

Discussion

Philip James, D.Sc., *Moderator*

Molecular Basis of the Hepatic Control of Cholesterol Metabolism

Glomset: Could you tell us about the relationship between the regulatory systems for cholesterol metabolism and the processing in the cell cycle? When one has a series of growing cells, there must be a very interesting coregulation of these genes to produce the cholesterol needed for new cell synthesis. Must not this occur somewhere in the middle of the G-1 phase of cell replication?

Brown: I know that your work on the cell cycle in that regard is classic. The mRNA of sterol regulatory element binding protein (SREBP) cleavage activating protein, also called SCAP, appears to be constitutive, but it is not an early gene product and the SCAP protein is present quite early.

Glomset: So then perhaps the protease is up-regulated?

Brown: Yes, it may be, but there may also be some change in the acute membrane response to a growth factor. There may be other mechanisms of regulation. We know that SREBPs are essential for cholesterol biosynthesis and for isoprenoid biosynthesis. There may, however, be other mechanisms that turn these pathways on, and the account I have given may not be the exclusive process.

Schaefer: I would like to make two points. First, when Dr. Brown's colleague, Dr. Goldstein, was working with Arno Motulsky on the genetic disorders associated with premature heart disease, familial combined hyperlipidemia was found to be much more common than familial hypercholesterolemia itself, and to this day we do not know the basis for this condition, although the apolipoprotein-E (apo-E) genotype is one of the genetic factors that does contribute to the elevated low-density lipoprotein (LDL) cholesterol levels, as shown in many populations, including the Framingham Heart Study. The second point is concerned with the huge variability in response to diet. If one places individuals on an average American diet and then transfers them to a NCEP (National Cholesterol Education Program) Step 2 diet in which saturated fat and dietary cholesterol intake is cut in half, there is evidently a huge variability in individuals' response to that diet. We recently showed¹ that the variation ranged from a 0% reduc-

tion in LDL cholesterol to about 55%. The variable response, as originally shown by Miettinen in Finland, relates to the apo-E genotype where there is substantial polymorphism. Not only does the apo-B bind to the LDL receptor, but apo-E also binds with an even higher affinity. After a fat-rich meal, the lipoprotein remnants rise rapidly in the blood, and recent assays for this remnant² suggest that this assay is a much better predictor than the triglyceride levels of heart disease. A substantial challenge, which we term the McDonald's challenge, involves providing two sausage-egg "McMuffin" breakfast sandwiches, which contain 50 g fat and 500 mg cholesterol. In response to this input, the remnant concentrations of cholesterol and triglyceride in the plasma go up 10-fold within about 4 hours. These atherogenic remnants are also regulated by the apo-E genotype.

If there is an apo-E genotypic substitution of arginine for cystine at residue 112, the LDL cholesterol levels of these subjects are substantially higher than those observed in apo-E3 and apo-E2 subjects. This mutation is common and therefore has a substantial effect on relative risk within the population. While the LDL receptor mutations of familial hypercholesterolemia are rare, about one in 500 individuals, the apo-E4 genotype is much more common. Experimentally, a knockout of the apo-E gene induces premature severe atherosclerosis and apo-E deficiency in humans, as we described many years ago. The presence of the apo-E4 allele results in a 50% increase in coronary heart disease morbidity both in men and in postmenopausal women. It is also associated with Alzheimer's disease and dementia from all causes. This may imply that this more general category relates to vascular disease, but that is only a hypothesis at present.

Brown: I agree that the apo-E genotype is a very important determinant of heart disease. One of the really interesting questions is how an LDL receptor mutation together with apo-E2 genotypic variation combines to enhance the risk of heart disease.

Schaefer: Eric Borwinkler showed that the postprandial response to a fat load, whether measured as triglyceride or retinol palmitate, was higher in E2 heterozygotes. Yet most people with this genotype do not have the type-3 dys- β -lipoproteinemia. Nevertheless, most E2 heterozygotes have elevated lipoprotein remnants postprandially

without the dys- β -lipoproteinemia.

Brown: The other genotypic abnormality that is common is in the apo-E4 genotype, in which elevated LDL levels occur. E4 has a somewhat higher affinity for LDL receptors, and it may be a better competitor and perhaps a more efficient down-regulator of these receptors. I am not satisfied with current explanations.

Friedman: How does cholesterol get into the endoplasmic reticulum (ER)?

Brown: That's a central problem. We do not know whether it is the cholesterol within the membrane itself that is interacting with SCAP or whether some cholesterol is bound to another protein outside the membrane. Since there is so little cholesterol outside the membrane, we believe that it must be the intrinsic cholesterol. The plasma membrane is already very loaded with cholesterol and would, therefore, operate as a poor sensor. Because the ER has the lowest concentration of cholesterol in the cell, a build-up of cholesterol would most likely be recognized within the ER. Simultaneously, with the suppression of SREBP processing, one observes that cholesterol esterification and the esterification enzyme cholesterol acyl transferase (acyl-CoH or ACAT) is located within the ER.

Kontula: Is the SREBP mutated in human disease?

Brown: We do not know. The only manipulation of SREBPs that we can make is currently in mice. I should also mention that when mice overexpress the dominant form of SREBP, there is an accumulation of fatty acids and cholesterol in the liver to 10 times the normal levels. Despite this, plasma lipid levels remain normal. In fact, LDL turnover studies suggest that LDL receptors are frugulated with increased clearance, so that, if anything, the plasma levels tend to be low. By using Triton WR-1339 to measure the very low-density lipoprotein (VLDL) secretion rate in these animals, we find that it is the same as in controls. This implies that SREBPs activate lipid storage in the liver without inducing lipid secretion. We do not know whether there are parallel observations in human studies.

Marckmann: I would like to know about regulation of the triglyceride-rich lipoproteins. How do they feature in relation to atherosclerosis?

Brown: Schaefer is more of an expert on this than I. The thing that fascinates me is the interaction between HDL and triglyceride metabolism. I think that the VLDL metabolism is crucial and we should not be looking in a static way at triglyceride levels.

Auwerx: I would like to add one more question about SREBPs. There is an SREBP-1a and SREBP-1c. If I understand your hypothesis correctly, 1a is a much better transcription factor than 1c.

Brown: Correct.

Auwerx: Would some of the variation in LDL cholesterol levels be explained by differential splicing or differ-

ential modulation of the SREBPs? Is there any evidence of this?

Brown: Not yet. We are monitoring two promoters that can produce SREBP-1. One of them is a much more active form than the other. There may well be differences in the induction of one rather than another, and we are looking at this in detail now.

Sørensen: Can you explain the impact of dietary saturated fatty acids on the cellular feedback relationships in the control of cholesterol metabolism that you have been describing?

Brown: John Dietchy did beautiful studies in Dallas where, with hamsters fed cholesterol with or without saturated fatty acids or polyunsaturated fatty acids, he observed that the combination of polyunsaturated fatty acids with cholesterol led to the cholesterol being very efficiently esterified in the liver by the ACAT enzyme. This esterified cholesterol builds up in storage droplets. If cholesterol is fed with saturated fatty acids, there is a partial inefficiency in the esterification of the cholesterol, so that a higher level of free cholesterol develops in the liver cell. It is this free cholesterol that then regulates the receptors and may affect lipoprotein synthesis as well.

Gustafsson: I would like to ask about the possibility of yet another mechanism involving the regulation of cholesterol levels, and this refers to nuclear receptors. Some years ago, Magnus Phal isolated a receptor that he called RD1. This was later re-cloned and renamed by David Mangelsdorf in Dallas as LXR α .³ What was shown recently was that an oxysterol would activate LXR α , and this we have confirmed. This may then have the effect of downstream events affecting cholesterol 7- α -hydroxylase, for example.

Brown: David Mangelsdorf, with whom I have daily contact, has shown that when he transfects cells with this LXR, he sees no difference in the regulation of the cholesterol biosynthetic enzymes. But in HEP2 cells, this clearly leads to overexpression of 7- α -hydroxylase. Therefore, he is now working on a gene knockout animal for LXR, and we can soon expect results about the control of 7- α -hydroxylase.

Marckmann: If you compare different plant oils, such as palm, olive, and sunflower oils, you obtain different effects on LDL cholesterol even though they do not contain any dietary cholesterol. Do you think that this is dependent on the cholesterol content of the chylomicrons generated by the intestinal cells?

Brown: Well, there is certainly cholesterol in the system, even if you do not feed dietary cholesterol. The hepatic cells are making cholesterol; therefore the fatty acid composition of the diet can affect plasma LDL concentration without the need to supply extra cholesterol in the diet. When I specify dietary cholesterol, I am using it as shorthand for cholesterol plus saturated fatty acids. There

measured their bone density by DEXA scanning. We see that there is a close correlation between the number of hours they spend watching television and the number of hours they spend outdoors and their bone density levels.

Eisman: Identical twin studies suggest that the impact of overall genetics seems to be modest. Body weight is one of the best predictors of bone density, but the issue is whether bone size or body size is more important. One also has to take into account the interactions between calcium intake and exercise. If there are a number of gene changes at different sites in the receptor, one can also have a combination of differences that I believe relate to the promoter region, and the overall effect may be different from one genetic population to another. Nevertheless, studies in both Japanese and Australasian Caucasians reveal the same overall effects, suggesting that these genetic variants go back a long way in evolutionary terms.

Sørensen: I would like to comment on Dr. Hallberg's discussion about identifying environmental influences by looking at the differences between monozygotic and dizygotic twins. The problem is that the whole approach is invalidated if one has a suspicion that the environmental influences are more similar in monozygotic than dizygotic twins. The other complicating factor is that we are increasingly concerned now with the potential genetic influences on behavior, and this is obviously affected by environmental circumstances. It may now be becoming much more difficult to disentangle genetic and environmental influences. It is also particularly a problem when one tries to translate one's understanding of the environmental contribution to issues of public health.

Eisman: I agree. Nevertheless, when one assesses identifiable environmental factors, such as diet and exercise, one can look specifically at the extent to which they cross-correlate with bone density. People may, of course, misreport their diet and exercise patterns. Nevertheless, the sharing of environmental circumstances does not seem to be enough to explain the differences in bone density between mono- and dizygotic twins. I do, however, agree that it is inappropriate to translate the quantitative significance of the twin model. Our main interest now is very much in genomic screening.

James: If you performed genomic screening in selected subgroups and undertook physiologic interventions with diet and exercise, surely one can gain some evidence about the interaction of the environment with these genetic polymorphisms?

Sørensen: The history of these polymorphisms in other fields is rather dismal, unless one can identify some change in expression or a mutation that relates to changes in physiology as well. We have seen a number of these association studies of genotype with an effect before, and they may mean very little.

Eisman: We now consider it important to relate ge-

netic differences to physiologic studies, such as calcium handling. In animal models we are also looking at the relationship between physiologic regulation and genomic differences.

Vitamin D and Calcium Homeostasis

Eisman: For the vitamin D receptor gene polymorphisms, we need to recognize that there are at least seven distinct studies showing differences in the effect of dietary calcium intake on bone density. We may also see differences in calcium absorption or responsiveness in terms of vitamin D metabolites in relation to polymorphisms. I totally accept that we do not know what the polymorphisms are in terms of their functional role, and this is important. There are functional differences in response depending on genotype, and in four of the studies there is evidence of heterozygous effects that are distinct from those of homozygotes. About 16% of the Caucasian population has one extreme form of genotype, and about 50% are heterozygous. I would therefore argue that in the United States we need to be discussing whether we should redefine the recommended dietary allowance (RDA) for calcium. Currently we are taking the soft option in the sense that we are arguing that it is far too expensive to undertake screening, so we propose that people spend a substantial amount of money on their food to enrich calcium intakes. The overall issue is what the total cost implications are of the disease in relation to genotype screening.

Genetic Control of Iron Metabolism

Kühn: Iron uptake through membranes is probably the facet of iron metabolism that is least understood. We do not know how iron gets out from the enterocyte, how it goes through the intestinal membrane, or how it is recycled after degradation of hemoglobin, so it is difficult to speculate about iron transport across membranes if we have not as yet isolated the molecules.

Halliday: It has been suggested that there is a receptor of low molecular weight for iron and that this could be one explanation for why iron leaves cells in excess in hemochromatosis and other iron overload conditions. Now, if you look at the possibility that aconitase activity is there to bind iron and perhaps to aid in its transport, then we may have a system whereby a low-molecular-weight ion is transporting the iron to a different part of the cell there to induce toxicity.

Kühn: One issue is the iron solubility mechanism within the cell. There could be several low-molecular-weight substances that may be important in the transport of iron into the mitochondrion. Actually, there is an issue as to whether cytoplasmic aconitase is involved. It is usually found in plants and bacteria.

Eisenstein: I would like to discuss the role of citrate

and its possible relationship with transferrin. One of the poorly understood aspects of iron metabolism is how iron gets from stored ferritin to transferrin in the serum and, since apotransferrin does not relate to ferritin and does not get through into cells, the iron needs to get out of the stores to the cell exterior to be charged on to transferrin. Is it conceivable that there is a role for citrate in tracking the iron to the site that will allow it to be transferred to transferrin? There are a number of examples in bacterial systems in which iron citrate is imported and taken out of cells. The same seems to happen in mammalian systems.

Brown: When MHC molecules are presented on the surface of cells, they induce a transport of molecules from inside the cell. Is there any evidence that there is an impaired discharge of iron from the hepatocytes in hemochromatosis?

Halliday: Not from the hepatocytes, but there is from the reticuloendothelial cells.

Worwood: Dr. Halliday, do you agree that there is no real evidence of a failure to release iron from reticuloendothelial cells?

Halliday: The only in vivo evidence was produced by Fillet¹⁰ in experiments conducted many years ago that could probably not be repeated now for ethical reasons.

Hallberg: I would like to comment on issues of interest to Mark Worwood and, in particular, about the value of transferrin as an indicator of iron stores. We have measured iron absorption, menstrual blood losses, and all kinds of indicators in the serum in 203 women, and we have found that by ranking them for the lowest iron requirement to the highest, the indicator that shows the first reaction to a depleted iron status is serum transferrin. There was a sudden induction in transferrin levels, probably suggesting that the liver had sensed the fall in its iron content and had induced an increase in transferrin synthesis. When studying iron absorption from the whole diet by labeling all meats with radioactive iron to the same specific activity and studying people's iron absorption for 1 or even 2 weeks, we find a beautiful exponential relationship between iron absorption and transferrin levels. There is a point at which iron absorption equals iron losses, and then there is no chance for any more iron going into iron stores. The level is set by the availability of iron in the diet, so this regulation is probably, from a biologic point of view, extremely important. The problem for man has usually been to survive in an environment in which there is a very high iron intake. There is also regulation at the other end of the spectrum to prevent iron deficiency, but this is much less efficient. So if one thinks about the molecular basis for the regulation of iron from the intestine, I wonder whether we are dealing with the balance of two regulatory protein systems?

Halliday: I wish I knew the answer, because in hemochromatosis the regulatory mechanism is not working. You

can, however, overload the normal biologic mechanism with pharmaceutical doses of iron so that the control systems only apply to a diet in which you have, in effect, a normal amount of iron. We really do not know the regulatory mechanisms.

Hallberg: When one studies dietary iron absorption, there is excellent regulation, but when one studies iron supplement use, there is no regulation—the dominant effect is actually the hunger of the bone marrow for iron. So we are not at all secure in our use of supplements; they indeed may be dangerous in the long run. I would also like to ask about the impact of liver transplantation. If one transplants livers from patients with hemochromatosis, do the recipients develop or maintain their chromotosis? If so, this would imply that there was a hepatic abnormality as well as an intestinal one.

Halliday: As I understand the latest data, there is as yet no evidence of hemochromatosis developing in recipients of livers from hemochromatotic patients. But we have to be careful because there is only a short period of experience so far and patients have been on immunosuppressive drugs as well, so this can hardly be deemed a normal situation. Therefore, I do not think that we can exclude a hepatic disorder in addition to an abnormality in intestinal absorption. We thought that we could exclude a hepatic role from the studies in rats, but I do not believe that these data are sufficiently conclusive.

Larsson: Some would suggest that when the intestinal cell divides in a crypt, it is conditioned by the environment of the serum and is therefore sensing the iron stores, but then as it moves up the cell, it experiences the availability of iron in the intestine. It can therefore synthesize the molecules necessary to cope with the appropriate transport of iron in a way that responds to the body's needs.

Kühn: It is clear that the discovery of the HLAH (human leukocyte antigen, hemochromatosis gene) system may be a significant breakthrough. One of the points that Dr. Hallberg made is that the molecular mechanism by which the down-regulation of iron occurs is still unknown. We have been looking at the expression of the HLAH system in response to iron in cell culture systems. We used the human intestinal subculture. We looked at normal intestinal cells and found a normal HLAH. When treated with iron, the HLAH level is up-regulated, so this may be a mechanism whereby the body can sense the amount of iron that it should transport. Perhaps individuals with mutations in the HLAH system are unable to regulate their protein in the same way to shut off iron transport. If you look at these cells in vitro and add iron, you can look at the transepithelial transport of iron and see the same phenomenon that one sees in the whole body; i.e., when you treat them with iron you observe a down-regulation in iron transport.

Halliday: So it is the regulatory process governing that normal protein that may be abnormal? So far these concepts do not explain the differences that we have seen, but we have yet to look at individual affected families to see precisely what is going on.

Wood: Could I just comment on two mouse knockout models, which may give some insight into this? We looked at the beta-2 microglobulin knockout and we found that if you feed these mice iron in the diet, they overload with iron very quickly. We also looked at a TAP-1 knockout because we thought that we would not have expression of the major histocompatibility (MHC) complexes on the surface, and we included in this the probable defective HLA system. We expected on this basis to find iron overload, but we did not, so this provides some insight into how that particular MHC-like protein is actually working.

Kühn: Do you know whether the HLA molecule is actually on the villus surface, and do you know whether it interacts with some other protein on that surface?

Halliday: There are a number of people who have looked for this protein, and it has been variously described as occurring in the crypt cells or as having much more specificity in its localization in the perinuclear area. I think it depends on the antibodies used, and I think we have to be careful at present in drawing conclusions about its localization.

Hernell: We looked at the biliary excretion of iron in normal and hemochromatotic patients and found a very good correlation between the iron content in the liver and the biliary excretion of iron in the normal patients, but in the hemochromatotic patients we observed a higher excretion rate but far less than would be predicted from the iron content of the liver.

Larsson: If one takes patients who are truly homozygous with the exact same mutation at both loci, then one can still find variations in the extent to which they display a disease. One example of this is sickle-cell disease and whether or not it is associated with α -thalassemia. The patient then may have no disease at all whereas another may have severe disease early in life. In the common occurrence of coexisting α -thalassemia genes in African people with sickle-cell anemia, the sickle-cell anemia is much moderated. If one increases the amount of fetal hemoglobin, then the impact of the sickle-cell disease is mild. In the case of fetal hemoglobin and sickle-cell disease, we have some direct insight into the physicochemical interaction and the way in which the modification works. This modification is terribly important because if we can identify modifying genes that relate to substantial diseases from mutations, we may be able to develop a drug to modify these genetic diseases.

Worwood: If we come back to the β -microglobulin gene, this is the only gene that seems to have been linked

definitely to iron transport. However, we need to recognize that in the knockout mice an awful lot of proteins are being affected.

Polymorphism in Relation to Iron Metabolism

Hallberg: If one looks at the menstrual losses of women, one finds that this is under very clear genetic control, as shown by studies on monozygotic and dizygotic twins. One can identify enzyme differences in the uterine mucosa to explain this. The distributions of iron requirements relating to menstrual losses are the same in Sweden, England, Canada, Burma, Egypt, and China, which implies that this varied genetic control of menstrual losses and, therefore, of iron requirement was developed very early in the human race. The mean iron requirement for women is 1.36 mg/day, but the 95th percentile is 2.85 mg, so one has a long upper tail. These women would not have had problems in the old days because they were physically active and consuming an appreciable amount of meat. There would therefore not have been any selective pressure in favor of those with smaller losses.

James: I have heard you, Leif Hallberg, disagree with expert panels who look at the non-Gaussian curve of iron requirements and simply change from taking the limit of 2 SD for specifying an RDA and instead arbitrarily take a cut-off limit affecting 85% of the population. The simple assumption seems to be that we have to rely on 15% being clinically identifiable and provide them with supplements.

Hallberg: Yes, and the trouble is that you cannot identify these women with excessive menstrual losses because, on an individual basis, they have always had this loss without realizing that it is unusually large. There is no simple clinical way of identifying these individuals other than doing blood tests.

Worwood: When we consider hemochromatosis, we have a problem that affects 10% or more of the population with the heterozygous form, and they may have higher iron stores. One needs a large study to discriminate the differences, but the heterozygous form of this disease may also be important.

Motulsky: Hemochromatosis is a very nice example of a very common inborn error of metabolism, with 10% of the population having the heterozygous form and one in 400 having the homozygous form. I agree that the heterozygous form may show different responses in the sense that in evolutionary terms, under conditions of potential iron deficiency, these women with the heterozygous hemochromatosis gene may actually have absorbed more iron and therefore been at a greater advantage.

Halliday: I think that we have to be careful before we consider the heterozygote as having an advantage. We have a problem in determining this because we are not presently able to accurately determine the heterozygous

state, although the discovery of the putative gene will help in that regard. We also need to accurately determine iron stores and consider the impact age has, since iron stores increase with age. These factors then need to be related to dietary iron intake. To obtain a meaningful answer, one has to have a reasonable population size, and with a study of 2400 individuals, we have seen that mean ferritin levels in males particularly are much higher than, for example, in the Cardiff population. When we look at family studies, we find a slightly increased level of iron in heterozygous individuals and we have some associated indices of iron status, but we cannot be sure, because we have not biopsied them, which is the only satisfactory way of determining iron stores.

Hallberg: I think there is indeed some confusion relating to serum ferritin. The r^2 for the correlation between iron stores and serum ferritin is 0.5, which indicates that only 50% of the variance in iron stores relates to ferritin levels. I would have thought that with such a marvelous sample and the new probes, you will now have the opportunity to do proper measurements with phlebotomy and blood volume measurements. If you do these carefully you can obtain very good measurements of iron stores.

Motulsky: But, hasn't this been done to a considerable extent in Utah, where they identified the heterozygotes in that population?

Hallberg: Yes, but they have not actually done the phlebotomy measurements that you absolutely need to obtain good measurements of iron stores.

Angelin: If 10% of the population is heterozygous for hemochromatosis, but 15% of women, by virtue of their menstrual losses, have too little iron, we need to think about these two problems when we consider the recommendations about fortification. We may soon have a gene that relates to uterine enzyme changes that are responsible for menstrual losses and a gene for hemochromatosis. Perhaps in 5 years' time we are going to be able to screen the population for these two genes.

Worwood: At a meeting at the U.S. Centers for Disease Control and Prevention (CDC) earlier this year, there was a proposal for the universal screening for hemochromatosis. We were asked to specify what was needed and what is the actual clinical penetration of these mutations—that is what we are missing—but there are very powerful groups pushing for mass genotype screening.

Bruce: But what is the cost of this type of screening together with the cost of introducing effective therapies by virtue of this screening? I think that in most countries today it is just not possible for this to be affordable on a large scale. Perhaps you could identify higher-risk groups who could then benefit from screening at a lower cost.

Worwood: The cost of genotyping for hemochromatosis will probably be less than the cost of the present iron studies that have been conducted on higher-risk

groups. If we are talking about a mass scale test, it could only cost a few dollars per person.

Motulsky: At the CDC meeting there was a push for iron studies within the population now. Unfortunately, there are so many false positives and false negatives from current tests that before one entered therapy, one would need to do a lot of liver biopsies to specify the condition. This is an extraordinary proposal for a population that is not coming to you with any particular complaint, so the committee finally came down in favor of the need for pilot studies. In practice, this newly identified gene is quite common, and hemochromatosis may be affecting one in 100 of the British population. But doctors generally think that it is an esoteric disease. We know that there is not a 100% penetrance, and we have to find out exactly what it is—50% or 60%?

Metal-Metal Interactions

Halliday: I would like to ask the people who spoke about calcium absorption whether they had considered the effect of iron on calcium absorption and the reverse, because, of course, the two do interact and there is some evidence to indicate that there is at least one similar pathway of absorption.

DeLuca: I think the interaction between iron and calcium on their own is actually minimal, but I do believe that when you throw in other dietary components, such as phytate, they become very much intertwined. My understanding is that metal carriers or the transport mechanisms are different.

Halliday: But we do not know the carriers for either calcium or iron transport, so I think it is too early to say that the carriers are different.

DeLuca: But if you study transport of calcium, iron does not compete with calcium.

Hallberg: They interact with both heme and nonheme iron. The mathematical interaction between calcium and iron has the form of one-in-five competitive binding, which I think is very interesting.

James: Are you talking about interactions at the luminal or cellular level?

Hallberg: At a cellular level, I think. All the luminal interactions involve such interactions as phytate with ascorbic acid, whereas calcium interaction with iron looks very different. Whether one is discussing heme or nonheme iron, one finds that calcium interferes with the absorption from both sources and in exactly the same way. This implies that it has to happen within the mucosal cell of the intestine where you have common pathways for the transfer of heme and nonheme iron into the body.

Wood: Most nutritional interactions happen when there is a significant imbalance between the molar ratio of two different metals. Under conditions of normal dietary patterns, I doubt that you will see an interaction between

the C282Y mutation rather than HLA status should provide confirmation of the findings of Bulaj et al., and it will also allow determination of the extent of the clinical expression of disease in true heterozygotes. A further key question is why there is marked variation in iron loading in hemochromatosis that is related to the ancestral haplotype, when the evidence so far suggests that the mutation in the HLA-H gene is identical in all patients with hemochromatosis, irrespective of haplotype. It is possible that, in addition to environmental effects on disease expression, other genetic factors affect the severity of iron overload. Thus, further study of heterozygous subjects identified on the basis of the C282Y mutation should be illuminating.

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