

Discussion

Philip James, M.D., *Moderator*

James:

First let us consider the physiological roles of essential fatty acids at different stages of life. Then we can discuss the requirements and ratios of *n*-6 and *n*-3 fatty acids in development, growth, and adult health. The effect of coexisting intakes of the saturated, the monounsaturated, and the isomeric fatty acids on essential fatty acid metabolism is perhaps worthy of mention. Then there is the question of whether there is a primordial sensory drive for fat and how this relates to appetite control. If fat can be reduced without sensory loss, then what is the minimum fat intake from an energy density point of view? And, finally, we ought to assess the role of dietary fat in relation to chronic disease and public health.

Physiological Roles of Essential Fatty Acids

Bergström:

Thirty years have passed since the structures of six prostanoids were established and their precursors shown to be the essential fatty acids. At that time we knew that they occurred in semen and that they had strong effects on the circulation. These findings led to an explosion of interest in the subject. Now drugs based on prostaglandins are in clinical use.

Essential fatty acids were certainly available long ago in the "primordial soup" of algae and other primitive organisms. It was shown many years ago that if you took arachidonic acid or compounds with the three corresponding double bonds and bubbled oxygen through them, they were oxidized and small amounts of prostaglandins could be isolated; this indicated that these compounds were highly active and certainly capable of being formed before there were any enzymes for these reactions. Later on, through evolution, the enzyme mechanisms emerged, but I think that the primitive origin of prostaglandins explains why this group of bioactive compounds has such a bewildering array of different activities in different animals throughout the animal kingdom; even in mammals there are quite large differences between different species.

It was discovered that a gorgonian had about 2% of its dry weight as a prostaglandin and that by two simple reactions it could be transformed into clinically useful prostaglandins. So every reputable company had divers in the Caribbean collecting it, and for two years material from the gorgonian was used for all clinical trials. Then industry began making analogues, and they made six to seven thousand. The natural prostaglandins are inactivated extremely rapidly at the site of the double bonds, so chemists began to block this inactivation with glyceryl groups or by changing the side chain, with early and clinically relevant success.

The field of prostaglandins is now recognized to be complex. Going from arachidonic acid, we have the E and the F and the V(1, 2, and 3) series; and then we have the thromboxanes and the prostacyclins, which affect coagulation. The E and the F compounds are crucial for pregnancy: the E compounds ripen the cervix at delivery, and the F compounds, produced locally, then take over during delivery.

The main field of clinical use is in human reproduction. By the time of delivery, E₂ is normally produced in the cervix to soften and widen the central channel; the same prostaglandin can be applied therapeutically to the cervix. Prostaglandins can also be used to induce labor, but the strongest drug available is the 15-methyl analogue for bleeding after delivery. In India, for example, 100,000 women die during childbirth—about 30,000 simply from bleeding. A quarter of a milligram of the relevant prostaglandin stops that. Another problem during pregnancy is high blood pressure, or toxemia, towards the end of pregnancy. A dehydration product of a prostaglandin E reduces blood pressure and starts the uterus contracting mildly so that within 24 hours the blood pressure is down and delivery is induced. These products of the essential fatty acids are being widely used, not only in improving the health and survival of pregnant women but also in agricultural research work where the timing of pregnancy is so important. In addition, the leukotrienes are physiologically effective in extraordinarily low concentrations.

Rosenberg:

What is the relationship between dietary intake of the precursor essential fatty acids and the endogenous production of these physiologically important compounds?

Bergström:

The only experimental work that I know of has been done on the prostaglandins of human semen. You can induce by diet some modification in the relationship between E_1 and E_2 , but otherwise it is impossible. Different prostaglandins occur in different cell types and at such low concentrations that a dependence on changing intakes of essential fatty acids is unlikely.

James:

And the inflammatory responses that are increasingly linked to EFA metabolism in terms of monocyte changes and in circulating prostaglandin-induced fever? Is the dietary supply of essential fatty acids again not an issue because the need for prostaglandins is so minute?

Bergström:

I agree that essential fatty acid intake may be important in that regard. However, a lot of the publications about blood levels of leukotrienes are false. Circulating levels in fact are negligible. But in some animals, prostaglandins really act as a circulating hormone to regulate heat production. That is the only case we know of because prostaglandins are inactivated so quickly in the lungs.

Katan:

What happens when you flood the body with the *n*-3 precursors? Would you expect to have an effect on the product made, or is the regulation so tight that it is not influenced by excess substrate?

Bergström:

It is not substrate-dependent. The level in semen, for example, stays very constant irrespective of what you eat.

Weber:

In the cardiovascular system, we have shown, by measuring major metabolites of the thromboxane and prostacyclin series, that you can manipulate this formation by diet.

Crawford:

About ten years ago, Jim Willis and I published some data in which we showed that, in rabbits, an essential fatty acid deficiency reduces circulating prostaglandin synthesis without influencing cell membrane composition. We suggested that there are two precursors for eicosanoid synthesis. If you have cell damage or cell stimulation, local eicosanoid synthesis may depend on membrane composition, which is quite different from the eicosanoid synthesis that occurs as a by-product of arachidonic acid catabolism by peroxidative systems. Swedish groups showed that you could only get eicosanoid synthesis from the free fatty acid and not from the phosphoglycerides. So, where does free arachidonic acid come from? Free arachidonic acid does not exist in the cell. If you inject free arachidonic acid into animals it is lethal. Sinclair showed in my laboratory in 1975 that if you put free arachidonic acid into cell systems in vivo it is immediately soaked up into phosphoglycerides and very little of it is left free. Eicosanoid synthesis comes from two sources: one from the membrane when phospholipase interacts with the cell membrane; the other involves biosynthesis from freely produced arachidonic acid through the conventional metabolic processes, e.g., from linoleic acid or dietary arachidonic acid. The amount of arachidonic acid that is actually produced from ingested linoleic acid is very small indeed. Most of the arachidonic acid is activated as the coenzyme derivative, so there is little free arachidonic acid for cyclooxygenase or lipoxidase transformation. So the metabolic origin of arachidonic acid is directly related to that coming from the dietary intake of essential fatty acids. The fact that it ends up in almost picomolar concentrations is irrelevant. What is present in the cell membranes has to be related to what was taken in the diet.

Gurr:

There seems to have been an underlying assumption that when we are talking about essential fatty acids we are only considering their role in terms of the conversion into eicosanoids. Can we distinguish roles for essential fatty acids in membranes which have nothing to do with eicosanoid production? I know Michael Crawford advised us against the overly liberal use of the word "fluidity," but the fluidity of membranes might affect physiological functions independently of eicosanoid production. This morning Katan told us about the introduction of double bonds with bends in the molecule. These could affect the physical properties and thereby the physiological properties. What he did not tell us was that beyond two or three double bonds the

shape of the molecule changes. The bent shapes of the linoleic acid give way to helical structures where the molecules start to pack together again like saturated fatty acids. So there may be some subtle roles in fluidity when rather large numbers of double bonds are introduced without effects on eicosanoid production.

Local changes in membranes can affect receptor conformation quite markedly. What I do not know is whether the total amount of polyunsaturated fatty acid in the diet makes that much difference to receptor responsiveness. We induced an increase in polyunsaturated fatty acid concentration in membranes with a rise in the ratio of cholesterol to phospholipid, and this tended to counteract the fluidity effects of the unsaturated fatty acids. I think we may have homeostatic mechanisms working to override the effects of diet. Professor Bergström was implying that diet has minimal effects because intrinsic cellular events tend to counteract the effects of diet.

Bjerve:

The role of membrane fatty acids is also crucial for normal brain structure and development in the preterm infant. We followed 21 consecutive preterm children entering the Children's Department at The University Hospital in Trondheim. At one year of age, the factors determining the Bayley psychomotor developmental index (PDI) were analyzed using multiple regression analysis. Gestational age, Apgar score, birth weight, and plasma phospholipid fatty acids were included in the model, which explained nearly 78% of the total variability, with a *p*-value of 0.0002. The only phospholipid fatty acids entering the model were the *n*-3 acids docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA). DHA entered the model with a positive slope of 0.613 (*p* = 0.002), while DPA entered the model with a negative slope of -2.10 (*p* = 0.041). The positive correlation between DHA and PDI suggests that DHA is necessary for normal development of human brain function. The negative correlation between DPA and PDI suggests that Δ 4-desaturase activity is important in determining *n*-3 requirements in the preterm infant. The results suggest that DHA may be specifically needed in preterm infants. When α -linolenic acid is the only source of *n*-3 fatty acids, we need to consider using far higher concentration than usual to allow enough DHA to be produced.

Björntorp:

Am I allowed to ask a question about adipose tissue and lipolysis? Fish oil decreases lipolysis in adipose tissue, but what is the mechanism?

Drevon:

Perhaps the *n*-3 fatty acids alter receptor sensitivity. We have also found that, after six to eight weeks of fish oil feeding, the fasting and nonfasting respiratory quotient (RQ) is higher than in lard-fed animals. We have monitored O₂ uptake and CO₂ output during the whole 24-hours cycle. Could that be linked to an increased sensitivity to insulin? Perhaps the insulin receptor is influenced by the *n*-3 fatty acids.

Nevertheless, I showed today that the LDL receptor in the peripheral blood mononuclear cells from persons ingesting 5 g of *n*-3 fatty acids for four months is not influenced. Freshly isolated cells with many different ligands fail to show a response to fish oil or corn oil. So that is at least one receptor which is not affected.

James:

Am I to understand that no formal studies have been done on glycerol turnover and the response to an insulin clamp with *n*-3 fatty acids to document the finer control of insulin sensitivity at the lipolytic level in vivo?

Drevon:

Storlien in Australia has been doing clamp studies with a constant level of insulin injected. They conclude that in the rat *n*-3 fatty acids increase insulin sensitivity, but they do not know what the mechanism is. They also wonder why you do not get the same RQ change in man as in the rat with feeding *n*-3 fatty acids. The amount of triglycerides in the muscle cells of humans is different from the rat. They find a difference in RQ in different kinds of rat muscle cells with a significant change in the red but not in white muscle cells. They wonder if the amount of triglycerides in the red muscle cells influences insulin sensitivity.

Gustafson:

It may be relevant to point out that arachidonate may be hydroxylated to compounds which serve as intracellular signal transducers. This has been suggested as the mechanism of action of dopamine in the pituitary when it modulates the secretion of prolactin. It is possible that cytochrome P450 has a role in oxidizing arachidonate in specific positions to form these signal transducers. Hydroxylated arachidonate metabolites are supposed to have a vasoconstrictor function in the kidney, for instance, and in the retina. They might be modulated by dietary intake of essential fatty acids.

Essential Fatty Acid Requirements

James:

We heard this morning that half the brain's essential fatty acids were accumulated by 40 weeks of pregnancy. With postnatal brain growth there is a further requirement as well as for all the needs for cell replication in the body. How should we determine requirements during growth? Dr. Crawford emphasized that specific fatty acids are needed for the early phase of development. I would have thought that the demand would be much greater in the second half of pregnancy rather than in the periconceptual period.

Gurr:

One way to determine fetal requirements is to take a factorial approach and look at how much the fetus accumulates in different organs as it grows. Or you can take a functional approach and ask, "What are these fatty acids required for and can we measure various biochemical or physiological functions?" and hope that that tells us something about requirements. If we use a factorial approach, we are ignorant about the degree to which the mother can adapt if she goes through a period when she is not eating very much essential fatty acid. Perhaps it does not matter because she can mobilize it from somewhere else. Then that throws out our assessment of how much she needs in the diet. If she mobilizes EFA from her adipose tissue, then that raises the question of the extent to which fatty acids in the adipose tissue are available.

Crawford:

There is a certain quantitative requirement which the fetus is going to express and this was discussed in 1977 and quantitated by an expert committee organized by the Food and Agriculture Organization and World Health Organization. They have already done the calculations. I think what is new is a paper by the Wynns in 1980 in which they pointed out that the Dutch famine, the postwar German food shortages, and the Norwegian food shortages all produced almost identical information on maternal nutrition. All three countries kept very precise records. If a woman became pregnant prior to the food shortage, regardless of country, and in the latter part of the pregnancy was exposed to severe food shortage, then she produced, by and large, reasonably healthy babies.

But those mothers who did manage to conceive during the period of food shortages had a three- to four-fold increase in the incidence of neural developmental disorders and congenital abnormalities in

all three countries, even if their diets improved subsequently during pregnancy. There are many examples in the animal kingdom of EFA deficiency leading to infertility. With anorexia nervosa women also become amenorrheic. Nature seems to prepare in advance to ensure that conception, and early brain development, which is so dependent on diet, only occurs in women who are reasonably fed. Within the first few weeks of conception, the rate of cell division is 2.5 million times the rate of cell division once you get to somewhere around 20 weeks of pregnancy.

James:

Although that may be true in terms of relative rates, in terms of mass supply, is later pregnancy more important?

Crawford:

Cell division dictates the requirement for mass supply. It is the number of cells that you create at the beginning which determines the subsequent growth velocity. The number of cells in the body is determined at an early stage and this is particularly true for brain cell division. If brain division is retarded at that stage, then the effect is permanent. If you start off with a good rate of cell division, then you have the appropriate number of cells for the rest of your life.

James:

So you have a critical stage of cell replication, but you believe that the supply thereafter is not so critical, despite the need to build membranes and so on, as the given number of cells that are replicating?

Crawford:

The supply after that has a lot to do with maternal health, because we know that the placenta is a pump which is pumping nutrients from the mother to the fetus.

James:

And there are special fatty acid binding-proteins in the placenta?

Crawford:

Absolutely right. The arachidonate concentration across the placenta is twofold, and the same applies to DHA. But there is very little α -linolenic acid on the fetal side of the placenta and virtually no eicosa-pentaenoic acid either. So the placenta selectively

picks out docosahexanoic acid and arachidonic acid as well as a wide variety of other nutrients that are needed. If you get good placental production, then you get good fetal production, almost regardless of the maternal nutritional status. However, at the end of the term the mother's status is bound to be important both for the last phases of fetal growth and for her own maternal health. Maternal health will be adversely affected by inadequate nutritional conditions in the latter part of pregnancy.

Connor:

There are two significant animal models. One is the mammalian model. If the diet of the mother is deficient for a couple of months, then the offspring show biochemical evidence of deficiency. That has been well shown in both the rat and the rhesus monkey. Another interesting model is the chick embryo. One can influence the fatty acid content of the egg by the diet fed to the laying hen so that the newly hatched chick can be either deficient in essential fatty acids or have them in excess. So this is a good model to determine requirements quickly because in 21 days you have the answer in the newly hatched chick.

Bruce:

I think that some of the questions were partly answered back in the 1960s and 1970s by the extensive studies done by Pauletti and Galli in Italy and by the group to which I belonged in Göteborg. We mapped in detail the development of the fatty acid patterns in the brain, liver, and muscles of the fetus in experimental animals. The brain was given priority, then the muscle phospholipids were next. We showed that there is a large pool of polyunsaturated fatty acids in the muscles which can be used if necessary. But we also found that it took almost three generations on moderate intakes before the fetuses stabilized their low levels of essential fatty acids.

Crawford:

Sinclair, when he was working with us, showed that if you put rats on too low an intake of essential fatty acids, they would not reproduce. So you had to find a gray area of intake where they would still reproduce but where by the third generation you had a 20% reduction in cell number at birth. Now the interesting thing about Pauletti and Galli's work and Bruce's work was that you could demonstrate learning deficits in this next generation. But if you repleted the diet of these deficient newborn pups you did not reverse the learning deficits. Although

you could reverse the chemical composition, cell division did not catch up. It parallels the data of Dobbing and Widdowson who looked at the effect of gross malnutrition on critical periods of cell division.

Pedersen:

There is one important question that may not be answered by the animal model, and that is the efficiency of conversion of α -linolenic acid into the very long-chain *n*-3 fatty acids, because we know that that deficiency is different in the rat and in humans. How efficiently can a woman convert α -linolenic acid into the very long-chain fatty acids, and do we need for reproduction, in addition to α -linolenic acid, docosahexanoic acid? We have the impression from Michael Crawford that we need marine fatty acids, but Dr. Connor only mentioned α -linolenic acid in relation to requirements.

Connor:

In our monkey model, with feeding linolenic acid from soybean oil we found a decline in DHA in the plasma and red cells. I think this decline in DHA in human infants has not occurred with breast-milk feeding, which provides the α -linolenic acid rather than the DHA. In the chick embryo we fed the hen linolenic acid and the egg yolk had mainly linolenic acid, but the development of the brain DHA was much retarded. However, if we fed EPA or DHA, then the level in the brain attained its maximum state. So I think there is probably a need for DHA in the diet. Now whether a woman with a store of linolenic acid will be slow in converting this to DHA during pregnancy is an open question.

Gebre-Medhin:

The few good studies on fetal growth charts suggest that maternal undernutrition has very little impact on total fetal growth before, say, week 35 or 36. Of course these are only gross indicators. The second point is that, as far as I know, there is very little difference in the rate of congenital malformations in, say, areas with very poor nutrition compared with rates in affluent societies. There is therefore no indication of a potential effect of EFA deficiency on congenital malformations in man. Thirdly, it seems as if severe malnutrition does not affect fertility. Had it been so, I think we would not have had this huge population explosion. Finally, I previously believed that anorexic patients could not become pregnant and develop a good fetus. But I am now working with three patients with florid anorexia who not only became pregnant, but the fetal growth

chart indicates near-optimal growth until about week 24 of pregnancy.

Maternal Supplementation

Garza:

In Guatemala, they are now going back to the villages where 15 years ago mothers were given various levels of protein and energy in a supplementation trial. They are finding very strong relationships between the level of supplementation, the length of supplementation, and the cognitive function of the children! Ernesto Pollitt has been primarily responsible for this work. Jerry Haas has also looked at physical size and work performance in the children of supplemented mothers and has found some strong correlations between the supplementation history and the work capacity of the children 13 and 15 years later. So if one looks at fetal growth charts and birth weights, it is very difficult to see any substantial changes occurring with supplementation, but 13 and 15 years later the functional outcomes in the children are dramatically changed. The magnitude of birth weight differences between control and experimental villages was only about 300 g, if I recall correctly, so that the differences could be detected only with fairly large numbers. This collaborative study is being done in Stanford, Davis, and Cornell.

James:

So this is different from Sally McGregor's work concept where children who are "relatively malnourished" from the age of about six months are then given both food and social stimulation over a period of one year. They show a remarkable improvement in developmental quotients which are then maintained without further intervention for a period of seven or more years.

Norum:

I wonder whether someone could comment on the data from the Faroe Islands where birth weights are higher than expected but toxemia of pregnancy is lower than in Danish or English women. Is this something to do with a better intake of *n*-3 fatty acids?

Dyerberg:

I only know that there is presently an ongoing study in Denmark which seems to indicate that the content of long-chain *n*-3 fatty acids in mothers' blood cell membranes is positively correlated with the

gestational age before delivery. The difference in age is of the order of three to seven days.

Crawford:

We have two independent correlations between umbilical cord blood levels of DHA and the head circumference of normal babies at birth. Then when we look at DHA in relation to the degree of prematurity, we find a similar link. There are reasonable correlations between DHA and the degree of gestation from 24 weeks to the 40-week, full-term babies. The blood of these premature babies does not contain any significant quantities of either eicosapentaenoic acid or α -linolenic acid; the placenta has selectively taken DHA out of the maternal system. Basically all that the baby has been fed with during fetal life is DHA.

Garza:

It is very difficult to interpret your two graphs because any relationship with head circumference may just relate to the fact that they are babies of different gestational ages.

Crawford:

No. They are appropriate in age when we are assessing head circumference, i.e., between about 38 and 42 weeks of gestation. The other data relate to prematurity, i.e., varying gestational periods. There are many other nutrients that correlate with early delivery.

Rosenberg:

Is the fatty acid content of human milk affected by the *n*-3 intake?

Connor:

Yes. Eskimo milk contains a lot more *n*-3 fatty acids because the mothers are eating seal oil. Milk composition can also be affected by linoleic acid intake; a high carbohydrate diet also induces a high saturated fatty acid content in the milk. But, regardless of the diet, there is always a basal component of both series of essential fatty acids.

Postnatal Nutrition

Bjerve:

The European Paediatric Society has recommended that the *n*-3 fatty acids should be about 5% of energy for premature babies. They have not specified whether there should be α -linolenic acid or whether

there is a specific requirement for any longer *n*-3 fatty acids, nor the need for any longer *n*-6 fatty acids other than linoleic acid. With the increasing numbers of premature births in Norway and in most other Western countries, the issue will be more and more important. We need to find methods to assess whether we need to add both arachidonic acid and, of course, the hexaenoic acid. My personal view is that the present European recommendations for preterms are inadequate. Dietary polyunsaturated fatty acids should be increased and we probably need to include the long-chain unsaturates, at least those from the *n*-3 series and possibly the *n*-6 long-chain fatty acids also.

Gebre-Medhin:

National nutritional guidelines are, of course, intended for the total population and they do not usually exclude children with respect to the proposed fat intakes. These guidelines state that the amount of food energy provided by fat should be between 30 and 35% of energy. Now the issue at stake at this time is, when should the unweaned child move from its fiber-free, high-fat diet down to this low-fat diet of about 30%? And in the practical implementation of these things, we do not have clear and satisfactory recommendations. Now we have the impression in Scandinavia—at least in Sweden—that there are some children who consume a fiber-rich diet to appetite and yet do not grow adequately. We now have some very striking evidence suggesting that a proportion of children will not be doing well on diets containing 30 to 32% of energy as fat.

One example is a young child who very quickly shows signs of failure to thrive. Linear growth and head circumference are affected when the child is consuming 31% of energy as fat. As soon as we raise the fat intake to 35 to 40%, we begin to see catch-up growth, both linear growth and weight gain. These data suggest, then, that the lower range for fat intake for children should probably not be less than 35% of energy during the first two years of life.

Bjerve:

I think we should try to evaluate the minimal amount of essential dietary components, including the *n*-6 and the *n*-3 fatty acids, in absolute terms. We have a long tradition of expressing these intakes as a per cent of energy. They are the only essential nutrients that we give in relative values. We have patients in Trondheim who have nearly 3% of total energy in the form of *n*-6 fatty acids but they have both biochemical and clinical signs of *n*-6 fatty acid

deficiency. So I think it is wise now also when we are reevaluating the preterms that we start discussing what the absolute requirement is for the *n*-3s and the *n*-6s because otherwise we will get confused again.

James:

That is a very intriguing suggestion, implying that as children, certainly from the age of two in Western societies, seem to be eating progressively less as they sit more in front of television, then their true needs for EFA will increase as a proportion of calories.

Isomeric Fatty Acids

Crawford:

These fatty acids can affect essential fatty acid metabolism in at least two ways: competition for desaturases of various kinds, and a direct effect on the desaturase itself. The ratio of nonessential to essential fatty acids may really matter. We may not be talking about an effect specifically of *cis* or isomeric fatty acids but about the effect of changing ratios between fatty acids which are either nonessential or essential. My reading has led me to favor the competitive effect rather than a specific direct effect on the desaturase enzyme. Thus saturated fatty acids of different chain length may affect the rates of processing through the desaturated pathways.

James:

I am told by colleagues that the isomers differ markedly from one another and that the impact of the isomer does not depend on its *trans*-configuration; some of the biologically most effective interactors with the desaturases are the unusual *cis*-isomeric fatty acids. That implies a selective effect rather than a mass action competitive effect. I have no data where people have assessed the processing and through-out of the essential fatty acid metabolism under the impact of intakes of saturated fatty acids or any other manipulation in vivo.

In other words, if you are trying to work out the rate of EFA metabolism, it is difficult unless you have appropriately-labeled fatty acids. Some groups, including our own, have spent time synthesizing appropriate fatty acids, but the kinetics and modeling of the system are difficult.

Gurr:

The only data that I recall are Lansey's data. He synthesized a whole range of *cis*- and *trans*-isomers

with the double bonds in different positions along the chain, and he showed that the incorporation of these unsaturated fatty acids with different structures into the 2 position of the phospholipids varied enormously according to the position of the double bond along the chain.

Bjerve:

We have data from nearly 160 individuals where we are recording the concentration of arachidonic acid and eicosapentaenoic acid in the plasma phospholipids. We get the expected increase in eicosapentaenoic acid with increasing fish intake and the expected decrease in arachidonic acid concentration in plasma phospholipids. But if we divide these individuals into two groups according to high or low levels of stearic, palmitic, or myristic acids, it turns out that those having above a median concentration of myristic acid do not reduce their arachidonic acid concentration when we increase the fish intake, whereas it is very much reduced in those having the low myristic acid concentration. We find no effect in relation to stearic acid and only a very slight effect with palmitic acid. How this translates to the diet is unclear, but there are clear differences in how the three saturated fatty acids within the lipoproteins affect the concentration of the products of *n*-6 and *n*-3 fatty acid metabolism or their incorporation within the plasma phospholipids. We have also found that there is a linear correlation between the increase in *n*-3 fatty acid concentrations in plasma phospholipids and a concomitant decrease in blood pressure. We have no measurements of the amount of prostanoids or eicosanoids produced.

Bergström:

You cannot correlate dietary intake to the composition of the phospholipids because the rates at which these different acids are incorporated are quite different.

James:

I guess that plasma phospholipids are a crude index of recent intake, but did this index respond when you manipulated the diet and there was a physiological change in blood pressure?

Bjerve:

We have only epidemiological data, but during an intervention study the relation between changes in *n*-3 fatty acids and blood pressure was the same as that found at the baseline correlation study, so we

concluded that the *n*-3 fatty acid intakes in Norway affect blood pressure.

Dietary Fats and Lipoprotein Metabolism

Andersson:

I want to comment on the different mechanisms involved in the cholesterol-lowering effects when manipulating dietary fat intake. Current ideas suggest that the LDL receptor is down-regulated by cholesterol and saturated fats. But what happens in the liver and gut has been little studied. Of course the classical sterol balance technique has been used for a long time, i.e., where you compare the cholesterol intake with the excretion of bile acids and cholesterol from the bowel. Unfortunately, this is a very crude technique because there are several problems. One is the transit time of the large bowel. Another is the degradation of bile acids and cholesterol in the large bowel. So one reason why we have failed to show clear changes in the classical sterol balance technique is that such a change must be more than 50% to be detectable. We have tried to overcome many of the problems by introducing the ileostomy model. Then you can compare the sterol and fat intake with excretion without the interference of the colon. The study must be done under metabolic ward conditions with collections of the ileostomy effluent every 2 hours in order to avoid degrading the bile acids and cholesterol in the collection bag. Subjects spend three to four days on each diet, and we randomize the order of the diets. These methods increase the precision and allow the detection of smaller changes in sterol balance. There is also an immediate effect of a dietary change. We have performed a series of studies with six to 11 subjects in each series. If you decrease total fat intake from 44 to 20% of energy, then sterol excretion increases. With polyunsaturated fat you get another pattern, with an increased excretion of both bile acids and cholesterol itself. And with monounsaturated fats, we saw a very significant increase in cholesterol excretion from the small bowel. These effects are immediate. By labeling the dietary cholesterol we will be able to see from which source the excreted cholesterol is derived.

Miettinen:

In our studies, when we carried out this type of change, there was a dramatic reduction in the efficiency of cholesterol absorption, indicating that dietary and biliary cholesterol absorption were reduced. This then reduced the amount of cholesterol returning from the intestine to the liver, so that the hepatic cholesterol content probably fell, thereby

activating the receptors for LDL apoprotein B₁₀₀. That is probably the reason for the reduction in serum cholesterol under those conditions.

Norum:

You are saying, Tatu Miettinen, that when you reduce the total fat, then the total cholesterol absorption goes down by a half. Is there any difference between the different types of diet you are using? And is there any effect on intestinal mucosal metabolism, e.g., in cholesterol esterification, or is it just the passage of cholesterol into the mucosal cell?

Miettinen:

Unfortunately I do not know yet if it is actually due to a change in intake of polyunsaturated or saturated fats, but there appears to be a straight link between the fat intake, either as a percentage of total calories or in absolute terms, and the cholesterol absorption efficiency: the higher the fat intake, the higher the cholesterol absorption efficiency. I consider that a low-fat intake makes it difficult to dissolve cholesterol into a micellar solution, and that would reduce its absorption. One might imagine that if a low-fat intake would reduce intestinal CAT activity, that might be related to cholesterol absorption too. These studies were conducted on normal solid food.

James:

So, as you drop your fat intake you are increasing starch intake so that any potentially resistant starch will sweep material into the colon and therefore potentially have some impact on cholesterol absorption?

Miettinen:

There was an increase in starch and sugar intake on the low-fat diet.

Crawford:

Before we move on to public health, may I refer to some very interesting information that Christian Drevon produced on the immune system? There has been a tacit assumption that many of the effects seen on feeding fatty acids have been eicosanoid-mediated. And what he suggested both with cyclooxygenase and lipoxygenase inhibitors, such as aspirin, was that this was not so. It seems to me something relating to the *trans* isomer story may be involved, i.e., the question of the structural integ-

rity of cell surfaces and the impact of the different shapes of fatty acids on the properties of cell membranes.

Some colleagues have been looking at the question of immune function in multiple sclerosis. And one of the things that they have been interested in is the electrophysiological activity of cell membranes in multiple sclerosis because the cells move in a different way in the electrophoretic field. Quite clearly a specific effect on electrophoretic mobility of cell membranes is induced by increasing the linoleic acid content of the membrane. This has nothing to do with eicosanoid function. As far as one can deduce, it is purely a function of cell membrane composition. So, I would like to hear a little bit more about this effect on T-cell behavior in relation to cell membrane composition.

Drevon:

The viability of cells decreases rapidly with the use of long-chain polyunsaturated acids but only when the cells are activated. So it is not just a question of adding the polyunsaturated fatty acids and killing off the cells. It has something to do with the activation process promoted by the antigen-presenting cells. It seems as if 10 minutes exposure of the cells to the different fatty acids is enough to inhibit T-cell proliferation in vitro. As far as the mechanism is concerned, the data suggest that the prostaglandins or leukotrienes do not produce the effect. I wonder if anybody here has studied apoptosis, the process where there is sudden fragmentation of DNA in the cell before the cell explodes. We have a feeling that that might be the reason why these activated T cells stop proliferating.

James:

Are you talking about a physiological effect? It sounds very pathological to me.

Gustafson:

What is known about apoptosis is that glucocorticoids can induce it by binding to a receptor that moves into the nucleus, probably to activate endonuclease. And that leads to cell death. In the same way dioxins can also induce the death of T cells. So I wonder whether the clofibrate receptor might be involved. If arachidonic acid is taken up by the T cells and perhaps converted into a hydroxylated derivative, this could bind to the clofibrate receptor if such a derivative of an essential fatty acid is an endogenous ligand of this clofibrate receptor. Transport into the nucleus could then activate cer-

tain genes such as the endonuclease genes and lead to apoptosis. That would be in parallel with what is known about glucocorticoids and T-cell death.

Fatty Acids and Public Health

Hjermann:

We now have the 15-year follow-up data on the Oslo study diet/antismoking trial. As you know, all the drug trials have not been able to show any reduction in total mortality, but in this study there is a significant effect on total mortality and of course also in coronary heart disease mortality. So we have been able to show for the first time in primary prevention that it is possible not only to prevent disease incidence but also coronary heart disease mortality and total mortality. [Number of deaths: CHD 24 vs. 48 ($p < 0.05$); total deaths 58 vs. 82 ($p < 0.05$), in treatment and control groups, respectively.]

Goodman:

I thought that there was a good review of many aspects of the topic this morning. I am very pleased to see the Oslo 15-year follow-up data. It should also be pointed out that the Coronary Drug Project in the U.S., after another decade of follow-up of the niacin group, showed a reduction in total mortality as well. So worry about the failure to see a fall in total mortality in coronary intervention trials is really a red herring. The clinical trials were not designed to look at total mortality and did not have a large enough data set, nor were they followed up for long enough. The recent meta-analysis, published in the *British Medical Journal*, was quite inadequate to address that question and received an unreasonable amount of publicity. There are so many interesting questions about dietary fats and control of lipoprotein and atherosclerosis risks. We reviewed the evidence on dietary fatty acids and thrombosis for the Diet and Health Report that was published by the National Academy of Sciences in 1989. The evidence against stearic acid is poor on normal intakes of stearic acid. The old evidence from in vitro studies, which Bill Connor could talk about in considerable detail, was an artifact with the stearate having physiochemical effects but not really reflecting thrombosis.

I would like to ask Martijn Katan his view on the extent to which there is genetic control of the body's response to saturated fat in terms of LDL cholesterol levels. There is a belief that in humans there are also genetic hyper- and hyporesponders to dietary cholesterol.

James:

And perhaps he could also consider the effect of different fatty acids on clotting?

Katan:

I think the problem is that we do not have a good in vitro assay which can be used for assessing fatty acids such as stearic acid in relation to thrombosis in vivo. The fibrinogen story is much more solidly based in human epidemiology than some of the platelet provocation tests.

Sandström:

We find a very significant effect on factor VII with changing the diet to a high-fiber, low-fat diet; the fatty acid composition does not seem to have much influence.¹ But when only the fat and not the fiber intake is changed there is no effect. So it seems to be the combination of a low-fat and a high-fiber diet that affects factor VII. We also see an effect on fibrinolysis using in vitro techniques, but not on circulating fibrinogen levels.

Kromhout:

I understand from Meade that there is no evidence that diet affects fibrinogen levels.

Hjermann:

In the Oslo study, we investigated the effect of this cholesterol-lowering, body-weight-lowering diet on hemostatic risk factors, and it turns out that the fibrinolytic capacity is easily increased by this diet, and that the factor VII-phospholipid complex level is lowered by this diet. These experiments on the hemostatic risk variables were done in separate experiments, account being taken of the effects of smoking on fibrinogen levels. There are also many dietary ways of influencing platelet activity.

Bjerve:

In the Tromsø study, when we control for smoking, there is a clear association between a decrease in fibrinogen concentration and increasing concentrations of dietary ω -3 fatty acids.

Gurr:

I have not heard mention of lipoprotein-a, which I understand is strongly related to the risk of coronary heart disease. No one has yet shown that dietary factors have any effect on lipoprotein-a.

Sandström:

We analyzed lipoprotein-a in all our subjects but could not see any effect of dietary change on this lipoprotein.

Katan:

In Wageningen, Seidell and Deurenberg have found that weight reduction in a well-controlled trial caused a fall in lipoprotein-a. That is rather preliminary, but very intriguing.

James:

What about the issue of the low essential fatty acid levels found in the fat depots of U.K. men who succumb to heart disease?

Connor:

I think we have to be very careful because the level of adipose tissue simply reflects the habitual diet, so it may be that the diet in Scotland is a little lower in linoleic acid. Certainly those people did not have essential fatty acid deficiency as classically described, so I think it is a correlation. There was also, I think, a low content of EPA in the diet and therefore in the adipose tissue.

Kromhout:

A trial with 2000 post-myocardial infarction patients showed a significant effect of using fatty fish two or three times a week. The trial therefore provided convincing evidence that a small amount of fish is of importance in relation to survival. We have to remember that, in practice, fish consumption seems to have secondary as well as primary preventive properties in relation to heart disease.

Hambraeus:

I have not heard anything about the effect of physical exercise and increased energy turnover on lipid metabolism. Exercise is one of the great regulators of lipid metabolism with relevance to public health since, in industrialized countries, we have reduced our physical activity and our energy turnover by about 20–25%. So this may be one of the major reasons for losing control of endogenous fat turnover. How much exercise do we need to induce a high energy turnover and what type of fat intake should we then have?

Jéquier:

During exercise of moderate intensity, the insulin level falls and lipolytic hormones such as epineph-

rine are secreted. These changes increase fatty acid turnover, and in long-time exercise of moderate intensity there is always a shift to increased lipid oxidation. High-intensity exercise uses more glycogen. Dr. Leibel alluded this morning to the question of whether one calorie of carbohydrate is equal to one calorie of fat. I would not challenge the thermodynamics, but I think it may be appropriate to say a few words about short-term nutrient intake regulation.

When normal-weight subjects receive only 100 g of carbohydrate on day 1 and then on the second day an enormous amount (800 g), the body can cope with approximately half of the load being oxidized during the 24 hours. This is measured using a respiration chamber, and under these conditions half the carbohydrate is stored as glycogen. The study has also been carried on for eight more days. From the third day on there is net lipogenesis. As far as energy balance is concerned, with a high load of carbohydrate, there is good evidence that there is an increase in sympathetic activity—and then perhaps one calorie of carbohydrate is not exactly the same as one calorie of fat because of this increased energy expenditure due to the stimulation of sympathetic activity which you do not see with excess fat.

In a different type of experiment, we have looked at the influence of a fat supplement in a two-day study. On the first day, the subject was in energy balance. The intake and oxidation rates were approximately 2800 kcal and they were also in nutrient balance, i.e., the same amounts of protein, carbohydrate, and lipid were oxidized as ingested. On the second day, subjects received 1000 kcal excess of fat as a mixed fat supplement. What is striking is that the amount of nutrient oxidized as protein and carbohydrate did not change at all, and there was no change in the lipid oxidation rate. So the whole fat supplement was stored! So dietary fat behaves very differently; if there is an excess of fat it will be immediately stored, at least during these short-term studies, whereas excess carbohydrate is stored for a short time as glycogen with an increase in energy expenditure. Over one to two days, carbohydrate balance is well-regulated in man, whereas fat balance is not at all well-regulated.

Leibel:

I do not think we have any disagreement about the effects of short-term overfeeding of any of these foodstuffs—carbohydrate or fat—but the only thing I am emphasizing is that when you look long term at the caloric equivalence of these foodstuffs, they really do seem to be quite comparable. Whatever happens to fat in terms of its being deposited pref-

erentially on short-term overfeeding, there seems to be no difference between carbohydrate and fat supplements in terms of energy balance when you look over a period of 50 to 80 days.

James:

So neither the sympathetic activation nor Danforth's proposition that thyroid hormone is increased somewhat on a high-carbohydrate diet is important? You do not think that either of those responses effectively changes energy expenditure such that a high-carbohydrate diet is effectively more thermogenic and "therefore more slimming"?

Eric, what about your and J. P. Flatt's concept that if one is on a fat intake that is sufficiently high to reduce the food quotient relative to the respiratory quotient then one is depriving individuals selectively of carbohydrate with food intake driven by a need to maintain carbohydrate stores. Therefore, a high-fat diet is a carbohydrate-deprived diet? So you then tend to overeat to obtain carbohydrate. Are high-fat diets particularly conducive to high energy intakes?

Leibel:

I would say yes, they are. Palatability is, I believe, the major effect of a high-fat diet and the reason for a positive energy balance. If fat is a promoter of weight gain and obesity, it is more likely to be through its effects on the hedonic characteristics of the food source than because of any mysterious effect on intermediary metabolism.

Jéquier:

There have been a number of recent studies, e.g., from Bouchard's group and from Dr. Tremblay, showing that if you offer a diet which is rich in fat and low in carbohydrate, the subject will eat spontaneously as much as 1000 kilocalories more a day. And when you look at the carbohydrate intake that they have with this high fat diet, it is rather low—in the range of 200 to 250 g per day—and these people are very active. Therefore, it seems as if there is also a need for a certain amount of carbohydrate. Obviously, the brain and some other tissues are using carbohydrate exclusively. So both aspects—the hedonic aspect of fat and the drive for carbohydrate—will play a role in the overall regulation of food intake.

Katan:

There is some problem in reconciling the short-term studies showing an association between high-fat di-

ets and obesity with longer-term trials where there is no really solid evidence that high-fat diets do cause massive weight gain. There is the National Diet Heart Study in the United States, which lasted one year, and had men on diets varying in fat content from 40 to 20% of energy. The differences in body weight gain between these men were really very small—maybe a kilogram or so over a year. I have also seen data from a long-term trial in Seattle showing similar results, i.e., putting men on diets varying from 36 to 22% of energy as fat over a year did not really cause much of a difference in weight gain.

Rössner:

When we came back from lunch, Dr. Mogren complained that we were slow eaters. Probably that is a good thing! Two weeks ago in Nice, Dr. Kraal from New York demonstrated, by using the Kissileff Universal Eating Monitor, where you measure the rate of eating over time, that there was a relationship between the speed at which people were eating and the amount of fat that infiltrated the liver. He had biopsied very obese individuals and could show that the faster they ate a liquid homogeneous meal, the more fat went into the liver. So maybe the microstructure of eating is important here. Can anyone tell us about the composition of the meal and the eating pattern during the meal itself—the effects on the slowing curve of intake during a meal and how this affects total intake?

Drewnowski:

Our data suggest that the basis for the behavior may be at any of three levels: metabolic, sensory, or attitudinal. So, on the one hand, yes, we have been looking at the metabolic aspects of food preference and we were quite impressed with the effect of a variety of gastrointestinal peptide hormones on the selection and preferences for dietary fat. That is at the metabolic level. At the sensory level, there is also evidence that preferences for fat may be learned, while preferences for sugar may well be innate. Preferences for fat are probably learned in very early childhood and may stay with a person for the rest of his life. Finally, from the sociocultural standpoint, preferences for foods high in fat are really part of the Western diet. So it could be any of those factors acting at any of the three levels that contribute in different ways to the food preferences of obese individuals.

James:

So you are implying that the attributes, as we see it, of fat are culturally dependent? We cannot there-

fore believe that the Chinese are incessantly miserable because they are on such a low-fat diet!

Drewnowski:

On the contrary, I think this is precisely what we should believe, because in cultural studies of different societies it becomes quite clear that people do not eat fat because they do not have any. Once they can afford it, they really do prefer a diet rich in sugar and fat. Socioeconomic studies also really show quite clearly that economic progress goes hand in hand with increased consumption of animal protein and meat. We were convinced for many years that whereas we in the West liked sugar and fat, the Japanese liked rice and pickles. But now the evidence is that preferences for sugar and fat really are universal.

James:

So it is not just the cultural pressures which link a particular type of eating pattern to affluence and *savoir-faire*?

Drewnowski:

Cultural factors determine the time at which the high-fat diet becomes available. So the American public has become used to a very high-fat diet and is very resistant to dietary guidelines that advocate replacing fats with fruits and vegetables. In practice, they are replacing one source of fat with another.

Rössner:

Is there energy compensation with the low-calorie diets when dietary fat is being manipulated?

Drewnowski:

This is a very good point and in fact there are very few studies which show what happens in the long term regarding caloric compensation. The latest studies, conducted with Olestra by some colleagues of mine, show that there is caloric compensation within 24 hours but that caloric compensation is not specific to fat. On the contrary, it happens in the evening and it usually involves carbohydrate snacks so the total number of calories is the same but the carbohydrate to fat ratio has changed and that may be beneficial to health.

Crawford:

I wonder if I could throw a spanner in the works about this. I remember visiting Professor Debry's

department in Nancy where many of the perceptions of food that the French wanted to test were quite different from the kinds of things that the Americans are testing. Debry had an indwelling cannula to measure continuously insulin production. He presented his test subjects with a variety of different plates and was able to show very conclusively that insulin production would be stimulated not by the nutrient content of the food but by the visual perception of the food before people even smelled or tasted it. I think it is interesting that one sometimes forgets in our obsession with discussing nutrients that there are things other than nutrients that are in food that drive people towards eating different types of food.

Jéquier:

There has also been another study by Dr. Lars Sjöström in Göteborg on this aspect, looking at the insulin response of obese people looking at and smelling good meals. His results were not so convincing. The hypothesis was that the fat people would have a greater surge of insulin, but I remember that this was not so.

Gebre-Medhin:

I agree with Dr. Drewnowski that if people can afford it economically, they would prefer a diet rich in fat and sugar. If you take a country like Ethiopia, the privileged group of people of the same ethnic origin, living in the same area, buy much more fat than the poor people.

Just a final comment about the distribution of food intake over the day. During a study in Uppsala we discovered that pregnant women put on much more weight when they eat their food later on in the day, say after 4 p.m.

Rössner:

We rarely see any obese patient who is fat from overeating breakfast. I do not know of any study that has tried to relate this to circadian rhythms or hormonal factors during the 24 hours or anything else, but in our experience, late eating—in Sweden, cheese sandwiches and snacks—is one of the culprits.

Leibel:

With regard to the timing of meals and a possible difference in risk of obesity, there are some studies that have been done by the group at St. Luke's which suggest a synergistic relationship between food intake and physical activity on thermogenesis.

That is, if you provide a meal and then exercise an individual, a greater fraction of those calories is burned off than if the individual simply eats the calories and is at rest. The effect of the two together is synergistic; that is, the sum of the two is greater than either individually. This might explain some of the phenomenology that was described earlier, i.e. if you eat late in the day when it is less likely that an individual will be exercising to any significant extent, those calories may, in fact, count a bit more than those that are taken in the morning.

James:

There are actually a couple of formal studies showing that if you take two meals a day or seven meals a day isoenergetically, then there is no discernible difference in 24-hours calorimetry and energy expenditure. That does not mean that if people actually exercised later on as part of the design of the calorimetry study, you could not have gotten a 1 or 2% effect which cumulatively could have been important, and we do not know whether, in fact, late eating is associated with a higher energy intake.

Norum:

Leif Hambraeus was talking about the public health aspect of exercise. We are not discussing exercise because I think it is so obvious that we should do more exercise. Exercise does all sorts of valuable things. It lowers blood lipids, it increases insulin sensitivity, it decreases blood pressure, and has many other effects. From a public health point of view, we should try to get the population to do more exercise. There is no reason not to say that.

James:

Do some of the benefits of exercise come from the reflex increase in food intake which then allows the consumption of protective factors in food such as the essential fatty acids you keep emphasizing?

Norum:

Yes, that is probably true.

Hambraeus:

My point was that if we have a high energy turnover, does it really matter what type of fat we eat? I am not sure we know that.

Katan:

That question is easily answered. Exercise may make you leaner and in that way you reduce your coronary heart disease risk, but whether a person has a high or low energy turnover does not influence his or her response to saturated fatty acids and cholesterol. The only way exercise might influence the issue is by reducing the percentage of energy taken. For instance, if you take 20 g of butter in a person with a low activity level, that is a larger percentage of energy than in a person with a high energy turnover. When expressed as a percentage of food intake, saturated fats have the same impact in active and sedentary people. The best example is the Finnish wood-cutters—very highly active people who had the highest mortality from coronary heart disease in the classic Seven Countries Study.

1. Marckmann P, Sandström B, Jespersen J. Effects of total fat content and fatty acid composition in diet on factor VII coagulant activity and blood lipids. *Atherosclerosis* 1990;80:227-33