27

Genetics of Energy and Nutrient Intake

Treva Rice, Louis Pérusse, and Claude Bouchard

Introduction

The study of the role of genetic variation on energy intake and nutrient intake is broad and has considerable public health implications. Genetic differences influence behavioral and biological affectors of food intake. They are also thought to impact on several nutritionally influenced risk factors (e.g., dyslipoproteinemia) and morbid conditions (e.g., diabetes). These issues have been the topic of much research in the past few decades, as evidenced by the multiple review articles cited in this section. In the behavioral domain, the questions are relatively simple. Do genes determine eating behaviors such as how much one eats, preferences for certain types of foods, and frequency or pattern of eating? The current research suggests that there is resemblance among family members for these behaviors, although it is unclear if they are determined by genes, shared environments, or both.[1] In the physiological domain the questions center on the physical and hormonal mechanisms leading to such things as taste preferences, hunger, and satiety. For instance, taste receptors are clearly genetically determined, although the gene(s) may not be all identified yet,[2] and a growing number of genes encoding hormones and proteins that regulate hunger and satiety have been identified recently.[3]

The genetics of energy and nutrition intake invoke complex issues from the fields of genetic and molecular epidemiology, involving both genetic and environmental interactions. Gene-gene (GxG) interactions occur when the effect of one gene is modified by or depends on the effects of another. For example, leptin is a hormone involved in the signaling between adipose tissue and the hypothalamus. The gene (LEP) that synthesizes leptin has been identified and mapped to chromosome 7. However, multiple factors influence the circulating levels of leptin, and some of these (such as insulin levels) have their own genetic determinants. Thus, the measurable levels of circulating leptin can be influenced by interactions with other genes.

Another complex issue from genetic and molecular epidemiology involves gene-environment interactions (GxE). In this situation, energy and nutrient intake are considered environmental factors which impact on other traits that may have a profound interest from a public health perspective. For example, the exposure of individuals with certain

genetic mutations to high-fat/cholesterol environments may predispose them to develop disease, while other individuals with alternative gene forms promoting genetic protection remain free of disease in the same high-risk environment. Such gene-diet interaction questions have been addressed in the fields of genetic and molecular epidemiology and constitute the major focus of this review.

This section does not claim to extensively review every topic relating to the genetics of energy and nutrient intake. Rather, an attempt is made to give an overview of the breadth of the problem, some interesting findings, and suggestions for further study. A short review of genetic and molecular epidemiology methods is followed by overviews of the familial factors underlying the behavioral aspects of macronutrient intake, and gene-diet interaction effects on risk factors for coronary disease.

Genetic and Molecular Epidemiology

Before investigating the complex issues of GxE interactions, it must first be established that the trait of interest is heritable, or that it runs in families. Familial resemblance for a trait arises when members within families are more similar than are unrelated pairs of individuals and may be estimated in terms of correlations (or covariances) among family members. Methods for estimating familial resemblance range from relatively simple to very complex.[4,5] However, the cause of the familial resemblance may be due to shared genes, shared environments, or both. In the case where the gene is not known, or not measured, familial resemblance is indexed by comparing the degree of phenotypic (trait) sharing among family members of varying degrees of relatedness. For example, sibling, parent-offspring and dizygotic (DZ) twins share 50% of their genes in common, monozygotic (MZ) twins share 100% of their genes in common, and spouse pairs share few or no genes in common if there is random mating for the trait under study. Depending on cohabitation effects, all of these relative pairs may share some degree of family environments.

Maximal heritability quantifies the strength of the familial resemblance. It represents the percentage of variance in a trait that is due to all additive familial effects, and can include both genetic and familial environmental sources. Depending on the complexity of the study design, this may be partitioned into separate estimates of genetic versus cultural (familial environmental) heritabilities. Each of the genetic and familial environmental sources may be partitioned further. For example, complex traits (phenotypes) may be due to one or more genes with moderate to major effects (oligogenic), many genes each having small effects (polygenic), and/or familial environments that are specific to certain relative pairs such as sibling, twin, or spouse.

In addition to these main effects of genes and familial environments, there may be interactions among these factors such as gene-gene (epistasis) and GxE. These effects are generally non-additive and thus may not be identified in heritability studies described above. Of major interest in this review are GxEs that arise when the phenotypic expression of a trait corresponding to a particular genotype depends, in part, on exposure to particular environmental factors. For example, GxE may occur if the fat mass response to dietary intervention depends on (or is modified by) an individual's genotype. In the hypothetical case of Figure 27.1, an absence of GxE is depicted in panel (a), where the body fat response to increasing fat intake is consistent across genotypes, with a simple mean shift by genotype. In contrast, GxE is present in panel (b), where some genotypes show very different patterns of fat mass accumulation with increasing levels of energy intake.

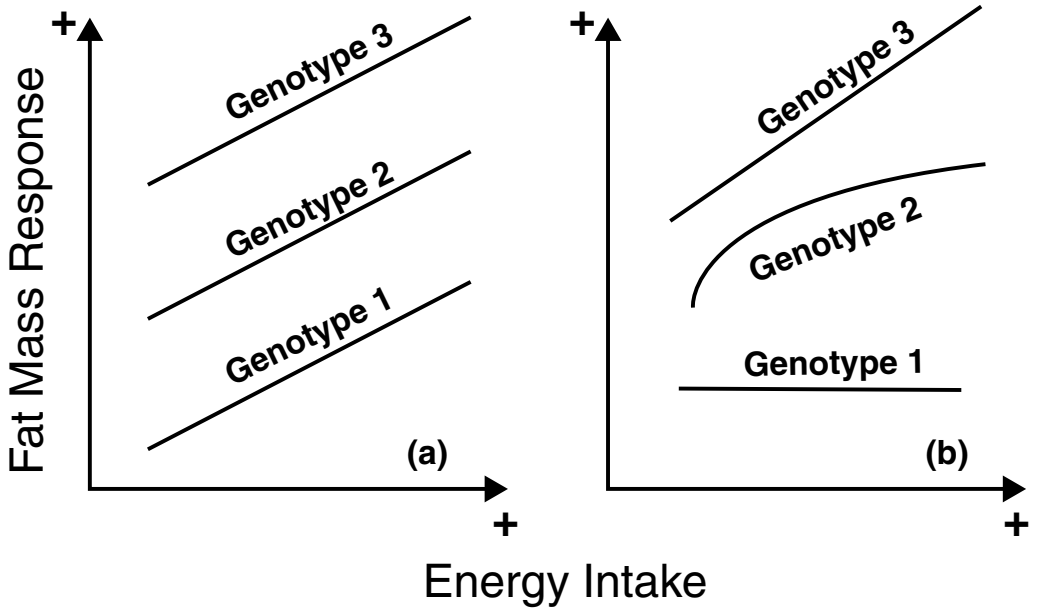

FIGURE 27.1
Hypothetical example of three genotypes plotted by energy intake (X-axis) and fat mass (Y-axis) values. In panel (a), there is no gene-diet interaction since the lines depicting the responses by genotype are parallel. In panel (b), gene by diet interaction is demonstrated, since there is a differential response in fat mass due to energy intake as a function of the genotype. That is, the genotypes respond differently.

Two major approaches are used to study GxE, unmeasured genotype (genetic epidemiology) and measured genotype (molecular epidemiology), as recently reviewed by Pérusse and Bouchard.[6] The twin methodology is a very useful unmeasured genotype approach for testing GxE. One important assumption underlying the twin method is that MZ and DZ twins have equal environmental covariances. Variances can be tested for significant differences across twin types using classical analysis of variance approaches. If the variances are different prior to but not after adjustment for pertinent environmental covariates, then there is indirect evidence of GxE interaction. Alternatively, the twins can be stratified according to degree of environmental sharing, and heritability estimates may be compared across the strata.

The above twin method is cross-sectional and therefore an indirect assessment of the GxE effects. Another unmeasured genotype approach that more directly indexes GxE is the twin intervention study, where MZ pairs (who share 100% of their genes in common) are challenged under standardized treatments (environments). A comparison of the within- and between-pair variances of the response to treatment provides an indication of whether genetic factors underlie the response. That is, a greater variability between- than within-pairs suggests a greater correlated response to environmental challenge for the same genotype.

The second major approach for detecting GxE involves measured genotypes,[6] with the preponderance of evidence for measured gene-nutrition interactions arising from intervention studies. This method is usually applied to association analysis of candidate genes. However, it can be applied to association or linkage analysis of candidate genes or genome scan data.[7] Linkage studies seek identification of loci that cosegregate with the trait (e.g., dietary response) within families, while association studies seek identification of particular variants that are associated with the response at the population level. In other words,

linkage analysis is often useful in localizing gene effects but requires family data, while association analysis can provide information about the functional variants that ultimately give rise to the observed phenotypic variability and may be applied to family or individual data. These complementary methods provide the means to probe the genome and describe the complex genetic etiologies underlying the responses to interventions. In association analysis of candidate genes, the phenotypic responses to intervention are compared among groups stratified by genotypes. If individuals with a particular allele form tend to respond to the intervention differently than do individuals with alternative allele forms, then there is evidence of GxEs. GxG interactions are simply incorporated in association analysis by including the main effects of multiple candidate genes, as well as the interaction terms between them.

Although association analysis may be applied to either individual or family data, the dependencies among related individuals should be considered, since failure to adjust for nonindependence can inflate the association evidence. Methods for dealing with this problem range from complex, such as bootstrapping[8] where the model is repeatedly fit to subsets of the data, to relatively simple, such as sandwich estimators.[9,10] The sandwich method asymptotically yields the same parameter estimates as ordinary least squares or regression methods, but the standard errors (and consequently hypothesis tests) are adjusted for the dependencies.

Familial Factors Underlying Macronutrient Intake

In the last few decades, the familial factors underlying macro- and micronutrient intake have been characterized. These studies differed in many respects, making direct comparisons difficult. For example, study designs (twin vs. family), statistical methods of analysis and how macronutrient intake was measured (diaries ranging from 3 to 9 days, 24-hour recall) and reported (absolute vs. percent of kcalories, and adjusted vs. unadjusted for covariates) varied.[1,11] The major conclusion drawn from a review of these studies is that there is familial resemblance, although the source of the resemblance is unclear. For example, deCastro,[12] using a twin design, reported that additive genetic effects accounted for 40 to 65% of the variance for each macronutrient examined, and that there was no contribution from familial environmental sources. On the other hand, two family studies [13,14] suggested that most of the resemblance (30 to 50% of the variance) was due to familial environmental factors. An early study, involving the largest sample sizes reported to date,[15] reflects the most probable answer to the question of genetic vs. environmental determination of food intake: people who live together come to resemble each other whether they are genetically related or not. This conclusion is based on the fact that correlations between genetically unrelated pairs of individuals who live together are as large as those for genetically related cohabitating individuals. Similar conclusions were drawn from both twin[12,16] and family studies.[13,14,17,18]

The magnitude of the familial effect for macronutrient intake generally centers between 30 to 50% in family studies, with higher estimates derived from many twin studies. However, as shown in Table 27.1, there is a considerable range in estimates across studies. Moreover, resemblance tends to be higher for nutrients expressed in percent of total caloric intake as compared to absolute amounts, both across studies and within studies that indexed both types of measures.[13,19] This suggests that the familial effect may be specific to food selection or preference (nutrient concentrations) rather than for amount of foods consumed. Results from twin studies [20,21] and from animal models showing differences in

TABLE 27.1

Range of Heritability Estimates (%) for Macronutrients*

Macronutrient	Genetic	Cultural	Combined
Total kcalories	0–78	23–45	24–51
Absolute values			
Protein	0–70	26–41	8
Total fat	0–56	26–50	9–24
Saturated fat	50–60		10–27
Monounsaturated fats			33–41
Polyunsaturated fats	10–40		3
Saturated/unsaturated ratio			3–12
Total carbohydrates	0–68	20–26	31–36
Simple carbohydrates	12–60		8–20
Complex carbohydrates	22–62		55–56
Sodium			10–71
Dietary Na/K			20–68
Urinary Na/K			18–53
Percent of total kcalories			
Protein	10–70	25–61	61
Total fat	18–48	8–51	27–54
Saturated fat			10–70
Monounsaturated fats			50
Polyunsaturated fats			47
Total carbohydrates	15–67	12–47	52
Dietary cholesterol			66
Sodium			51
Potassium			24
Calcium			52

* Estimates extracted from References 12-14, 16-19, 102.

macronutrient selection among various mouse strains[22] also suggest that food preferences are partly explained by genetic factors. From this review it appears likely that there are substantial familial effects underlying nutrient intake, but whether this effect is due to genetic, familial environmental, or both factors is unclear.

Gene-Diet Interactions

While an appropriate diet is recommended for reducing the risks of many common diseases, it is well known that there is a great deal of variability in people's responses to dietary change. For example, atherogenic diets may carry little risk for some people, but for others dietary changes generally have a good outcome. While the causes for this heterogeneity across people are not completely understood, there is convincing evidence that genes play a role. Phenylketonuria (PKU), an inborn error of metabolism causing an accumulation of phenylalanine in the blood leading to mental retardation, is a classic example. Restriction of dietary phenylananine in individuals who are homozygous for the mutation reduces the phenylalanine accumulation and mental retardation.

The following summary of gene-diet interactions is organized around two general topics: 1) adiposity and 2) lipids and lipoproteins. A summary of some of the genes that may interact with dietary intake to influence these phenotypic domains is given in Table 27.2. Most of the molecular work investigating specific genes has centered on the latter domain.

TABLE 27.2

Summary of Measured Gene-Diet Interactions

Gene*	Cytogenic Location*	Intervention**	Response Phenotype	Study
MTHFR	1p36.32	Cross-sectional, folate	Homocysteine	Jing Ma et al., 1996[92]
TNFR2	1p36.23	High fat	Wt, insulin, leptin in mice	Schreyer et al., 1998[39]
		Cross-section, diet-treated diabetic vs nondiabetic	BMI, leptin	Fernandez-Real et al., 2000[40]
Dob1	1p35-1p31	High fat	Mice bred for diet response	West et al., 1994a, 1994b[29,30]
LEPR	1p22.3	Overfeeding	Fasting insulin, leptin, HDL-c	Ukkola et al., 2000b[46]
HSD3B1	1p12	Aging (longitudinal)	Skinfold sum	Vohl et al., 1994[103]
APOB	2p22.3	Crossover, high vs low saturated fat and cholesterol	CH, LDL-c	Friedlander et al., 2000[59]
		Crossover, saturated vs monounsaturated fat	ApoAI, LDL-c, ApoB, HDL-c	Dreon et al., 1994; 1995[88,89]
		Crossover, high saturated vs NCEP vs high monounsaturated	TG	Lopez-Miranda et al., 2000[83]
IRS1	2q36.3	Optifast wt loss program: diet, exercise, and support group	Wt loss	Benecke et al., 2000[38]
Dob2	3p21	High fat	Mice bred for diet response	West et al., 1994a, 1994b[29,30]
PPARG	3p25.2	Aging (longitudinal) obese vs lean	BMI change	Ek et al., 1999[49]
FABP2	4q26	Crossover, insoluble vs soluble fiber	Total cholesterol, LDL-c, ApoB	Hegele et al., 1997[45]
UCP1	4q28.2	Low kcalorie	Wt and BMI loss	Fumeron et al., 1996[104]
		Low kcalorie + exercise	Wt loss	Kogure et al., 1998[44]
GRL	5q31.3	Overfeeding	Wt, AVF, SBP, CH	Ukkola et al., 2000a[36]
ADRB2	5q32	Overfeeding	Wt, leptin, SF8, OGTT insulin area, abdominal total fat	Ukkola et al., 2000c[37]
		Crossover, saturated vs monounsaturated fats	TG	López-Miranda et al., 2000[83]
LEP	7q31.33	Low kcalorie	Leptin	Mammes et al., 1998[45]
		Low kcalorie	Wt loss	Oksanen et al., 1997[105]
LPL	8p21.3	Low kcalorie	TG, VLDL-tg, ApoB	Jemaa et al., 1997[74]
		Crossover, high saturated vs low saturated and high polyunsaturated	CH	Humphries et al., 1996[75]
		Crossover, high vs low saturated fat and cholesterol	TG, HDL-c	Friedlander et al., 2000[59]

ADRB3	8p11.22	Aging morbid obese vs normal wt	Wt gain	Nagase et al., 1997[53]
			Wt gain	Clément et al., 1995[54]
		Optifast wt loss program: diet, exercise, and support group	Wt loss	Benecke et al., 2000[38]
Dob3	8q23-q24	High fat	Mice bred for diet response	West et al., 1994a, 1994b[29,30]
Dob1	9p13	High fat	Mice bred for diet response	West et al., 1994a, 1994b[29,30]
UCP3	11q14.1	Cross-sectional, morbidly obese vs nonobese	BMI, max BMI, Wt, diabetes	Otabe et al., 1999[43]
APOCIII	11q23.2	Crossover, saturated vs monounsaturated fats	CH, LDL-c, Apo B	López-Miranda et al., 1997[82]
APOAI	11q23.2	Crossover, saturated vs monounsaturated fats	LDL-c	López-Miranda et al., 1994[78]
APOAIV	11q23.2	Reduced fat	HDL-c, TG	Dreon et al., 1994; 1995[88,89]
		High cholesterol	LDL-c	McCombs et al., 1994[79]
		Crossover, NCEP vs average	LDL-c, HDL-c, TG	Mata et al., 1994[80]
		Crossover, saturated vs monounsaturated fats	CH, LDL-c, Apo B	Jansen et al., 1997[81]
CETP	16q21	Cross-sectional, alcohol	HDL-c	Fumeron et al., 1995[96]
LDLR	19p13.2	Fiber	ApoB, total cholesterol, LDL-c	Hegele et al., 1993[84]
APOE	19q13.32	Reduced fat	LDL particle size	Dreon et al., 1994; 1995[88,89]
		Crossover, low vs high cholesterol	HDL-c, CETP	Martin et al., 1993[86]
		Crossover, low vs high fat	HDL-c subclasses and LDL particle size	Williams et al., 1995[87]

* MTHFR = 5,10-methylenetetrahydrofolate reductase (NADPH); TNFR2 = tumor necrosis factor alpha, receptor 2; Dob1 = Dietary obese; HSD3B1 = hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1; APOB = apolipoprotein B; IRS1 = insulin receptor substrate 1; Dob2 = Dietary obese; PPARG = peroxisome proliferative activated receptor, gamma; FABP2 = fatty acid binding protein 2; UCP1 = uncoupling protein 1; GRL = glucocorticoid receptor locus; ADRB2 = beta 2 adrenergic receptor; LEP = leptin; LPL = lipoprotein lipase; ADRB3 = beta 3 adrenergic receptor; Dob3 = Dietary obese; Dob1 = Dietary obese; UCP3 = uncoupling protein 3; APOCIII = apolipoprotein C-III; APOAI = apolipoprotein A-I; APOAIV = apolipoprotein A-IV; CETP = cholesteryl ester transfer protein, plasma; LDLR = low-density lipoprotein receptor; APOE = apolipoprotein E.

** Cross-sectional = different subjects across groups; Crossover = same subjects with repeated measured across groups; NCEP = National Cholesterol Education Program diet (total fat < 30%, saturated fat < 10%, cholesterol intake < 300 mg/d)

Regarding the twin intervention method, one series of studies dominates the literature. This was an overfeeding experiment involving MZ twin pairs. In the long-term experiment, 12 pairs of male MZ twins were submitted to a 1000 kcal surplus diet 6 days a week for a period of 100 days.[23] The nutrient content of the diet was 50% carbohydrate, 35% lipid, and 15% protein. During the course of the protocol the excess energy intake was 84,000 kcal. In the short-term experiment, 6 pairs of male MZ twins were given the same protocol for a period of 22 consecutive days[24] In both the long-term and short-term experiments, a variety of physical and metabolic measurements spanning the phenotypic domains listed above were measured before and after the dietary interventions. Genetic epidemiology results from both the long-term and short-term studies have been reported in several publications and provide evidence for GxE interaction. More recently, association studies of the responses to overfeeding for a few candidate genes have been reported.

Adiposity

It is not surprising that overfeeding has an adverse effect on adiposity. In general, an increase in caloric intake leads to an increase in adiposity, and this effect appears to be more pronounced in some individuals, depending on genotypes. Evidence for these conclusions arises from several sources using different study designs.[6,25-27]

More direct evidence of GxE on adiposity was obtained in both the short-term[24] and long-term[23] MZ intervention experiments. In the long-term intervention experiment, there was a mean increase in body mass of 8.1 kg, with a threefold difference between the lowest and highest gainers, ranging from 4 to 12 kg.[23] The variability in response for weight was at least three times greater (F-ratio of 3.4) between unrelated individuals than within twin pairs, suggesting a significant genotype–overfeeding interaction. Similar magnitudes of results were found for body mass index (BMI), percent body fat, fat mass measured with underwater weighing, and subcutaneous fat measured by summing across six skinfolds. Moreover, the variance was six times greater between than within pairs for abdominal visceral fat (measured with computed tomography scan) after adjusting for total fat mass. These findings indicate that some individuals tend to store fat predominantly in selected fat depots in response to caloric surplus primarily as a result of genetic factors. In another MZ twin intervention study,[28] the opposite dietary treatment was conducted. Fourteen pairs of female MZ twins were strictly supervised for 28 days on a very low calorie diet. The diet provided for 1.6 MJ/day and included 37 g protein, 50 g carbohydrates, and 3.8 g fat. Significant diet-induced reductions were seen for several measures of body composition, including weight, BMI, percent fat, fat mass, abdominal fat, and several skinfold measures. Moreover, the variance was 11 to 17 times higher among unrelated individuals than between twin pairs, suggesting a significant GxE interaction effect. Together, these twin studies suggest that the changes in body composition due to dietary intervention (both overfeeding and underfeeding) are due in part to the genotype.

Some of the evidence for gene-diet interactions on adiposity comes from the measured genotype approach. Given the obvious connection between food intake and obesity, there is nevertheless a paucity of measured gene-by-diet interaction studies of obesity. West et al. provided early evidence of gene-diet interaction on obesity in mice.[29,30] Nine strains of mice were selectively bred for their responses to a high-fat diet; there was a sixfold difference in adiposity gain between strains that were sensitive (AKR/J) and resistant (SWR/J) to weight gain. Three dietary obese loci (Dob1, Dob2, and Dob3) were found to underlie these differences, and they have been mapped to syntenic human chromosomes on 1p35-p31 and 9p13 for Dob1, 3p21 for Dob2, and 8q23-q24 for Dob3. No studies were found reporting linkage or association of these genes to dietary responses in humans. However, linkage of nearby anonymous markers (D1S476, D1S200, D1S193,

D1S197 on chromosome 1 and D8S592 and D8S556 on chromosome 8) with several adiposity measures (BMI, sum of skinfolds, fat mass, percent fat, and leptin) has been reported.[31,32]

The lipoprotein lipase (LPL) gene has been implicated in gene-diet interactions in several reports. LPL plays a role in the regulation of plasma lipoprotein composition and concentrations, and in partitioning triglycerides between the adipose tissue for storage and the skeletal muscle for oxidation, and is thus an obvious candidate. Overexpression of LPL in skeletal muscle of transgenic mice was shown to protect against diet-induced obesity.[33] While no studies were found linking the changes in adiposity following dietary intervention with LPL in humans, it has been related to lipid responses (reviewed below). Moreover, the Hind III polymorphism was shown to modulate the relation between visceral fat and plasma triglycerides,[34] providing evidence of pleiotropy (i.e., a single gene impacting on multiple traits).

The glucocorticoid receptor locus (GRL) is also involved in the regulation of LPL activity and lipolysis, and glucocorticoids are insulin antagonists.[35] The Bcl I variant of the GRL locus was associated with the overfeeding response in weight and abdominal visceral fat in the MZ twin overfeeding study.[36] Individuals homozygous for the 2.3 kb allele had a greater increase in response to overfeeding. A similar result was noted for plasma total, LDL cholesterol, and systolic blood pressure responses in the same report, suggesting pleiotropy and that the GRL locus has an impact on the overall atherogenic profile response to overfeeding.

Another genetic factor related to lipolysis is the adrenergic system. Adrenergic receptors (ADR) can stimulate (B1, B2, B3) or inhibit (A2) lipolysis by modulating triglyceride breakdown in the adipocytes. Data from the MZ overfeeding study was used to investigate the effects of ADRA2, ADRB2, and ADRB3 polymorphisms on adiposity and fat distribution responses to overfeeding.[37] Results indicate a significant GxE effect for ADRB2 on weight, plasma leptin, sum of skinfolds, and insulin area under the OGTT (oral glucose tolerance test) curve. Greater weight gain in response to overfeeding occurred in Glu27Glu/Gln27Gln than Gln27Gln carriers of the ADRB2 gene. As with the LPL and GRL loci, ADRB2 impacted the response to overfeeding for multiple traits (i.e., pleiotropy). There were too few subjects with the rare alleles for the ADRA2 and ADRB3 loci in this study for a comprehensive investigation. However, in another study the response in obese women to a weight loss program was investigated for rare mutations at both the ADRB3 (Trp64Arg) and IRS1 (Gly972Arg) loci.[38] Carriers of both rare mutations lost less weight and had a higher frequency of type 2 diabetes than noncarriers. Thus, there is evidence of both pleiotropy (i.e., these loci affect both body composition and insulin levels) and oligogenic effects (i.e., multiple genes affect body composition) between these two genes.

Pleiotropy was also observed for the tumor necrosis factor alpha receptor, which may play a key role in the metabolic syndrome involving both diabetes and obesity. TNFA is expressed in adipose and muscle tissues and blocks the action of insulin. In a study of mice lacking one of the TNF receptors (TNFR2), weight, insulin, and leptin level responses to diet were all modulated by the TNFR genotype.[39] Although this marker has not been reported in gene-diet studies of humans, the presence of the A2 allele was seen to predispose subjects to obesity, higher leptin levels, and insulin resistance.[40] It is interesting to note that the TNFR2 locus is closely linked to the Dob1 (dietary obese) locus on chromosome 1p (Table 27.2).

The above findings for TNFR2, ADRB2, and ADRB3 suggest that each impacts on both adiposity and insulin responses to diet. Insulin is a lipogenic hormone regulating transcription of lipogenic genes, and can act directly or in conjunction with glucose metabolites. In animals, insulin inhibits food intake via receptors in the hypothalamus. The insulin response to diet was examined in the long-term MZ twin overfeeding experiment, in

which an OGTT was administered.[41] The between-pair variance in response to overfeeding was 2.5 to 5 times higher than the within-pair variance for measures of fasting insulin and glucose and insulin sensitivity from the OGTT, suggesting gene-diet interactions. Thus, some individuals are more prone than others to modify their insulin and glucose levels and perhaps insulin sensitivity in response to overfeeding.

The number of studies looking for the genes underlying adiposity is currently in a rapid growth stage. For example, 48 different candidate genes have been associated with obesity-related phenotypes in the past few years, as recently reviewed by Pérusse et al.[42] Of these, at least seven candidates (HSD3B1, IRS1, PPARG, UCP1, LEP, ADRB3, and UCP3) were associated with changes in adiposity over time, although only four these (LEP, UCP1, UCP3, and IRS1) were investigated for responses to dietary intervention. All of these markers are good candidates for GxE interactions. The uncoupling proteins have a role in releasing stored energy as heat. UCP3, which is abundant in skeletal muscle tissue, was recently associated with weight change in the morbidly obese during diet therapy.[43] The G polymorphism of the UCP1 gene was also associated with weight loss after a treatment program that included a low-calorie diet and exercise in obese Japanese women.[44] Leptin is a hormone secreted primarily by adipose tissue and is generally considered to act as a satiety signal in a feedback loop with the brain. Several mutations in the LEP gene were associated with plasma leptin responses to dietary intervention in one study.[45] Those authors concluded that LEP may be a gene regulating the variability of responses to nutritional environments rather than for obesity per se. In the long-term MZ twin overfeeding experiment, the Gln223Arg variant of the leptin receptor (LEPR) was associated with several metabolic variables,[46] including plasma leptin, insulin, and HDL-c, but not body composition measures. The insulin receptor substrate 1 (IRS1) gene has a role in controlling cellular growth and metabolism, and was associated with longitudinal changes in BMI.[47] As previously outlined, rare mutations at both the IRS1 and ADRB3 loci led to less weight loss and higher type 2 diabetes in response to a weight loss program in obese women.[38]

The remaining markers listed above are also good candidates in gene-diet interaction effects on obesity, although few reports regarding gene-diet interactions were found. For example, the peroxisome proliferator-activated receptors (PPARs) are expressed in adipose tissue, and the gamma subtype (PPARG) has been implicated in adipose cell function, including lipid composition of the membrane and sensitivity to insulin.[48] PPARG was linked to longitudinal changes in BMI.[49] The adrenergic system (discussed above) has a role in regulating energy balance through thermogenesis and lipid mobilization in adipose tissue. The beta 3 adrenergic receptor (ADRB3) is thought to play a minor role in catecholamine-induced lipolysis. However, reports of linkage or association of ADRB3 to obesity and weight changes in humans have been inconsistent.[50-54]

Lipids, Lipoproteins, and Apolipoproteins

The lipid, lipoprotein, and apolipoprotein response to dietary intervention is the most extensively studied area of those reviewed in this section. A great deal of evidence[55-59] suggests that plasma lipid level responses are under genetic control. Individual differences in the plasma lipid profile response to dietary fats and cholesterols are found in several species, including mouse,[60] rat,[61] and monkey.[62,63] Some individuals are quite sensitive to changes (high-responders) and others are relatively insensitive (low-responders), as confirmed in a meta-analysis of 27 studies.[64] For example, early evidence of environmental (including dietary) effects on total cholesterol (CH), high density lipoprotein-cholesterol (HDL-c), HDL-c subfraction 2 (HDL2-c), and low density lipoprotein-cholesterol (LDL-c) using the twin design was reported by O'Connell et al.[65] Heritability estimates, although remaining significant, were decreased after adjusting for environmental factors, and by

stratifying the sample based on nutritional variables. In another study of children with elevated LDL-c levels, the effect of a nutrition-education program was investigated.[66] Greater reductions in plasma total and LDL-c were observed in children with less family history of coronary heart disease.

Evidence for gene-diet interactions on HDL-c subfractions (HDL1-c, HDL2-c, and HDL3-c) were also reported in a baboon population.[67] The baboons were measured under a basal diet and again after being fed a high cholesterol and saturated fat challenge diet. The results suggested that there were both pleiotropic effects (i.e., the same gene(s) influencing multiple traits) and GxE interactions. The authors concluded that although a similar set of genes influenced the variation in each of the three subfractions under both diet conditions, the expression of the genes influencing HDL1-c and HDL2-c were altered by the high-fat diet (i.e., a GxE interaction).

Additional evidence of GxE effects come from the short-term MZ twin overfeeding intervention study. Plasma responses in CH, triglycerides (TG), LDL-c, HDL-c, and the HDL-c/CH ratio were investigated.[68] Although overfeeding induced significant changes only in CH and LDL-c, there were large interindividual differences in the responses of all of these variables. GxE interactions were detected for TG, HDL-c, and HDL-c/CH. It was noted that TG changes were negatively correlated with HDL changes, and that the correlated responses may be related to the susceptibility to develop hypertriglyceridemia, which is known to be under genetic control and related to changes in insulin concentrations.

Much of the evidence for GxE interactions on lipids, lipoproteins, and apolipoproteins involve the measured gene approach.[59,69-71] Genetic variations in several apolipoprotein genes (A-I, A-IV, B, CIII, E), the LDL receptor (LDLR) and LDL subclasses (patterns A and B) have been implicated in the dietary response of lipids.

The LPL gene discussed above, involved in partitioning exogeneous triglycerides between storage and oxidation, has been associated with plasma lipid levels and CHD risk.[72,73] In humans, several mutations have been implicated in the gene-diet interaction. For example, the Hind III polymorphism was associated with variability in plasma cholesterol, LDL-c, LDL-triglyceride, and Apo B responses to diet.[70,74,75] The N291S mutation showed a significant effect on TG and HDL-c responses to diet.[59] Other evidence from a MZ twin study (non-intervention) suggests GxE involvement of the Ser447Ter mutation.[76] Intrapair variances were different across twin types for CH, TG, and HDL-c levels, although the environmental source using this method is not specified. The authors suggested that this LPL variant acts as a restrictive variability gene, so that individuals without the mutation are more susceptible to fluctuations in plasma cholesterol and HDL-c.

Several of the apolipoprotein genes have been implicated in gene-diet interactions.[71,77] The APO A-I, A-IV, and C-III complex of genes is involved in lipid metabolism. A mutation in the A-I gene promoter region (G $\rightarrow$ A) was associated with the plasma LDL-c response to a high monounsaturated fat diet.[78] Apo A-IV is an intestinal glycoprotein with two allele forms (A-IV-1 and A-IV-2); its synthesis is stimulated by dietary lipids and it may act centrally to inhibit food intake. Although conflicting reports are found, individuals homozygous for the A-IV-1 allele generally have lower HDL-c and higher TG.[71] In a crossover intervention study, subjects consumed a low-cholesterol diet for two weeks, then three weeks of a high-cholesterol diet.[79] In the high-cholesterol diet condition, plasma LDL-c increased more in the A-IV-1 group than the A-IV-2 group, with no change in HDL-c or TG levels for either genotype. Similar results were found in men (but not women) in another report combining data from three intervention studies.[80] In another study,[81] an A $\rightarrow$ T mutation in position 347 affected the total CH, LDL-c, and Apo B responses to a high fat diet. Lipid changes due to dietary intervention were found to be similar for the Apo C-III gene. For example, the SstI polymorphism interacted with diet to produce genotype-dependent responses in total CH, LDL-c, and Apo B levels.[82] Thus, for this cluster

of apolipoprotein genes located on chromosome 11q within 1 cM of each other, there is consistent evidence of a gene-diet interaction effect on LDL-c, although the results for HDL-c and TG are not as clear.

The APOB gene is involved in the synthesis and secretion of chylomicrons and very low density lipoprotein (VLDL), and is a ligand for the interaction of LDL-c with the LDL receptor. Several variants have been associated with lipid responses to dietary intervention. While there are inconsistencies in the literature, genetic variations at both the Mspl and XbaI RFLPs have been reported to influence the plasma Apo A-I, LDL-c, Apo B, and HDL-c[71] and TG[83] responses to dietary fat and cholesterol. Moreover, an insertion/deletion polymorphism of the APOB gene was related to the lipoprotein response to increases in dietary fiber.[84]

Several studies investigated the role of APOE polymorphisms in the response of plasma LDL-c levels to dietary interventions.[85] Apo E is a protein associated with several lipoproteins, mediates the lipoprotein interaction with specific cell surface receptors, and has an important role in CH and TG metabolism. APOE has three common isoforms (E2, E3, and E4), with E3 the most common. APOE represents the most widely studied candidate, and although there are conflicting results in the literature,[55,71,85] most conclude that carriers of the E4 variant respond well to dietary intervention. Several possible explanations were suggested for the differences across studies; for example, expression of the response in absolute versus fractional levels. Since individuals with the E4 phenotype usually have higher initial plasma LDL-c levels, there is likely to be a larger absolute change, while the fractional change may be consistent across APOE phenotypes. Additional factors leading to inconsistencies across studies include low sample sizes leading to reduced power for testing hypotheses, and the sex ratio of subjects, since dietary responsiveness differs between sexes. Other factors include whether the intervention protocol reduced dietary fat, cholesterol, or fiber.[86,87] For example, a meta analysis of 16 studies showed that a greater lipid response in carriers of the E4 allele was only found when the dietary modification reduced total fat intake, irrespective of dietary cholesterol.[70] In another study, the increase in dietary fiber was associated with greater reductions in LDL-c in carriers of the E2 allele. Other studies also suggest a difference in the APOE gene association with plasma LDL-c response, depending on LDL particle size.[88,89] That is, the diet-induced change in LDL-c levels may not be due to reduced particle number but rather to a shift from larger cholesterol-rich LDL particles to smaller, denser LDL particles.

The LDL particles vary in size, density and lipid content. Subjects with small, dense LDL particles (subclass pattern B) exhibit higher levels of TG and Apo B and lower levels of HDL-c compared to subjects with a predominance of larger LDL particles (pattern A). Population studies have shown that about 30 to 35% of adult men exhibit the more atherogenic pattern B which is associated with a threefold higher risk of myocardial infarction. This lipoprotein phenotype is under strong genetic determination, with heritability levels of about 50% and evidence of a major gene effect.[90] The plasma lipoprotein response to changes in dietary fat in relation to the LDL subclass pattern was investigated in a dietary crossover experiment.[88,89] In this study, 105 men were randomly assigned to either a high fat (46%) or low fat (24%) diet for six weeks and then switched to the alternate diet for an additional six weeks. Subjects were categorized as pattern A (n=87) or pattern B (n=18), and the lipoprotein responses were analyzed as the changes from the high- to low-fat diets. After this dietary intervention, pattern B subjects exhibited a threefold greater reduction in LDL-c compared to pattern A subjects, while only men with pattern B exhibited a reduction in Apo B levels. These group differences were independent of BMI, Apo E phenotype, and plasma lipid levels. The decrease of LDL-c observed in pattern A subjects was due primarily to a shift in LDL particle mass from larger to smaller cholesterol-

depleted LDL, without a change in LDL particle number. This shift in LDL distribution with the low fat diet induced expression of pattern B phenotype in 36 of the 87 pattern A subjects who did not express it on a high fat diet. Thus, in response to a low fat diet 41% of the pattern A subjects exhibited the more atherogenic pattern B lipoprotein profile. The results of this study provide a good example of genotype-diet interaction and show that dietary recommendations may not be equally good for every individual in the population.

LDLR mediate cholesterol uptake and are located on cells of many tissues. LDLR polymorphisms within the exon have been related to reductions in plasma concentrations of ApoB, total, and LDL cholesterol response to dietary fiber,[84] but not to the response of LDL-c concentrations to dietary fatty acids.[91]

MTHFR is an enzyme involved in folate production and in remethylation of homocysteine. Elevated levels of homocysteine are due to enzymatic deficiencies or to low intake of vitamins B_6, B_{12}, and folic acid, and are risk factors for coronary heart disease. Gene-diet interactions on homocysteine levels have been reported.[92] A MTHFR polymorphism was associated with increased homocysteine levels, but only in men with low folate intake. Thus, low folate intake may increase the risk of hyperhomocysteinemia in subjects with the MTHFR mutation. The MTHFR locus is closely linked to both the TNFR2 and Dob1 loci involved with adiposity responses to dietary intervention.

The FABP2 gene produces the intestinal fatty acid binding protein. It plays a role in absorption and intracellular transport of saturated and unsaturated long chain fatty acids.[93,94] The FABP2 T54 allele has been associated with insulin resistance and an atherogenic metabolic profile. In a crossover study of the effects of dietary soluble and insoluble fiber, the T54 allele was associated with a significant decrease in total and LDL cholesterol and Apo B during a period when the diet was high in soluble fiber.[95]

Finally, the cholesteryl ester transfer protein (CETP) gene mediates the transfer of cholesteryl ester from HDL-c to triglyceride-rich lipoproteins. It also has a role in reverse cholesterol transport and in the catabolism of HDL-c. CETP isoforms were associated HDL-c levels and risk for myocardial infarction, but only in subjects who drank 25 g/day of alcohol.[96] Thus, there is evidence of a gene-alcohol interaction effect on HDL-c levels.

Gene-Gene (GxG) Interactions

Interactions between genes also have a role in determining the susceptibility to diseases. Gene-gene interactions occur when the impact of a gene is mediated by genetic variation at another gene locus. For example, it has been suggested that variation in total CH and LDL-c is influenced by interactions between the linked LDLR and APOE genes.[97] The cholesterol-raising and lowering effects of the E4 and E2 alleles, respectively, were seen only in individuals with a particular LDLR genotype. These two genes are located about 40 cM apart on chromosome 19p13.2-p13.32. Another example of GxG was reported by Helbecque et al.[98] A significant interaction between the VLDL receptor genotype (VLDLR) and the Apo E phenotype was found for plasma TG levels. Interactions among GRL, LPL, and ADRA2[99] were also reported. GRL and ADRA2 interactions were detected for LDL-c levels, while GRL and LPL interactions were found for HDL-c levels. Interestingly, none of the main effects were significant. This is a classical example of GxG interaction, where there is no association in the presence of either locus separately, but jointly they have an effect. Although the exact mechanism is not clear, the interaction may influence rates of lipolysis and release of free fatty acids (FFA) from adipose tissue.

Other examples of GxG interactions are found in the body composition domain. For example, indirect evidence for two pleiotropic loci affecting fat mass and BMI was reported by Borecki et al.[100] using segregation analysis. One locus apparently affected extreme overweight, while the other influenced variation only in the "normal" range. Evidence for multiple loci affecting body composition has also been explored using the measured gene approach. Since each of the LPL, GRL, and ADRA2 loci had similar effects on several correlated body composition traits, the hypothesis of GxG interaction was investigated.[101] Previous studies had reported that the ADRA2 Dra I variant and the GRL Bcl I variant were each associated with abdominal fat. When the three candidates (GRL, ADRA2, and LPL) were considered simultaneously, significant interactions on overall and abdominal adiposity were observed that accounted for a small but significant percentage of the variance.

Only one study was found investigating GxG interactions for responses to dietary intervention, involving the APO A-I and A-IV loci.[81] Male subjects were fed three consecutive diets, each lasting for four weeks, which differed in amounts of saturated and monounsaturated fats. The G → A mutation in APOAI and the 347Thr/Ser mutation in APOAIV were examined. Each locus showed a gene-diet interaction effect on responses in total cholesterol, LDL-c, and Apo B levels. However, the GxG effect on the response was not significant, resulting in a simple additive effect of the two loci on the lipid responses.

Conclusions

This section is not intended to be an exhaustive summary of the genetics of nutrition. Rather, we have attempted to show the broad scope of behavioral and physiological factors underlying the genetics of nutrition. The general findings may be summarized as follows. First, there are familial factors underlying food intake and preferences. However, whether this effect is due to genes, familial environments, or some combination of both is not clear. Second, it is obvious that nutrition plays an important role in the development of certain diseases leading to morbidity and mortality such as obesity, dyslipidemia, and diabetes, and that genes underlie this effect to some extent. Third, there are multiple complex etiologies that lead to increased risk for these diseases.

A great deal of work remains to be done on several fronts. First, very little was found regarding gene-diet interactions for many of the peptides and hormones[3] that have been implicated in food intake. Some of these include cholecsytokinin (CCK), glucagon-like peptide 1, agouti-related peptide, CART, corticotropin releasing factor (CRF), pro-opiomelanocortin (POMC), opioids, neuropeptide Y (NPY), and others. These inhibit food intake, while others stimulate appetite and thus may contribute significantly to the responses to energy intake. More extensive candidate genotyping of existing intervention data would be helpful in this regard. Second, it is highly unlikely that the genes identified to date are the only ones affecting the traits discussed here, even in the lipid domain, for which much is already known. While candidate gene studies are useful in confirming the effects of these known genes, linkage analysis of genome scan data are needed in order to locate novel chromosomal regions that may lead to identification of new genes. In this regard, large-scale diet intervention studies of family data are needed. While this may be impractical in human populations, genome scans from intervention studies of closely related species such as the baboons are feasible. Third, in addition to gene-diet interactions, models that incorporate the possibility of other complex etiologies such as pleiotropy and

oligogenic and epistatic actions are needed. Since candidate genes for lipids (e.g., LPL) may also influence other traits such as diabetes and obesity, we should not limit our candidate gene investigations to one type of trait. This field is ripe for an explosion of studies that probe the genome and describe the complex genetic etiologies underlying responses to nutrition. It is obvious that nutrition plays a large role in several traits of public health interest such as those involved in the metabolic syndrome and discussed here. An understanding of the factors involved in this syndrome should take nutritional factors into account.

References

1. Pérusse L, Bouchard C. In: *The Genetics of Obesity* (Bouchard C, Ed) Boca Raton: CRC Press, 1994, p 125.
2. Prutkin J, Fisher EM, Etter L, et al. *Physiol Behav* 69:161; 2000.
3. Smith GP. *Neuropeptides* 33: 323; 1999.
4. Rice TK, Borecki IB. In: *Genetic Dissection of Complex Traits* (Rao DC, Province MA, Eds) San Diego: Academic Press, 2001, p 35.
5. Rao DC, Rice T. In: *Encyclopedia of Biostatistics,* Vol 4. (Armitage P, Colton T, Eds) Sussex, UK: Wiley, 1998, p 3285.
6. Pérusse L, Bouchard C. *Nutr Rev* 57: 31S; 1999.
7. Borecki IB, Suarez BK. In: *Genetic Dissection of Complex Traits* (Rao DC, Province MA, Eds) San Diego: Academic Press, 2001, p 45.
8. Effron B. Philadelphia, PA: *SIAM*. 1982
9. Huber PJ. Proceedings of the 5th Berkeley Symposium on Mathematical Statistics and Probability. Berkeley, CA: University of California Press, Vol I: 221; 1967.
10. White M. *Econometrica* 48: 817; 1980.
11. Woods SC, Schwartz MW, Baskin DG, Seeley RJ. *Ann Rev Psychol* 51: 255; 2000.
12. DeCastro JM. *Physiol Behav* 54: 677; 1993.
13. Pérusse L, Tremblay A, Leblanc C, et al. *Am J Clin Nutr* 47: 629; 1988.
14. Vauthier J-M, Lluch A, Lecomte E, et al. *Int J Epidemiol* 25: 1030; 1996.
15. Garn SM, Cole PE, Bailey SM. *Hum Biol* 51: 565; 1979.
16. Fabsitz RR, Garrison RJ, Feinleib M, Hjortland M. *Behav Genet* 8: 15; 1978.
17. Patterson TL, Rupp JW, Sallis JF, et al. *Am J Prev Med* 4: 75; 1988.
18. Oliveria SA, Ellison RC, Moore LL, et al. *Am J Clin Nutr* 56: 593; 1992.
19. Wade J, Milner J, Krondl M. *Am J Clin Nutr* 34: 143; 1981.
20. Reed DR, Bachmanov AA, Beauchamp GK, et al. *Behav Genet* 27: 373; 1997.
21. Faith MS, Rha SS, Neale MC, Allison DB. *Behav Genet* 29: 145; 1999.
22. Smith BK, Andrews PK, West DB. *Am J Physiol* 278: R797; 2000.
23. Bouchard C, Tremblay A, Després J-P, et al. *N Engl J Med* 322: 1477; 1990.
24. Bouchard C, Tremblay A, Després J-P, et al. *Prog Food Nutr Sci* 12: 45; 1988.
25. Leibel RL, Bahary N, Friedman JM. *World Rev Nutr Diet* 63: 90; 1990.
26. Weinsier RL, Hunter GR, Heini AF, et al. *Am J Med* 105: 145; 1998.
27. Pérusse L, Bouchard C. *Ann Med* 31S: 19; 1999.
28. Hainer V, Stunkard AJ, Kunesova M, et al. *Int J Obes* 24: 1051; 2000.
29. West DB, Waguespack J, York B, et al. *Mamm Genome* 5: 546; 1994.
30. West DB, Goudey-Lefevre J, York B, Truett GE. *J Clin Invest* 94; 1410; 1994.
31. Chagnon YC, Pérusse L, Lamothe M, et al. *Obes Res* 5: 115; 1997.
32. Chagnon YC, Rice T, Pérusse L, et al. *J Appl Physiol* 90: 1777; 2001.
33. Jensen DR, Schlaepfer IR, Morin CL, et al. *Am J Physiol* 273; R683, 1997.
34. Vohl M-C, Lamarche B, Moorjani S, et al. *Arterioscler Thromb Vasc Biol* 15: 714; 1995.
35. Cigolini M, Smith U. *Metabolism* 28: 502; 1979.
36. Ukkola O, Rosmond R, Tremblay A, Bouchard C. *Atherosclerosis* 157: 221; 2001.

37. Ukkola O, Tremblay A, Bouchard C. *Int J Obes* (in press).
38. Benecke H, Topak H, von zur Mühlen A, Schuppert F. *Exp Clin Endocrinol Diab* 108: 86; 2000.
39. Schreyer SA, Chua SC Jr, LeBoeuf RC. *J Clin Invest* 102: 402; 1998.
40. Fernandez-Real J-M, Vendrell J, Ricart W. *Diabetes Care* 23: 831; 2000.
41. Oppert J-M, Nadeau A, Tremblay A, et al. *Metabolism* 44: 96; 1995.
42. Pérusse L, Chagnon YC, Weisnagel SJ, et al. *Obes Res* 9: 135; 2001.
43. Otabe S, Clement K, Dubios S, et al. *Diabetes* 48: 206; 1999.
44. Kogure A, Yoshida T, Sakane N, et al. *Diabetologia* 41: 1399; 1998.
45. Mammes O, Betoulle D, Aubert R, et al. *Diabetes* 47: 487; 1998.
46. Ukkola O, Tremblay A, Després J-P. *J Intern Med* 248: 435; 2000.
47. Lei H-H, Coresh J, Shuldiner, et al. *Diabetes* 48: 1868; 1999.
48. Zeghari N, Vidal H, Younsi M, et al. *Am J Physiol* 279: E736; 2000.
49. Ek J, Urhammer SA, Sørensen TIA, et al. *Diabetologia* 42: 892; 1999.
50. Hegele RA, Harris SB, Hanley AJG, et al. *Diab Care* 21: 851; 1998.
51. Gagnon J, Mauriege P, Roy S, et al. *J Clin Invest* 98: 2086; 1996.
52. Mitchell BD, Cole SA, Comuzzie AG, et al. *Diabetes* 48: 1863; 1999.
53. Nagase T, Aoki A, Yamamoto M, et al. *J Clin Endocrinol Metab* 82: 1284; 1997.
54. Clement K, Vaisse C, Manning B St. J, et al. *N Engl J Med* 333: 352; 1995.
55. Humphries SE, Peacock RE, Talmud PJ. *Clin Endocrinol Metab* 9: 797; 1995.
56. Simopoulos AP. *Biomed Env Sci* 9: 124; 1996.
57. Ordovas JM. *Proc Nutr Soc* 58: 171; 1999.
58. Ellsworth DL, Sholinsky P, Jaquish C. *Am J Prev Med* 16: 122; 1999.
59. Friedlander Y, Leitersdorf E, Vecsler R, et al. *Atherosclerosis* 152: 239; 2000.
60. Kuan SI, Dupont J. *J Nutr* 119: 349; 1989.
61. Van Zutphen LFM, Den Bieman MGCW. *J Nutr* 111; 1833; 1981.
62. Eggen DA. *J Lipid Res* 17: 663; 1976.
63. Rudel LL. *J Am Coll Nutr* 16: 306S; 1997.
64. Hopkins PN. *Am J Clin Nutr* 55: 1060; 1992.
65. O'Connell DL, Heller RF, Roberts DCK, et al. *Genet Epidemiol* 5: 323; 1988.
66. Dixon LB, Shannon BM, Tershakovec AM, et al. *Am J Clin Nutr* 66: 1207; 1997.
67. Mahaney MC, Blangero J, Rainwater DL, et al. *Arterioscler Thromb Vasc Biol* 19: 1134; 1999.
68. Després J-P, Poehlman ET, Tremblay A, et al. *Metabolism* 36: 363; 1987.
69. Abbey M. *Cur Opin Lipidol* 3: 12; 1992.
70. Ordovas JM, López-Miranda J, Mata P, et al. *Atherosclerosis* 118: 11S; 1995.
71. Dreon DM, Krauss RM. *J Am Coll Nutr* 16: 313S; 1997.
72. Jemaa R, Tuzet S, Portos C, et al. *Int J Obes* 19: 270; 1995.
73. Abbey M, Belling B, Clifton P, Nestel P. *Nutr Met Cardiovasc Dis* 1: 10; 1991.
74. Jemaa R, Tuzet S, Betoulle D, et al. *Int J Obes* 21: 280; 1997.
75. Humphries SE, Talmud PJ, Cox C, et al. *Quart J Med* 89: 671; 1996.
76. Thorn JA, Needham EWA, Mattu RK, et al. *J Lipid Res* 39: 437; 1998.
77. Ordovas JM, Schaefer EJ. *Br J Nutr* 83: 127S; 2000.
78. López-Miranda J, Ordovas JM, Espino A, et al. *Lancet* 343: 1246; 1994.
79. McCombs RJ, Marcadis DE, Ellis J, Weinberg RB. *N Engl J Med* 331: 706; 1994.
80. Mata P, Ordovas JM, López-Miranda J, et al. *Arterioscler Thromb* 14: 884; 1994.
81. Jansen S, López-Miranda J, Salas J, et al. *Arterioscler Thromb Vasc Biol* 17: 1532; 1997.
82. López-Miranda J, Jansen S, Ordovas JM, et al. *Am J Clin Nutr* 66: 97; 1997.
83. López-Miranda J, Marín C, Castro P, et al. *Eur J Clin Invest* 30: 678; 2000.
84. Hegele RA, Zahariadis G, Jenkins AL, et al. *Clin Sci* 85: 269; 1993.
85. Kesäniemi YA. *Cur Opin Lipid* 7: 124; 1996.
86. Martin LJ, Connelly PW, Nancoo D, et al. *J Lipid Res* 34: 437; 1993.
87. Williams PT, Dreon DM, Krauss RM. *Am J Clin Nutr* 61: 1234; 1995.
88. Dreon DM, Fernstrom HA, Miller B, Krauss RM. *FASEB J* 8: 121; 1994.
89. Dreon DM, Fernstrom HA, Miller B, Krauss RM. *Arterioscler Thromb Vasc Biol* 15: 105; 1995.
90. Rotter JI, Bu X, Cantor RM, et al. *Am J Hum Genet* 58: 585; 1996.
91. Friedlander Y, Berry EM, Eisenberg S, et al. *Clin Genet* 47: 1; 1995.

92. Jing Ma, Stampfer MJ, Hennekens CH, et al. *Circulation* 94: 2410; 1996.
93. Baier LJ, Sacchettini JC, Knowler WC, et al. *J Clin Invest* 95: 1281; 1995.
94. Hegele RA. *Clin Biochem* 31: 609; 1998.
95. Hegele RA, Wolever TMS, Story JA, et al. *Eur J Clin Invest* 27: 857; 1997.
96. Fumeron F, Betoulle D, Luc G, et al. *J Clin Invest* 96: 1664; 1995.
97. Pedersen JC, Berg K. *Clin Genet* 35: 331; 1989.
98. Helbecque N, Dallongeville J, Codron V. *Arterioscler Thromb Vasc Biol* 17: 2759; 1997.
99. Ukkola O, Pérusse L, Weisnagel SJ, et al. *Metabolism* 50: 246; 2001.
100. Borecki IB, Blangero J, Rice T, et al. *Am J Hum Genet* 63: 831; 1998.
101. Ukkola O, Pérusse L, Chagnon YC, et al. *Int J Obes* (in press) 2001.
102. Heller RF, O'Connell DL, Roberts DCK. *Genet Epidemiol* 5: 311; 1988.
103. Vohl M-C, Dionne FT, Pérusse L, et al. *Obes Res* 2: 444; 1994.
104. Fumeron F, Durack-Bown I, Betoulle D, et al. *Int J Obes* 20: 1051; 1996.
105. Oksanen L, Ohman M, Heiman M, et al. *Hum Genet* 99: 559; 1997.