intake were positively associated with iliac skinfold thickness. A model replacing only dairy intake with mean calcium intake and with all the same independent variables in the model explained 15.3% of the variation in iliac skinfold thickness. A similar trend was observed when iliac skinfold thickness was replaced with body weight as the dependent variable in the regression analysis. These findings suggest that dairy and calcium may be focal food groups and nutrients in maintaining body fat and weight during adolescence. [Supported by USDA, Grant # 9900700].

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PAPER

Dairy food consumption and body weight and fatness studied longitudinally over the adolescent period

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OBJECTIVE: Although research suggests that adolescents, particularly girls, may avoid dairy products due to concerns that these foods are 'fattening,' the longitudinal relation between consumption of dairy foods and relative weight status during adolescence has not been explored. Using data from the MIT Growth and Development Study, a longitudinal study designed to assess the metabolic, dietary, and behavioral factors that predict changes in body composition with growth and development in girls during the adolescent period, the current analysis was undertaken to examine the relation of dairy food intake with relative weight status and percentage body fat (%BF).

SUBJECTS: A total of 196 nonobese premenarcheal girls 8–12 y old were enrolled between 1990 and 1993. Girls were followed until 4 y postmenarche.

MEASUREMENTS: At each annual follow-up visit, data were collected on %BF by BIA, body mass index (BMI) *z*-score, and dietary intake (assessed by FFQ). The present analysis is limited to the 178 girls who have at least three annual visits and who have valid anthropometric and food frequency data. In all, 1198 individual measurements were analyzed.

RESULTS: At study entry, participants had a mean (s.d.) BMI *z*-score of −0.27 (0.89), a mean (s.d.) %BF of 23.4 (4.7), and obtained 19.9% (9.2) of daily calories from dairy foods. Linear mixed effects modeling indicated no relationship between BMI *z*-score or %BF and measures of dairy food or calcium consumption.

CONCLUSION: Avoidance of dairy foods due to a possible association with relative body weight is not supported by these findings. We find no evidence that dairy food consumption is associated with BMI *z*-score or %BF during adolescence, but further research specifically designed to address this question is needed.
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Keywords: dairy; calcium; adolescence; longitudinal analysis; body composition

Introduction

The attainment of an optimal peak bone mass during adolescence is important for the prevention of osteoporosis later in life. Studies in children and adolescents have shown that calcium consumption, in the form of supplements and as dairy foods, positively influences bone mass, making it an important modifiable risk factor.[1–4] Although dairy foods provide the majority of calcium in the diets of children and adolescents,[5–8] inadequate calcium intake among American youth has been well documented.[6,9,10] An analysis of dietary data from four US Department of Agriculture (USDA) surveys showed that calcium consumption among 11–18 y olds decreased significantly from 1100 mg in 1965 to 960 mg in 1994–1996.[11] Several factors may explain the decrease in calcium intake among children, chief among them a decline in milk consumption, an increase in juice and soda consumption, and an increase in the number of meals children eat away from home, which is of concern because the calcium density of restaurant or fast food meals is lower than for home-prepared foods.[12]

The increasing prevalence of unhealthful dieting practices may also adversely affect dairy food consumption, especially among adolescent girls.[13–15] Research indicates that adolescents may reduce dairy food consumption due to fears about weight gain and misperceptions that milk and other dairy

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foods are fattening.[14,16,17] Teenage girls have cited losing weight as a reason for reducing dairy food consumption.[18]

In contrast to the notion held among adolescent girls that dairy foods will cause weight gain, recent research suggests an inverse relation of dairy food and calcium consumption with weight or fatness measures. Cross-sectional analyses conducted among adults have shown that calcium intake is a significant negative predictor of BMI and percent body fat (%BF).[19–21] Longitudinal analyses conducted in adults have shown that increased levels of calcium or dairy foods are negatively associated with changes in body weight.[21–23] Among children, a study of the relation between nutrient intake and body composition in preschool children found that calcium and dairy food consumption were significant negative predictors of %BF.[24] *In vitro* and animal studies have led to the development of a proposed mechanism by which calcium might affect the regulation of body weight. These laboratory data suggest that increasing dietary calcium may lessen diet-induced adiposity by modulating adipocyte intracellular Ca^{2+} and thereby regulating lipogenesis and lipolysis.[20]

Although a few prior studies report the use of longitudinal study designs, change in body composition measures was typically assessed using a simple pre/postcomparison, which fails to give a complete description of the pattern of change over time. Longitudinally collected data requires special techniques for analysis because observational studies often have unbalanced designs and/or missing data, and because repeated measurements taken on the same individual are correlated with each other. The objective of this analysis was to examine the relation of dairy food consumption with changes in weight status and body fat in girls from preadolescence through adolescence, using annual data from a 10-y longitudinal study of growth and development in girls. Our hypothesis was that consumption of dairy foods would not be associated with either increases or decreases in body weight over the adolescent period.

Methods

Study sample

The data for this analysis derive from the Massachusetts Institute of Technology (MIT) Growth and Development Study, a prospective study designed to examine the relation of energy expenditure to growth and development in girls from preadolescence to adolescence. Girls ($n=196$) were recruited between the fall of 1990 and the spring of 1993 from public schools in Cambridge and Somerville, Massachusetts, the MIT summer day camp, as well as through contact with family and friends of faculty. At study entry (baseline), all girls were between 8 and 12 y old, premenarcheal, and nonobese based on a triceps skinfold thickness (TSF) $\leq$85th percentile for age and sex according to NHANES I.[25] All participants were in good health as assessed by physical examination and medical histories. On the anniversary of their baseline visit, participants returned for measurements every year until 4 y postmenarche (study exit). If girls had not started menses at their annual visit, they were encouraged to telephone when they experienced their first menstrual period. Because most girls did not call, they were queried regarding menarche at each follow-up visit until menarche was reported. The study was approved by the Committee on the Use of Humans as Experimental Subjects at MIT and by the Human Investigations Review Committee of the New England Medical Center.

Dietary assessment

All participants completed a Willett semiquantitative food frequency questionnaire (FFQ) at each annual follow-up visit. The questionnaire was specially designed for children based on a validated semiquantitative FFQ for adults. Similar to the adult version, the questionnaire was designed to be self-administered; however, participants were given verbal and/or written instructions on how to properly complete the forms. The 116-item FFQ was based on recall of diet in the past year. Dairy food categories as listed on the FFQ included skim/low-fat milk, whole milk, cream, sherbert or ice milk, ice cream, ice cream sundaes, milkshakes, yogurt, cottage or ricotta cheese, cream cheese, and other cheeses (e.g. American, Cheddar, etc). The individual dairy foods were classified into categories for further analyses: (1) low-fat dairy (skim milk, yogurt, cottage cheese, ice milk/sherbert); and (2) full-fat dairy (whole milk, cream, ice cream, sundaes, cheese, cream cheese and milkshakes). Pizza was not considered in our analysis of daily servings of dairy or percentage of daily kilocalories from dairy foods, but it was included in our analysis of dairy calcium. Serving sizes were of natural units or typical servings sizes. When completing the FFQ's, participants indicated how often, on average, they had consumed the amount of each food item in the past year. The nine response categories available ranged from 'never or less than 1 per month' to '6 or more per day'.

The food composition database used to calculate levels of intake for calories and nutrients was based on publications from the USDA, laboratories, and manufacturers. Calories and nutrient intakes were calculated by multiplying the frequency of consumption by the nutrient composition for the portion size for each specific food listed. Calories and nutrients were then summed across all foods to obtain total levels of all calories and nutrients for each individual. Servings of specific dairy foods were converted into daily servings and total daily servings of dairy foods was calculated by summing across all dairy foods. The percent of daily kilocalories from dairy foods was calculated by adding the calories from each individual dairy food and dividing by total daily kilocalories. Calcium from dairy foods was calculated by adding the amount of calcium from each individual dairy food.

Other analyses conducted in this cohort indicate that the FFQ provides a reasonable estimate of dairy intake. We found

that dairy food consumption estimated from the FFQ correlated well with dairy food consumption reported on 7-day diet records at baseline and study exit (Spearmans' *R* at study exit for skim milk = 0.68; whole milk = 0.50; cheese = 0.49; ice cream = 0.43; correlation is between FFQ estimate and diet record estimate, unpublished observations).

Anthropometry

At each annual follow-up visit, height and body weight were measured in the morning. Height was measured to 0.1 cm with a wall-mounted stadiometer. Weight was measured in a hospital gown using a Seca scale accurate to 0.1 kg. Body mass index (BMI) was calculated as weight in kilograms/height in meters squared. BMI *z*-score was calculated using the revised Centers for Disease Control and Prevention (CDC) growth reference standards.[26] Bioelectrical impedance analysis (BIA) was used to measure resistance (*R*) and reactance after an overnight fast or 2-h postprandial (Bioelectrical impedance analyzer, BIA 101, RJL Systems, Clinton Township, MI, USA). The accuracy of the machine was checked before the measurement with a 500 Ω resistor supplied by the manufacturer. Measurements were taken with the subject supine and electrodes were then placed on the dorsal surface of the right foot and ankle, and right wrist and hand. A current was applied at a frequency of 50 kHz. Percentage body fat (%BF) was estimated using prediction equations developed in this cohort, using measures of total body water (TBW) by isotopic dilution of $H_2{}^{18}O$ as the criterion method. Separate equations were used depending on the menarcheal status of the participant. We found that %BF estimated from our equation closely approximates %BF estimated by $H_2{}^{18}O$ in our cohort.[27]

Physical activity and inactivity measures

Participants completed a questionnaire at each annual follow-up visit designed to identify usual patterns of physical activity. Participants were presented with two 24-h timetables (school day and weekend day) and asked to recall, on an hourly basis, their participation in five types of activities during each time block: sleeping or lying down, sitting, standing, walking, vigorous activity (exercising, playing, or being involved in sports). In addition, participants completed a similar grid on which they reported, on an hourly basis, television viewing time (including time spent watching videos or playing video games). The average daily time spent in each activity was computed as a weighted average of the school day and weekend day reports. Average daily time spent walking and in vigorous activity were combined and weighted by their intensity (using an MET value) to create an activity index, which was calculated as 2.5*walk+5.5*vigorous. Average daily time spent sleeping or lying down, sitting, and standing were combined and weighted by their intensity (using an MET value) to create an inactivity index, which was calculated as 1.5*sit+1.5*stand+1.0*sleep. Information on the reliability of this physical activity assessment protocol has been published elsewhere.[28] In this cohort, the correlation between baseline nonresting energy expenditure and baseline physical activity index was 0.29.

Analysis

Dietary variables

The exposure of interest was dairy food intake, which was expressed in several different ways. First, total dairy food consumption was assessed using the following variables: (1) daily servings of dairy foods; (2) percentage of daily kilocalories from dairy foods; and (3) daily calcium (mg) from dairy foods. In addition, we also considered the percentage of calories from low-fat dairy foods and the percentage of calories from full-fat dairy foods. To better approximate normality, we took the natural log of dairy food servings and the square root of calcium (mg) from dairy foods. These transformed variables were used in all analyses. In our analysis of the percentage of daily calories from low-fat dairy, quartiles of consumption were used because the data exhibited significant non-normality.

Data exclusions

Dietary exclusion criteria were used to omit annual visits when participants left more than 12 items blank on the FFQ or when daily energy intake was less than 500 kcal or greater than 5000 kcal as calculated from the FFQ. In addition, participants with less than three annual visits were excluded. Therefore, this analysis includes data from 178 (91%) participants, representing 1198 data points, with an average of 6.7 measurements per girl.

Statistical analysis

Paired *t*-tests were used in the simple comparison of changes between baseline and exit. Generalized additive modeling (GAM) was used to visualize the relation between BMI *z*-score or %BF and our exposure measures. Although this technique ignores the correlation structure in repeated measurements, these plots allow one to visualize the general pattern of the relation and to assess the appropriateness of a linear model. GAM models were run separately for each outcome and predictor (data not shown).

Linear mixed effects modeling (LME) was used to evaluate the longitudinal relation between relative body weight or body fatness and dairy food consumption. As we had two outcomes (BMI *z*-score and %BF) and five exposure variables (daily servings of dairy food, percent daily calories from dairy foods, dairy calcium, percentage of calories from low-fat dairy, and percentage of calories from full-fat dairy), 10 separate LME models were evaluated. The applied mixed effects model consists of two parts: fixed and random effects. Fixed effects describe a population intercept and population

slopes for a set of considered covariates, which include exposures and confounders. Random effects describe individual variability in the outcome and changes over time. By considering individual random slopes and intercepts, this model allows us to examine the influence of covariates on the change in outcome over time. The LME model also accounts for the correlation between repeated measurements on the same subject and the different numbers of measurements per subject.

To control for possible confounders in the relation between either %BF or BMI *z*-score and the measures of dairy food consumption, the following strategy was used. Longitudinal models were evaluated to determine which potential covariates were significant predictors of both dairy food consumption and either %BF or BMI *z*-score. The following variables were considered: physical activity index, inactivity index, parental overweight (defined as at least one parent with a BMI >25), race/ethnicity (coded as two dummy variables for black individuals and 'other' with white individuals as the reference category), daily servings of fruits and vegetables, percentage of daily calories from sugar-sweetened soda, percentage of daily calories from snack foods, percentage of daily calories from protein, percentage of daily calories from carbohydrates, and percentage of daily calories from fat. For models with BMI *z*-score as the outcome variable, age was expressed as chronological age; for models with %BF age was expressed relative to age at menarche.[29] With the exception of age and parental overweight, we included as covariates only those variables that were significant predictors of both the exposure and the outcome. This approach allows us to maximize the number of covariates and avoid the risk of overparamaterizing the model. Parental overweight was included in all models due to its strong longitudinal relation with BMI *z*-score and %BF. Data were analyzed using SAS (Version 8.0, SAS Institute, Cary, NC, USA) and S-PLUS (Version 4.5, MathSoft Inc., Seattle, WA, USA). Alpha was set at 0.05 for all analyses.

Results

Characteristics of study sample

Characteristics of the cohort at baseline and study exit are shown in Table 1. The cohort was predominantly white (74%) with an average age of about 10 y at baseline. The mean (s.d.) BMI *z*-score was −0.27 (0.89) at baseline, reflecting the study entry criteria. Significant increases in %BF and BMI *z*-score were observed between study entry and study exit. The correlation between BMI *z*-score and %BF was 0.75 (over all available time points). At baseline, the mean

Table 1 Characteristics of the cohort at study entry and 4-y post menarche (study exit)

	Study entry (n = 166)		*Study exit (n = 141)*		*Paired comparison (n = 132)*
Characteristic	*Mean (s.d.)*	*Median*	*Mean (s.d.)*	*Median*	*P-value*
Outcomes					
BMI *z*-score	−0.27 (0.89)	−0.25	0.02 (0.79)	0.07	<0.001
% body fat by BIA	23.4 (4.7)	23.1	27.6 (3.8)	27.8	<0.001
Dairy food consumption					
Dairy foods (serv/day)	3.1 (1.6)	2.9	2.6 (1.6)	2.4	0.02
% daily kcal from dairy foods	19.9 (9.2)	18.9	18.8 (8.8)	19.0	0.37
Dairy calcium (mg)	808 (400)	827	659 (432)	549	<0.001
% daily kcal from low-fat dairy[a]	9.2 (7.9)	7.1	10 5 (8.5)	8.5	0.13
% daily kcal from full-fat dairy	10.7 (8.8)	8.1	8.3 (5 7)	7.2	0.13
% daily kcal from milk[a]	11.6 (8.0)	10.1	8.7 (7.7)	6.8	<0.001
% daily kcal from cheese[a]	3.2 (2.9)	2.5	4.0 (3.0)	3.4	0.002
% daily kcal from yogurt[a]	1.5 (2.0)	0.99	2.8 (4.0)	1.3	<0.001
% daily kcal from ice cream[a]	3.6 (3.8)	2.9	3.1 (3.2)	2.5	0.14
Covariates					
Age (y)	10.0 (0.93)	10.0	16.9 (1.0)	17.0	<0.001
TV (h/day)	3.5 (2.5)	3.0	1.8 (1.5)	1.4	<0.001
Activity index	15.2 (6.8)	14.7	10.6 (6.2)	9.7	<0.001
Inactivity index	24.4 (2.3)	24.3	25.9 (2.6)	26.4	<0.001
Daily kilocalories	2021 (669)	1933	1723 (655)	1640	<0.001
Daily servings of fruits and vegetables	5.3 (3.1)	4.6	4.8 (2.6)	4.4	0.10
% of daily calories from soda[a]	2.7 (3.9)	1.6	4.0 (4.6)	2.5	0.002
% calories from fat	30.1 (5.3)	30.5	27.9 (6.2)	27.6	<0.001
% calories from protein	15.6 (3.0)	15.6	16.3 (3.4)	16.4	0.04
% calories from carbohydrates	56.3 (7.1)	55.8	57.6 (8.2)	57.7	0.08

[a]Paired comparison based on nonparametric Wilcoxon signed rank test.

(s.d.) number of dairy food servings was 3.1 (1.6) servings and the percentage of daily kilocalories from dairy foods was 19.9% (9.2). Daily servings of dairy foods and calcium from dairy foods decreased significantly between baseline and study exit. At each cross-sectional age from age 9 to 16 y, milk contributed approximately 50% of total servings of dairy foods. The contribution from skim milk remained relatively constant at each age while the contribution from whole milk decreased substantially with age (data not shown).

Longitudinal analyses

Dairy food consumption and age. Our longitudinal models of the relation between dairy food consumption and age indicate a significant decrease in dairy food consumption with increasing age. Each of our four measures of dairy food consumption decreased significantly over time. For example, based on the slope and intercept from our LME model, over a 6-y period between the ages of 10 and 16, daily servings of dairy foods would decrease by about 15%, or approximately half of a serving (intercept = 1.54; slope of log(dairy) = −0.02, $P<0.001$). Over the same time period, percent of daily calories from dairy foods would decrease by 1.6% on average (intercept = 22.5; slope of % kcal from dairy = −0.28).

Table 2 Results from linear mixed models predicting BMI *z*-score from dairy food and calcium consumption

	Estimate	*P-value*
Model 1[a]		
Intercept	−1.3	<0.001
Age	0.05	<0.001
Log daily servings of dairy food	0.017	0.65
Model 2[a]		
Intercept	−1.3	<0.001
Age	0.05	<0.001
% daily kcal from dairy food	0.0008	0.65
Model 3[b]		
Intercept	−0.54	<0.001
Age	0.05	<0.0001
Square root dairy calcium	0.0017	0.44
Model 4[a]		
Intercept	−1.3	<0.001
Age	0.05	<0.001
Log % calories from full-fat dairy	−0.007	0.76
Model 5[a]		
Intercept	−1.3	<0.001
Age	0.05	<0.001
Quartile of % calories from low-fat dairy	−0.005	0.74

[a]Adjusted for daily servings of fruits and vegetables, quartile of percentage calories from soda, percentage of calories from protein, and parental overweight, [b]Adjusted for daily servings of fruits and vegetables, quartile of percentage calories from soda, grams of protein, daily kilocalories, and parental overweight, [c]Adjusted for quartile of percentage calories from soda, grams of protein, daily kilocalories, and parental overweight.

Table 3 Results from linear mixed model predicting %BF from various measures of dairy food and calcium consumption

	Estimate	*P-value*
Model 1[a]		
Intercept	23.7	<0.001
Age relative to menarche	0.54	<0.001
Log daily servings of dairy food	0.18	0.51
Model 2[b]		
Intercept	23.3	<0.001
Age relative to menarche	0.58	<0.001
% daily kcal from dairy food	0.013	0.32
Model 3[c]		
Intercept	28.8	<0.001
Age relative to menarche	0.55	<0.001
Square root dairy calcium	0.026	0.13
Model 4[b]		
Intercept	23.0	<0.001
Age	0.57	<0.001
Log % Calories from full-fat dairy	0.19	0.23
Model 5[a]		
Intercept	23.9	<0.001
Age	0.55	<0.001
Quartile of % calories from low-fat dairy	0.07	0.57

[a]Adjusted for physical activity index, percentage of daily calories from protein, and parental overweight, [b]Adjusted for percentage of daily calories from protein, and parental overweight, [c]Adjusted for physical activity index, grams of protein, daily kilocalories, and parental overweight.

Relation between dairy food consumption and changes in BMI z-score. After adjusting for covariates, we found no statistically significant relation of dairy food consumption, expressed as servings per day, percent of daily calories, or calcium from dairy foods, with BMI *z*-score (Table 2). In addition, no significant relation between the percentage of calories from low- or full-fat dairy and BMI z-score was observed (Table 2).

Relation between dairy food consumption and changes in %BF. There was no significant relation between daily servings of dairy foods or percentage of daily calories from dairy foods and %BF (Table 3). In addition, no significant relation between the percentage of calories from low- or full-fat dairy and %BF was observed (Table 3).

Discussion

The role of dairy food and calcium consumption in relation to weight control has received increased attention in the scientific and popular press. Our data do not support the hypothesis that a higher intake of dairy foods influences body weight or fatness changes during adolescence. Using longitudinal data collected annually over a 10-y period, we

observed no significant relation between dairy food intake and changes in BMI *z*-score or %BF over the adolescent period.

To our knowledge, this is the first truly longitudinal analysis of the relation between dairy food consumption and body weight status conducted in children to date. Other studies have generally shown either an inverse relation of dairy or calcium consumption with measures of body fatness and weight or no effect of dairy or calcium consumption. There is considerable heterogeneity among the studies published in this area thus far. Different methodologies used to assess body composition (DEXA *vs* BIA) and dietary intake (diet record, diet recall, and FFQ) may explain discrepancies among studies. In addition to heterogeneity in the assessment of dietary intake and body composition, studies vary in the number and type of variables used as covariates. For example, there is an inconsistent expression of calcium intake across published reports. It has been expressed as energy-adjusted calcium (Ca (mg)/daily calories),[22] protein-adjusted calcium,[21] and as a separate variable with energy intake included in the model as a covariate.[20] In addition, studies vary in the extent to which other dietary covariates are included in regression models.

Observational studies in children have reported significant inverse relations of dairy and calcium consumption with measures of body composition. Carruth and Skinner[24] conducted an analysis of longitudinal intakes (24–60 months) of dairy and calcium intake in relation to body fatness at 70 months in 53 preschool children. They concluded that calcium and dairy food consumption, expressed as calcium equivalents, were significant negative predictors of %BF and fat mass. In their analysis, however, intake was expressed as an average of the intakes at selected time points, rather than considering the pattern of change over time. Furthermore, their models, using %BF and fat mass as outcomes, were adjusted for BMI; the rationale for this choice is not clear, and would seem to make their results difficult to interpret. A case–control study of the predictors of obesity among 7 to 11-y-old Puerto Rican children observed that obese girls currently consumed fewer dairy foods than control girls, although the association was of borderline statistical significance ($P=0.054$) and no relation was observed among boys.[30]

The relation between dairy food consumption and body weight or fatness has also been examined with experimental designs, with varied results. In a randomized controlled trial (RCT) to evaluate the effects of dairy foods on bone and body composition in 48 pubertal girls, Chan *et al*[4] observed no significant differences between the groups in %BF or body weight after 12 months of follow-up. Results of another study by Chan *et al* (published only in abstract format) indicated that when 50 children aged 2–8 y with low calcium intakes (<800 mg daily) were assigned to either a dairy supplemented group or a control group for 6 months, children in the control group gained body fat during the study, while children in the dairy group had no significant change in body fat.[31] In a randomized open trial of milk intake on energy and nutrient intake and body weight among older adults, subjects in the milk group gained 0.6 kg more than the control group ($P<0.01$). The authors hypothesized that the weight gain may have been due in part to the timing of the study, as subjects were enrolled in the study during the fall and winter.[32] A 16-week randomized trial in which obese patients were assigned to a control diet (800 kcal), an isoenergetic diet of milk only, or milk plus one designated food only, those on the milk-only diet lost more weight than patients on the milk plus food diet, and significantly more weight than patients on the control diet. The authors hypothesize that the weight loss effect of the milk-only diet may be due to its novelty.[33] Although an experimental design is optimal for isolating the effects of dairy or calcium on body weight regulation, such studies may not provide definitive evidence if they are conducted over a short period of time.

In a prospective cohort analysis to examine the association between dairy food intake and insulin resistance syndrome (IRS) among 3157 young adults, researchers found an inverse association between the development of obesity (BMI $\geq 30\,kg/m^2$) and dairy food intake among those who were overweight at baseline (BMI $\geq 25\,kg/m^2$).[23] In another prospective analysis, Lin *et al*[22] conducted a secondary analysis in 54 young women aged 18–31 y who were participating in a 2-y exercise intervention.[22] They found that intake of calcium (adjusted for daily calories) and vitamin A predicted changes in body weight and %BF (at 2 y); the coefficient of calcium was negative and the coefficient of vitamin A was positive. In addition, they observed an interaction between calcium and energy intake such that calcium predicted change in body weight only at lower energy intakes.[22] Because their analysis used a calcium intake that was averaged across all diet records, the pattern of calcium intake in relation to body composition change is not elucidated.

Finally, Davies *et al*[21] re-evaluated data from five clinical studies with a primary skeletal endpoint to explore associations between calcium and body weight. In two cross-sectional studies, they found a negative association between BMI and calcium intake, where calcium intake was expressed as a calcium-to-protein ratio. In two longitudinal studies with weight change as the outcome, they observed no significant effect when the studies were evaluated separately; when they were combined, however, there was a significant inverse relation between calcium and change in body weight.[21]

We believe our approach has several strengths. Our analysis relies on a large number of annual measurements taken repeatedly over the adolescent period. The analytic approach selected allows us to capitalize on the richness of these data by characterizing individual variation relative to the population mean while taking into account the correlation between repeated measurements on the same subject and different numbers of measurements per subject.

Furthermore, additional analyses conducted in this cohort indicate that both the FFQ and BIA provide good estimates of dairy intake and percent body fat, respectively (see Methods). Percentage body fat was estimated using a prediction equation developed in this cohort, using measures of TBW by isotopic dilution of $H_2{}^{18}O$ as the criterion method.[27] Finally, we believe that type II error is unlikely, given the statistical power of the analysis. Our prestudy sample size estimates were based on a simple prepost change in body fatness. Since the additional time points in a longitudinal analysis provide greater precision, we repeated the power calculations to account for the additional measurements on each subject.[34] For daily servings of dairy and percentage of daily calories from dairy, we need at least 80% power to detect a difference in BMI z-score slope of 0.05 and over 99% power to detect a difference in %BF slope of 0.50%.

Our analysis also has some important limitations. All dietary methodologies are subject to measurement error and differential reporting of food intake is a concern in any study examining the relation between self-reported food intake and body weight. Indeed, many studies that have observed either no difference in energy intake between the obese and nonobese, or lower energy intakes among the obese have been criticized for this reason. Perks *et al*[35] compared energy intake estimated by FFQ with total energy expenditure (TEE) measured by doubly labeled water (DLW) in children and adolescents. They found that energy intake reported on the FFQ and energy expenditure by DLW were similar. However, discrepancy in energy intake was related to body weight and percentage body fat.[35] Differential reporting of specific foods is more difficult to study. However, we believe that dairy foods are more accurately reported than other foods on an FFQ because they tend to be eaten habitually and in readily quantified amounts. Dairy food consumption in this cohort appears higher than dairy food consumption reported in a nationally representative survey. Data from CSFII, based on diet recall, indicate adolescent females aged 12–19 y consumed an average of 269 g of milk and milk products per day, compared to 428 g of dairy products consumed in our cohort at study exit based on FFQ.[9] As a result of the differences in methodology, it is not possible to assess directly whether dairy intake in our cohort is representative of girls nationally. Lastly, our exclusion at baseline of any girls who were already overweight allows us only to examine the role of dairy foods on weight or fatness changes in initially normal weight preadolescent girls. Thus, we cannot address the influence of dairy consumption on weight and fatness changes in initially overweight girls.

An important consideration in assessing the results of these observational studies and intervention studies designed with primary skeletal endpoints is that none were designed with the intention of studying the relation between dairy food intake and body composition. Indeed, as noted by Heaney *et al*,[36] there have been few studies published that explicitly test the effect of calcium intake on body weight. Results of a 24-week RCT in humans, presented in abstract format, found that obese subjects who were supplemented with calcium or dairy products lost more weight than subjects randomized to a lower calcium diet. In addition, the weight loss effect was greater among subjects randomized to the dairy food group than the calcium supplement group.[37] Our results add another piece of evidence to the extant body of knowledge, which remains mixed. Further elucidation of the role of calcium in the regulation of body weight necessitates carefully designed observational, or perhaps experimental, studies conducted over longer periods of time.

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PAPER

The role of dietary calcium and other nutrients in moderating body fat in preschool children†

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OBJECTIVE: To assess preschool children's food consumption (24–60 months) and relate these findings to body composition at 70 ± 2 months.
DESIGN: A longitudinal study of children's dietary intakes for selected nutrients and servings of dairy products.
SUBJECTS: Fifty-three white children participating in a longitudinal study (2–96 months) of children's food practices and growth.
MEASUREMENTS: Using in-home interviews and trained interviewers, 18 days of dietary data and measured height and weight of each child at 6 month intervals were collected. Body composition was determined by dual energy X-ray absorptiometry.
RESULTS: Dietary fat was 30–33% of energy with saturated and monounsaturated fat intakes > 10% and polyunsaturated < 10%. Adjusting for body mass index (BMI), GLM models to predict percent body fat (%BF) or grams of total fat (gTF) with mean longitudinal calcium intake (%BF: $R^2 = 0.51$, $F = 7.88$, $P < 0.0001$; gTF: $R^2 = 0.51$, $F = 9.84$, $P = 0.0001$) or total servings of dairy products (%BF: $R^2 = 0.47$, $F = 6.93$, $P < 0.0001$; gTF: $R^2 = 0.47$, $F = 8.31$, $P < 0.0001$) as independent variables gave significant results. Higher mean longitudinal calcium (mg/day) intakes and more servings/day of dairy products were associated with lower body fat. Males had significantly less body fat ($P = 0.01$) than females.
CONCLUSIONS: Higher longitudinal intakes of calcium, monounsaturated fat, and servings of dairy products were associated with lower body fat.
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Keywords: calcium; preschool children; dietary fat; body fat; milk/dairy foods

Introduction

The importance of preschool children meeting their energy and growth needs is counterposed against the increasing number of overweight children in the United States.[1] In a study of 146 children over a 3 y period, the modifiable factors of dietary intake and physical activity accounted for more of the variance in body mass index (BMI) of children than the obesity status of the parents.[2] Modification of children's dietary fat intake has been especially targeted because of epidemiological data indicating that dietary fat intake early in life increases an individual's risks for obesity and other diseases in adulthood.[3] In parents' efforts to meet current guidelines[4] for dietary fat intake of children as no more than 30% of energy by age 2, some higher fat foods, such as dairy products and meats, may be limited or omitted from children's diets. Dairy products are the most nutrient dense source of calcium in children's diets.

Recent human and animal studies indicated that a higher calcium intake was associated with reduced body fat or less gain of body fat over time. In a study of adipose cells in transgenic mice, high calcium, medium dairy, and high dairy diets reduced lipogenesis, stimulated lipolysis and reduced body fat accumulation at equivalent levels of energy intake.[5]

The authors are conducting a longitudinal study of healthy white children (2–96 months) with the initial purpose of documenting their feeding practices and growth patterns. Families of middle and upper socioeconomic status (SES) were purposefully recruited to limit the potential negative effect on children's food intake and health status associated with lower SES and limited access to health care.[6] In addition, approximately 50% of infants born in the United States are white and of middle/upper SES[7] and many of the recommended nutrient intakes are based on this population.[8]

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Extrapolation of the calcium studies using adult subjects or mice to other population groups raises a question of whether longitudinal intakes of dietary calcium and dairy products moderate accretion of body fat in young children. When longitudinal nutrient intakes and energy are adequate to promote growth, are there other dietary components that may influence the amount of body fat at 70 months, an age that some children experience an adiposity rebound?[9] Our study objectives were to determine longitudinal intakes (24–60 months) of the energy macronutrients (protein, fat and carbohydrate), servings of dairy products, and intakes of selected micronutrients found in dairy products (eg, calcium, vitamin D) and then relate these longitudinal dietary intakes to children's body composition at 70 (±2) months.

Methods

Subjects

The study sample was composed of 53 children (29 males and 24 females) who represented a subset from a larger longitudinal study of 72 children living in the United States.[10] Nineteen children did not participate in the body composition assessment: seven parents declined, seven parents and children were not in the geographical area at 70 (±2) months and five children could not be scheduled within their time frame because of illnesses or scheduling conflicts at the medical site. The protocol for bone density measurements was approved by the medical center's institutional review board.

Diet

Two Registered Dietitians (RD) conducted in-home interviews with mothers when their children were 24, 28, or 32 and 28, 32, or 36 months (two interviews/child in the third y); all children were seen at 42, 48, 54, and 60 months of age. Interviews were done within ±10 days of the child's birthday. Three days of dietary intake (2 week days and 1 weekend day) were collected at each of six interviews providing 18 days of dietary data/child. The same interviewers saw the same children throughout the study period, and they probed for information and checked the completeness of mothers' responses at each interview. Also, mothers had been interviewed since their children were 2 months of age, and by 24 months they were trained in keeping accurate diet records and giving complete responses.[11]

Anthropometry and body composition

The children's height and weight were measured at each of the six in-home interviews, using a Center for Disease Control protocol previously described.[12] Body composition was assessed at 70 (±2) months, using DEXA (Model QDR Hologic 2000). All DEXA scans used low density software. Both anthropometric and dietary data were collected simultaneously from 24–60 months; however, the measurement of body composition at 70 months provided data about the cumulative physiological effects of longitudinal energy and nutrient intake on body composition. No follow-up body composition measurements were done for those 12 children who could not meet the 70 months time frame or the seven who declined participation in the body composition assessment.

Analyses

Diet. Nutritionist IV software (version 4.1) was used to calculate the average nutrient intake/day for each child at each interview (3 day dietary data for each of six interviews). For most foods consumed by children in this study, the nutrient database for saturated fats was complete; however, it was less complete for other components of dietary fat. When a food had incomplete listings for monounsaturated and polyunsaturated fats, a generic food with more complete listings was used, based on a match for energy/nutrient distribution and amount of saturated fat. Nutrient intakes for each child at each time period (3 days of dietary records/recalls) were averaged for each time period to give six representative days. Using SAS PROC MEANS,[13] these representative days were used to calculate group means at 27 months ($\bar{x}$ time of interviews at 24, 28, or 32 months), 34 months ($\bar{x}$ time of interviews at 28, 32, or 36 months), 42, 48, 54, and 60 months. The group means were compared to the Recommended Dietary Allowance (RDA)[8] and the Dietary Reference Intake (DRI)[14,15] for the ages 1–3 and 4–6 y. Student's *t*-test was used to determine significant gender differences in energy and nutrient intakes from 24–60 months.[13]

To calculate the total number of servings from the milk/dairy products, serving sizes were based on the calcium equivalent for 8 oz (240 ml) of fluid milk. For example, if a food contained one-half the calcium equivalent, then it was considered half a serving. For each child, the total number of servings were computed from the three days of food intake at each of six interview times. The three day data set was averaged with SAS PROC MEANS[13] to give a representative day for the number of servings of milk/dairy products for six representative days

Anthropometry. Using SAS PROC MEANS,[13] group means were computed for the children's BMI (kg/m^2) at 60 months, total body fat (g, %) derived from DEXA measurements at 70 months, and the parents' BMI. Tertiles were formulated based on percent body fat (% BF), and significant anthropometric and body composition differences between the highest and lowest tertiles were determined by Student's *t*-test.[13] Anthropometric results were compared to normative percentiles for age and gender.[16]

Mean height, weight, and BMI by gender at 60 months were computed for the 19 children who did not have body composition assessments.[13] Significant anthropometric differences between the 53 children who participated

and the 19 children who did not participate in the DEXA measurements were determined with Student's *t*-test.

Statistical modeling. General Linear Models (GLM) were developed using body fat (%) or total body fat (g) as the dependent variable, and the independent variables were the children's gender, BMI, parents' BMI, and longitudinal mean intakes of protein, carbohydrate, dietary fat (including saturated, monounsaturated and polyunsaturated fat), calcium, vitamins D and A, and riboflavin. Preliminary analyses with PROC R Squared (SAS) were used to determine which combination of variables explained the most variance in body fat and which variables to investigate with GLM models. Correlation matrices showed that parents' BMI was significantly related to child's BMI ($P=0.05$).

Longitudinal nutrient intakes were based on 6 representative days of dietary intake (3 day data set averaged at each of 6 interview times). Each child's nutrient intake on these representative days were averaged for a mean intake (g or mg/day) that was used as an independent variable in developing the GLM models. Similarly, mean longitudinal servings of dairy products used as an independent variable in the models were derived from a representative day (average of 3 day dietary data set) at each of 6 interviews, and then the 6 day averaged to give a mean longitudinal intake (number of servings/day) used in model building. The other independent variables were the same whether calcium or servings/day of dairy products were used in the models.

Results

Anthropometry and body composition

Table 1 describes anthropometric and body fat indices by gender for 53 children. Using normative growth percentiles[16] the males' mean linear height (cm) exceeded the weight percentile. For females, the mean weight and height were within the 50–75th percentiles. Males were taller than the females, but BMI for both genders was at the 50th percentile or slightly higher (norm = 15.4 *vs* males' BMI = 15.7). Both increased lean body mass and less body fat of males *vs* females are reflected in the BMI. As shown by the DEXA results, males had significantly lower percent body fat ($P=0.01$) and more lean body mass ($P<0.0001$) than females. The range of body fat (%) for each gender was about 2-fold (females = 14.3–30.8%; males = 11.9–30.8%). The difference between g body fat by gender was non-significant ($P=0.36$), and the ratio of fat free mass (g) to fat mass (g) was 4.5 for males and 3.7 for females.

Table 1 Anthropometric and body fat indices of 53 preschool children

Indices	*Males* n = 29		*Females* n = 24	
Height (cm)[a,b]	112.1 ± 3.0[c]	(75th)[d]	108.9 ± 4.6	(50th)
Weight (kg)	19.7 ± 2.1	(50–75th)	18.3 ± 2.0	(50–75th)
BMI	15.7 ± 1.2	(50–75th)	15.4 ± 1 0	(50th)
% BF_{DEXA}[e,f]	17.9 ± 3.8		20.8 ± 4.4	
BF (g)	3888.6 ± 1116.2		4192.7 ± 1262.5	
Range of %BF	11.9–30.8		14.3–30.8	

BMI = body mass index; BF = body fat; DEXA = dual energy X-ray absorptiometry.
[a,b]Height and weight of 5 y old children
[c]mean ± s.d.
[d]Normative percentiles for 5 y old children.[16]
[e]BF of children at 70 ± 2 months.
[f]Males had significantly lower %BF than females ($P=0.01$).

To further explore the diversity of body composition and examine extreme values within the group ($n=53$), tertiles were developed with males and females combined to form two groups that represented the highest and lowest percent of body fat. Table 2 shows that children in the highest body fat group (%) were not significantly different in height from those in the lowest body fat group, but they had significantly greater BMI, body weight (kg), and body fat (%, g). The mean BMI of the highest tertile was at the 75th and the lowest tertiles were slightly higher than the 25th percentile (norm = 14.6 *vs* 15.0).[16]

The comparison of the 19 children who did not participate and 53 participants in the study showed there were no significant differences in height (cm), weight (kg), or BMI of females. Weight (kg) and BMI of males in the two groups were not significantly different. However, males ($n=29$) in

Table 2 Anthropometric differences in preschool children whose body fat (%)[a] was in the highest and lowest tertiles[b] compared to the group

	Group (n = 53)	*Highest tertile* (n = 17)	*Lowest tertile* (n = 17)	P[b]
BMI (kg/m^2)	15.6 ± 1.1[c]	16.2 ± 1.09	15.0 ± 0.82	0.0007
Height (cm)	110.6 ± 4.1	111.6 ± 3.9	110.5 ± 3.2	0.3750
Weight (kg)	19.1 ± 2.1	20.2 ± 1.9	18.3 ± 1.5	0.0024
$\%BF_{DEXA}$	19.2 ± 4.3	24.0 ± 3.9	15.4 ± 1.7	< 0 0001
BF (g)	4026 ± 1183	5356.7 ± 1073.3	3021.8 ± 410.4	< 0.0001

BMI = body mass index; BF = body fat; DEXA = dual energy X-ray absorptiometry
[a]BF of children measured at 70 ± 2 months.
[b]Difference between highest and lowest tertiles (Student's t-test).
[c]mean ± s.d.

the group of 53 children were significantly taller ($P=0.006$) than males ($n=8$) in the group of 19 children ($\bar{x}=112.1\pm3.0$ *vs* 108.4 ± 3.9).

Energy and macronutrient intakes

Energy (kcal/day, kJ/day), fat as percent of energy, components of dietary fat, protein, vitamins D, A, and E, calcium and cholesterol for 24–60 months are shown in Table 3. Mean energy intakes exceeded the allowance of 1300 kcal/day (kJ = 5439) for children 1–3 y, but intakes were less than the 1800 kcal/day (kJ = 7531) for

Table 3 Energy and nutrient intake of preschool children (24–60 months)

		Months					
Nutrient	*RDA/DRI*[c]	*27*[a]	*34*[b]	*42*	*48*	*54*	*60*
Energy (kcal)	1300/1800						
[kJ]	[5439±7531][d]						
M		1410±346[e]	1473±277	1530±293[f]	1577±334[f]	1540±338[f]	1710±348
		[5899±1448]	[6163±1159]	[6402±1226]	[6598±1397]	[6443±1414]	[7155±1456]
F		1332±300	1394±412	1316±269	1363±334	1352±317	1568±493
		[5573±1255]	[5832±1724]	[5506±1125]	[5703±1397]	[5657±1326]	[6561±2063]
Total fat (g) (% of energy)							
M		48±14	49±10	52±15	54±12[g]	53±14	58±15
		(31)	(30)	(31)	(31)	(31)	(31)
F		47±13	49±18	44±14	46±14	48±15	57±25
		(32)	(32)	(30)	(30)	(32)	(33)
Saturated fat (g)							
M		17±6[e]	19±4	19±6	20±6	20±6	22±7
F		16±5	18±6	17±5	17±6	18±6	22±12
Monounsaturated fat (g)							
M		15±5	16±4	18±6	20±5[g]	19±5	21±6
F		15±6	16±6	16±5	17±5	17±6	20±8
Polyunsaturated fat (g)							
M		7±3	7±2	8±3	9±2	9±3	9±3
F		6±2	7±3	7±3	8±3	8±4	9±3
Saturated/poly fat ratio							
M		2.4·1	2.7:1	2 4:1	2.2 1	2.2:1	2.4.1
F		2.7:1	2.6.1	2 4:1	2.1.1	2 3.1	2 4:1
Protein (g) (% of energy)	16/24						
M		47±15[e]	50±11	51±15	53±15	53±16	57±14
		(13)	(14)	(13)	(13)	(14)	(13)
F		47±15	48±18	49±17	47±13	49±11	51±16
		(14)	(14)	(15)	(14)	(14)	(13)
Vit D (μg)	5						
M		3.6±2.3	4.0±2.0	4.4±2.1	4.7±2.5	4.8±2.3	4.7±2.3
F		4.3±2.6	4.4±2.5	4.3±2.8	4.3±2.1	4.6±2.3	4.2±2.7
Vit A (μg) RE	400/500						
M		697±393	783±425	613±236	664±387	727±304	728±357
F		811±650	782±583	603±367	660±522	678±283	772±341
Vit E (mg α TE)	6/7						
M		3.1±1.9[e]	3.2±2.1	2.9±1.7	3.3±2.5	3.6±2.9	3 2±2.8
F		3.5±1.6	4.2±2.7	3.1±2.4	2.7±2.0	3.5±3.5	3.3±3.4
Calcium (mg)	500/800						
M		823±364	791±252	846±277	844±356	949±287[h]	968±340[h]
F		789±312	808±375	774±315	698±224	787±227	751±343
Cholesterol (mg)[i]							
M		139±86	155±83	143±72	165±98	155±74	164±75
F		130±63	143±72	141±75	139±68	144±43	168±96

[a]27 months: mean age for dietary intakes at 24, 28, or 32 months.
[b]34 months: mean age for dietary intakes at 28, 32, or 36 months.
[c]Recommended Dietary Allowances (1989)/Dietary Reference Intake (1997, 2000) for children 1–3 and 4–8 y
[d]mean ± s.d. for kcal and for kJ [4.184×kcal].
[e]mean ± s.d.
[f]Gender differences for energy at 42, 48 and 54 months ($P=0.008$, $P=0.02$, $P=0.04$).
[g]Gender difference for total fat ($P=0.03$), monounsaturated fat ($P=0.03$) at 48 months.
[h]Gender difference for calcium at 54 ($P=0.03$) and 60 ($P=0.02$) months.
[i]No RDA/DRI for cholesterol or fat.

children, 4–6 y.[8] From 24–60 months, the amount of fat and protein as percent of energy vary minimally. Children in the study did not gradually decrease their fat intake over time, but had mean intakes of ~31% by 12 months of age.[11] The protein intakes were 2 to 3 fold the RDA which reflects both animal and dairy consumption.

Males had higher mean intakes of energy compared to females at 42, 48, and 54 months, dietary fat and monounsaturated fat at 48 months, and calcium at 54 and 60 months (Table 3). Although mean intake of dietary fat over time was about 31% of energy, the amount of saturated fat exceeded the recommended 10% to achieve the energy from fat/saturated fat ratio of 30:10 by age 5. The saturated to polyunsaturated fat ratios (24–60 months) as shown in Table 3 also suggest that the study children had limited intakes of vegetable oils and consumed more of the foods that contained saturated fats.

Restricting fat in the diets of preschool children may negatively influence nutrient intakes of vitamins A, D, and calcium, because these nutrients are found in milk/dairy products that also contain fat, such as whole and reduced fat milks and cheeses. However, results of this study indicate that the dairy foods were not limited, and the most frequently consumed foods that contained larger amounts of fat included: cheeses, 2% fat milk and ice cream. As shown in Table 3, mean intake of vitamin D over time was > 90% of Adequate Intake (AI) and vitamin A intakes consistently exceeded the recommended allowance for children. Mean calcium intakes (24–60 months) ranged from 791 ± 252 to 968 ± 340 mg/day for males and 698 ± 224 to 808 ± 375 mg/day for females. These intakes meet or exceed 95% of the new AI for calcium for children, 1–3 and 4–6 y of age.[14]

Over the study period, vitamin E intakes ranged from 48–51% of the recommended amount.[8,15] In general, children in the study consumed limited amounts of foods, salad dressing, or vegetable oils that are good sources of vitamin E. Some ready-to-eat foods, such as french fries and fried chicken, may have been prepared with vegetable oils that provided vitamin E. By using generic coding when vitamin E values were not available from the vendors, the vitamin E values better reflect dietary intake. French fries and products of animal origin (eg meats, cheeses, ice cream) were major sources of dietary fat consumed by children in this study. Although there is no recommended allowance for cholesterol, mean intakes were within the normal range of 100 mg/1000 kcal.[8]

Statistical modeling to relate body fat with dietary intake

Using GLM procedures, models with calcium or dairy products as independent variables to predict body fat (% or g) are shown in Tables 4 and 5. The children's percent and g body fat adjusted for BMI was positively related to mean longitudinal intakes of dietary fat and protein and negatively related to calcium and monounsaturated fat intakes (Table 4). These results suggest that higher mean longitudinal intakes of calcium and monounsaturated fat were associated with lower body fat at 70 months. Gender was negatively related to percent body fat, but not g body fat. Mean longitudinal intakes of polyunsaturated fat, saturated fat, carbohydrate, and father's BMI did not contribute significantly to the models with calcium. Also, vitamins D and A were not significant in any models with either calcium or servings of dairy products as independent variables to predict body fat of the children.

The GLM models with dairy products, (mean longitudinal servings/day) as the independent variable are shown in Table 5. Adjusting for BMI, the variability in percent body fat was significantly and negatively related to mean longitudinal servings/day of dairy products and gender, and positively related to protein. For the model using total g body fat, higher mean longitudinal servings/day of dairy products and monounsaturated fat intakes were negatively associated with body fat (g). Gender, protein, and dietary fat did not con-

Table 4 Regression analyses of longitudinal nutrient intakes (24–60 months) with body fat of preschool children[a]

	Regression Coefficients ($\beta \pm SEE$)						
Models[b]	*BMI*	*Gender*[c]	*Calcium*[d]	*Protein*[d]	*Dietary fat*[d]	*Monounsaturated fat*[d]	R^2
$\%BF_{DEXA}$	2.05 ± 0.41	− 2.64 ± 0.94*	− 0.015 ± 0.004	0.250 ± 0.087	0.619 ± 0.261	− 1.79 ± 0.72	0.51
	F = 25.26	F = 7.79	F = 15.24	F = 8.21	F = 5.63	F = 6.21	F = 7.88
	$P < 0.0001$	$P = 0.008$	$P = 0.0003$	$P = 0.006$	$P = 0.02$	$P = 0.02$	$P < 0.0001$
BF g, DEXA	704.55 ± 110.00	NS	− 3.78 ± 0.97	61.08 ± 23.12	178.65 ± 70.06	− 521.83 ± 191.92	0.51
	F = 41.02		F = 15.08	F = 6.98	F = 6.50	F = 7.39	F = 9.84
	$P < 0.0001$		$P = 0.0003$	$P = 0.01$	$P = 0.01$	$P = 0.009$	$P = 0.0001$

BF = body fat; BMI = body mass index; DEXA = dual energy X-ray absorptiometry.
[a]BF of children measured at 70 ± 2 months; $n = 53$, 29 males and 24 females.
[b]General Linear Models (GLM) procedures to predict BF (%, g).
[c]Males had significantly less % BF than females ($P = 0.01$).
[d]Nutrient intakes based on a 3 day dietary data set averaged at each of the 6 interviews to give 6 representative days/child. Longitudinal nutrient intake/day refers to an average of the 6 representative days.
*$P = 0.01$; males had less fat.

Table 5 Regression analyses of longitudinal dairy food and nutrient intakes (24–60 months) with body fat of preschool children[a]

	Regression Coefficients ($\beta \pm SEE$)						
Models[b]	BMI	Gender[c]	Dairy products[d]	Protein[d]	Dietary fat[d]	Monounsat fat[d]	R^2
% BF_{DEXA}	2.10±0.43	−3.41±0.94	−3.54±1.04	0.163±0.078	0.465±0.255	−1.21±0.68	0.47
	F=24.32	F=13.12	F=11.52	F=4.36	F=3.34	F=3.16	F=6.93
	$P<0.0001$	$P=0.0007$	$P=0.001$	$P=0.04$	$P=0.07$	$P=0.08$	$P<0.0001$
					NS	NS	
BF g, DEXA	703.44±115.38	NS	−907.06±284.60	38.36±21.32	136.48±69.74	−368.41±185.05	0.47
	F=37.17		F=10.16	F=3.24	F=3.83	F=3.96	F=8.31
	$P<0.0001$		$P=0.003$	$P=0.08$	$P=0.06$	$P=0.05$	$P<0.0001$
				NS	NS		

BF = body fat; BMI = body mass index, DEXA = dual energy X-ray absorptiometry.
[a]BF of children measured at 70±2 months; $n=53$, 29 males, 24 females.
[b]General Linear Models (GLM) procedure to predict BF (%, g).
[c]Males had significantly less % BF than females ($P=0.01$)
[d]Number of servings for 3 day averaged at each of the 6 interviews to give 6 representative days/child. Longitudinal dairy product/nutrient intakes/day refer to an average of the 6 representative days.

tribute significantly to the g body fat model. The result that gender was significant in the percent body fat models but not significant in the total g body fat models appear contradictory but can be explained by gender differences; percent body fat of males was lower ($P=0.01$), and their lean body mass was higher than females ($P<0.0001$), and there was no significant gender difference in total g body fat.

These results with dairy products in the model for total g body fat are consistent with those with calcium in the model (ie higher mean longitudinal intakes of calcium and monounsaturated fat were associated with lower percent and g body fat). In addition, the results with dairy products are consistent with the methodology used for determining serving sizes from the milk/dairy group, which was based on calcium equivalents in an 8 oz (240 ml) serving of milk. In this study, dairy foods were the major dietary source of calcium and monounsaturated fat in the children's diets.

Discussion

Mean longitudinal intakes of calcium (mg/day) and monounsaturated fat were significantly and negatively related to lower body fat (%, g). Similarly, mean longitudinal intakes of dairy products (number of servings/day) and monounsaturated fat intakes were associated negatively with body fat (g), but monounsaturated fat was not a significant predictor of percent body fat ($P=0.08$). When dairy products compared to calcium were in the predictive models, results for dietary protein, fat, and monounsaturated fat showed similar trends, but some values did not reach statistical significance (eg, $P=0.08$, $P=0.06$). However, dairy products contain these nutrients, and this may affect how they contributed to the models. Our findings support the relationship between higher calcium intakes or dairy products and lower body fat as previously reported in human and animal studies.[5] Similarly, the results that protein intakes were positively associated with increased adiposity of children in this study has been reported by other investigators.[17–20]

Although there are no adequate reference data for fat free mass, fat mass, and percent of body fat of preschool children,[21] small studies have reported mean body fat for males (ranges ~18–20%), and for females (~24–26%) who were of comparable age to children in this study.[22,23] Our results and those of other small studies[21–23] suggest that the physiological gender differences in body fat are observed in preschool children.

Our results show a consistent pattern of fat intake as ~31% of energy, and total energy intake was apparently adequate as reflected by normal growth in these children compared to their age/gender cohorts. Similar results about growth patterns and reduced dietary fat have been reported in preschool Hispanic children, Australian children, and Finnish children.[24–27]

In a survey comparison of children's consumption patterns from 1986–1994, dietary intake from the milk/dairy group differed by income categories.[28] Children in the highest income group maintained calcium intakes by using low fat and/or fat free milk products. The families in our study were primarily of middle and upper SES, and a majority of the children consumed 2% of fat free milk by 60 months of age, which probably contributed to their 31–33% of energy intake from fat. Our findings about dairy products and body fat may have implications for any population of children who report high intakes of beverages other than milk.[29]

A possible mechanism to explain why calcium was a significant factor in predicting body fat (% and total g) of preschool children in this study has been studied in transgenic mice and in human adipocytes.[5] In transgenic mice given various amounts of non-fat dry milk and calcium supplements, a high dairy calcium diet resulted in stimulated lipolysis 5.2-fold over the basal diet. Using *in vitro* analyses, human adipocytes responded to both 1,25-$(OH)_2$-D and parathyroid hormone with dose responsive increases

in intracellular Ca^{2+}. The investigators suggested that low Ca^{2+} diets increased adipocyte intracellular Ca^{2+} while higher Ca^{2+} diets suppress the calcitrophic hormone response. The increased dietary calcium reduced intracellular Ca^{2+} and decreased triacylglycerol accumulation through increased lipolysis.[5]

In a randomized controlled clinical trial of reducing diets in adult outpatients, those maintained on a milk-based diet for 16 months had greater weight loss (7.0 *vs* 1.7 kg) than patients maintained on a conventional hypocaloric diet that was isocaloric to the milk-based diet. The investigators suggested that greater compliance with the novel milk-based diet contributed to the greater weight loss.[30] However, the possible down regulation of lipogenesis and up-regulation of lipolysis with the milk-based diet *vs* the conventional hypocaloric diet is an alternate explanation for the greater weight loss.

It is unknown whether similar inhibition of lipogenesis and enhanced lipolysis occurs in children who consume diets that contain higher amounts of calcium and/or dairy products. If this mechanism to suppress fat acquisition occurs in preschool children with calcium intakes that meet or exceed the reference intake, then fat as 30% of energy as currently recommended[4] could result in a lower amount of body fat and a decreased potential for adiposity in childhood. However, if preschool children meet current US dietary guidelines for energy intake from fat by limiting their intakes of milk/dairy products, dietary calcium intake over time will be reduced. Results of this study also raise the question of whether other components of milk/dairy foods are contributing to the relationships between higher calcium intakes and lower body fat. For example, would a calcium supplement produce the same effect on acquisition of body fat in preschool children as the dairy products observed in this study? The adverse effect of chronically low calcium intakes on body composition of preschool children should be studied further, using longitudinal dietary patterns to establish the role of milk/dairy products in moderating the acquisition of body fat.

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