GENERAL DISCUSSION:

DR. POLLAK: We heard a lot about the pregnancy status of the cows and their milk this afternoon. We didn't hear much about actual measurements of steroid hormone levels in people after milk intake. Does anyone have any data with respect to that? I think it might be easier to measure the steroids in human serum than it is to measure the steroids in cow milk.

DR. RICH-EDWARDS: I didn't mention this yesterday but we looked at the estradiol levels after the one week on/one week off - milk in the Boston pilot study in prepubertal girls as well as before and after the month of milk introduction to the Mongolian children and we didn't see any change in estradiol levels in either study.

DR. BAUMRUCKER: There could be partitioning into the adipose depots. Nutritionists learned a long time ago that a snapshot of blood levels is difficult to interpret with respect to what is happening at the tissue level. That doesn't mean that it's not there somewhere in a compartment.

DR. RICH-EDWARDS: Fair enough.

DR. WILLETT: I think there are limitations in this Boston study. It was really done as a pilot study as Janet (Rich-Edwards) mentioned. Although I think the blood level is germane. We do see that the blood levels are quite predictive in adults, at least, of future breast cancer risk. They may not be exactly representative of what is going on at the tissue level, but it looks like there is some correlation. One of the other limitations in this Boston study was that it was only one week long. Does anybody know how long the exposure needs to be before you reach a steady state? As Janet said, in Boston we had a lot of trouble getting kids to stay off milk for one week.

DR. RICH-EDWARDS: I think the more important issue that I tried to bring out in my presentation is if over time you end up stimulating the somatotropic axis. Is it possible that in a mid to longer-term intervention you wind up provoking endogenous production of the reproductive steroids? And that I think really requires a much longer-term study than the pilot study we did.

DR. DANBY: Time is of the essence with hormones. It takes a long, long time to grow hair in old men's ears, 50 to 70 years of chronic stimulation. I'm not surprised at all that low dose, long term hormonal stimulation is necessary to turn on cancer in the breast or in the prostate. So it really is a matter of long-term efficacy at the receptor and all sorts of hormones may play in. You mention weeks, 5 years may not be enough. We need longitudinal studies.

DR. WILLETT: I think the animal studies by Drs. Sato and Ganmaa (Davasaambuu) and Dr. Clarke were very interesting and important. There is really a fundamental question here that we're struggling with. Are the amounts of hormones in milk sufficient to be biologically important? I will talk a little bit about that tomorrow with some of the epidemiological data. So we'll hear some more pieces of that tomorrow. But that is really a very fundamental question and there are related issues, the amounts of intake, bioavailability, metabolism and excretion, for example. I do not think that those animal models where milk is consumed as the primary liquid in the diet are outside the realm of human experience. Infants do that and a lot of young kids drink milk as their primary fluid, too. So it's not like a ten fold dose beyond the range of human intake. It is in the range of human consumption. The fact that these animal models of milk intake show effects on the uterus and on mammary tumors is pretty interesting, even though we're not exactly sure which hormones are involved. In fact, it's so interesting it seems that it ought to be looked at in quite a few different models and I wonder if anyone else has done that or read about that?

DR. POLLAK: I have a little preliminary data that's relevant to that question. We tried an experiment where we were interested in the possibility that there is some other hormone in milk that is absorbed rather than IGF-I. We share Jeff Holly's view and the view of some others, but not all of us, that the possibility of absorption of IGF-I in adults is low. And yet of all the uncertainties that we've reviewed today, there seems to be very little doubt that there is small but highly reproducible increases in IGF-I among milk drinkers. So if it's not absorbed, what's going on? How come the IGF-I levels go up a little bit? We were considering the possibility that maybe we absorb a growth hormone secretagogue, something that is in the milk that stimulates endogenous IGF-I production. Maybe there is a physiological reason for that in evolution?

As a very preliminary step we cultured pituitary cells in the presence of milk and it is perhaps not surprising but milk does stimulate pituitary growth hormone production. Now since the milk is such a crude mixture of thousands of substances, I'm not reading much into that. It may be a meaningless result. The only thing I'd read into that is that it is perhaps worth further experimentation. We've talked a little bit at this meeting about ghrelin and other milk constituents that are very poorly characterized that may or may not be absorbable. Having heard all the discussions, I think one of the take home messages is how little we know. Yet we can anchor ourselves in certain facts I think there was little controversy about. And one of those facts for which there was little controversy was the more milk you drink the higher your IGF-I levels, not by a lot but reproducibly by a little. I don't think anyone has data to the contrary in adult humans. To my knowledge I think everybody who has looked in a rigorous fashion has found that for contemporaneous milk consumption. I believe the epidemiologic studies have made that observation and there is some preliminary data from the Harvard group in terms of intervention studies that at least provide early data that is consistent with that.

DR. WOLK: In Sweden we have a relatively high consumption of milk and in men between the ages of 45 to 79 we could not see an association between milk consumption and serum IGF-I levels. We could see an association very clearly with protein, with zinc, with red meat and fish protein sources but not with milk.

DR. POLLAK: All right. I've learned something. So even one of the things that I thought we had a bit of certainty about is not quite so certain.

DR. HOPPE: In most of the studies an association has been seen between milk and dairy intake and serum IGF-I levels.

DR. POLLAK: So I guess we revise it to the majority of the studies but not all the studies.

DR. LIU: The question is did those studies also look at insulin's effects or somehow isolate the effects from insulin?

DR. POLLAK: No, I don't think that's been looked at. So there is a lot of undone work.

DR. LIU: For a long time there has been speculation that this is related to insulinotropic effects. Some polypeptides have been shown to enhance the insulin response and some of the proteins in

milk have been shown in animals to be able to induce the effects of insulin.

DR. BAUMRUCKER: One of the things that I'm concerned about is how can these studies rule out the impact of nutrition; it's a confounding effect. Milk has been advertised as this wonderful food and that is what we are seeing, the nutritional effects. So it's confounding so many things. Does somebody have a design that can get rid of that confounding effect so that you can really establish that there is something other than all these "gemisches" (mixtures) in milk?

DR. WILLETT: Yes, that is one of the important questions and it is addressed in some ways in the Danish trial in kids (Hoppe). There was a control group given an equal number of grams of protein from milk or meat and it wasn't quite perfectly controlled but it did suggest that the effect was independent of protein. Also in Dr. Olsen's Danish National Birth Cohort study where the numbers were huge, there was a very distinct difference between protein from milk versus protein from other sources, from meat and other animal and vegetable proteins. It looked like there was a real difference.

DR. BAUMRUCKER: Yes, but that is what I'm arguing. All protein sources are not equal. I don't, at the moment, know how to design an experiment that gets past this but until we do get past that barrier you're going to have arguments about this.

DR. WILLETT: There are different levels. A first hypothesis was that it was protein in general and those studies do address that. Then you get down to specific amino acids or specific fatty acids. As Janet (Rich-Edwards) maybe implied, we have put in a proposal to do a study in Mongolia that would really be macronutrient controlled. Because I think it is desirable to get a firmer answer to that question. There is still a pretty good suggestion that there is a milk specific effect independent of macronutrients, but a bigger, longer-term study is what would need to be done.

DR. RICH-EDWARDS: With better nutrient controls. We're working hard right now to come up with more than just the macronutrient match, but to get as close as we can to the micronutrients. And something several people have pointed out is the absence of the hormones in infant formula. I'm beginning to wonder if maybe we should try to build a milk substitute based on infant formula to as closely match the micro and macro nutrients of cow's milk, but without the hormones. Right now we're working hard on a rice-based substitute but it's really quite a tricky business to match all the amino acids and fatty acids.

DR. WILLETT: You can think of different levels of control. Some of that may be over-matching too. Maybe it is the amino acid mix and that is pretty important to know.

DR. RICH-EDWARDS: My understanding is there is no way you can really strip the hormones and growth factors from milk before disturbing the very nutritional proteins that make it milk. But, does anybody know another way?

DR. BAUMRUCKER: One at a time you might be able to strip them. To take them all out, no. And autoclaving does denature most of the growth factors, I think. That's what I've seen in the literature but again what does that do to the milk proteins or other things? I don't know whether that is valid.

DR. COLLIER: Has anyone ever done a study where lactose is added to water to see if lactose itself, because it is a potent energy source, would drive up insulin?

DR. LIU: There actually have been studies that tested this hypothesis in very limited numbers of subjects that showed that it is not lactose that induces the insulin response, but specific proteins. Caseins and also lipoproteins were responsible in those studies.

DR. COLLIER: So lactose won't invoke an insulin response?

DR. LIU: Yes lactose would, but they control for it. They can separate out the effects. And in those studies it is the proteins. Another thing to consider is the matrix. Drinking milk as a whole would most likely invoke a sequence of physiological responses in the gut. These include displacement or substitution effects, e.g., in delaying gastric emptying or delaying glucose absorption simply because of the fats in the milk. Those effects actually have been found to also have a profound impact simply because glucose is such a sensitive signal to invoke a hormonal response.

DR. ROGERS: I think we can actually, with a certain kind of model, produce milk that doesn't have measurable steroid hormones. For example, we could produce milk for experimental purposes of course, from cows where we removed all the female reproductive organs and some other things. Maybe that would allow you to test this.

DR. RICH-EDWARDS: Maybe for a small study.

DR. ROGERS: Well we can get a lot of milk that way.

DR. POLLAK: Would castrated cows be able to lactate?

DR. ROGERS: Absolutely.

DR. LIU: There's another piece of observational data that I may have time to show tomorrow. We just recently finished a 10-year prospective study looking at the association between milk and diabetes as well as plasma measures of sex steroid hormones. We looked at estradiol, testosterone, SHBG (sex hormone binding globulin) as well as DHEAS (dehydroepiandrosterone). We have some very interesting findings. While endogenous hormones are no doubt markers for increased risk of type 2 diabetes, when we looked at dairy products there was absolutely no association between those hormones in 400 adult women. In fact dairy intake is actually protective for type 2 diabetes and I think it has to do with effects of the glucagon-like peptides on insulin response as well as those displacement effects.

DR. DANBY: My interest is mainly in the 5 alpha-reduced hormones, and progesterone and so forth, but mainly the 5 alpha-reduced sex hormones because they go on to form DHT (dihydrotestosterone) which produces acne. That's my main interest. If you were to give cows a hefty dose of dutasteride, which blocks the 5 alpha-reductase, the cows would no longer produce DHT, 5 alpha-androstenedione, or 5 alpha-pregnandione. That would at least eliminate that portion of the hormones in milk.

DR. MORCOL: Can't you make knock out cows?

DR. ROGERS: You might be able to, but I can't.

DR. THATCHER: I think those Mongolian cows that Dr. Sato showed us are definitely animals that have very low hormones. Also in many Bos indicus animals you have to pre milk with a calf

to get them to let the milk down. Otherwise you can't milk them. That might be the reason they put the calf on first. Also 5 liters per day of milk is a pretty low amount of milk. I agree with Dr. Sato's interpretation that those cows are probably not pregnant, that they were being milked at the time of the year when they were not pregnant. They were in very poor condition and so I'm sure they're like an anestrous animal, which would be a good source of milk for no steroid hormones.

DR. POLLAK: Well it certainly sounds like the difference between the Mongolian milk and the American milk is much bigger than the difference between American milk plus or minus rbST treatment. The putative rbST (recombinant bovine somatotropin) effect seems to be either zero or small whereas Mongolian versus US seems to be rather impressive.

DR. THATCHER: In terms of the product that is produced, I would agree. On the other hand, Japanese Holsteins and North American Holsteins are the same type of cow. I'm still a little perplexed by the tremendous drop in fertility before the School Milk Law was put in place in Japan. That is the biggest change I could see from Dr. Sato's slide and then there is really no change and then a slight decline. I think there are some confounding effects there that one should be concerned about.

DR. POLLAK: Though factually correct, the data do not necessarily reflect cause and effect. Is there a consensus that the steroid content of milk is substantially higher if the milk comes from pregnant cows? We had a lot of discussion about how the dairy practice is changing and whether or not we really are getting a lot of milk from pregnant cows and how that may be changing almost as we speak. But putting that aside for the moment, let's just say we deliberately took milk from a pregnant cow as compared to an American cow that was not pregnant. Is there consensus that the difference in steroid concentration would be large?

DR. THATCHER: The cow is not like a sheep. The sheep has a placental source of progesterone that goes up in I think it's about 70 to 80 days. So you get a placental kick-in of progesterone that the cow doesn't have. The change in cow progesterone over the course of pregnancy, there is some data to suggest it goes up slightly. But if it goes up at all it's gradual and it's only probably about a nanogram or so.

DR. POLLAK: What about all of the estrogens?

DR. THATCHER: My experience measuring estrone, estrone sulfate, is that they'll start to go up at about 90 to 100 days of pregnancy; you'll start to see a rise in estrone sulfate and then it goes up exponentially as you get into the third trimester of pregnancy. As Gary (Rogers) said, the cow is not pregnant for 157 days into lactation so you're basically into 5 months of lactation and then you have another 3 months before it goes up in the blood. Now the Heap data that Dr. Sato refers to are levels in the milk. Heap originally collected that data for two reasons. One is to diagnose pregnancy and the levels are so variable that it is not a good diagnosis of pregnancy. The other is to determine the incidence of twins, twice the placental units so more estrone sulfate. Estrone sulfate definitely goes up, but a lot of the peripheral tissues don't have sulfatase. The mammary gland in the pig I think does. Doesn't it Bob?

DR. COLLIER: Yes it does.

DR. WILLETT: It does in humans, too.

DR. THATCHER: So that is kind of a way to target estrone sulfate to tissues that require estrogen. You need estrogen to grow a mammary gland and you need to be pregnant to be able to grow a mammary gland to initiate lactation to get the milk.

DR. NEVILLE: I don't think you need estrogen necessarily for the pregnancy-induced growth of the mammary gland. You need prolactin and you need progesterone. And progesterone is also more closely related, from the HRT studies, to breast cancer than estrogen. Women can still take unopposed estrogens, if they don't have a uterus. So there's been some mention of progesterone and I'm just wondering what role it's playing? The estrogen seems a little shaky to me but what is going on with progesterone?

DR. POLLAK: I guess what we're asking for from the dairy scientists is can you tell us exactly how the steroid concentrations in milk change as the pregnancy progresses? Leave aside for the moment the controversy of the changing farm management practices of whether we use that milk. On demand, could you give us milk by milking late in pregnancy that would have a lot of estrogen or a lot of progesterone?

DR. ROGERS: We could try. Probably for sure on the estrogen content because you can milk a cow right before they calve.

DR. THATCHER: Progesterone is going to go up at 5 to 7 days after ovulation and it's going to stay up until the cow calves.

DR. COLLIER: I think it's important to remember that the shelf milk that we buy in the store comes from milk that has been pooled from thousands of cows. So it represents the average concentration across sometimes hundreds of farms. So it is a pool of milk from cycling, non-pregnant cows, early pregnant and mid pregnant cows, etc. The shelf milk in California is going to be very similar to the shelf milk in New York, because it represents the average of a large population of cows. You get an average across all of these physiological states. If you buy milk today or a week from today you're going to find the same hormone amounts in terms of estradiol and progesterone.

DR. POLLAK: But is it possible, for the sake of argument, that maybe the steroid content of milk was higher say 15 years ago before all the problems with fertility lengthened the period of time needed to get the cows bred back during lactation? It sounds to me from the dairy industry perspective, that you'd like to get that milk from late pregnancy but because of all the fertility and health problems from genetic selection for high milk production, it's just not a good idea to milk those cows that late in pregnancy.

DR. COLLIER: That question is about the change in techniques over time. We have a much better way of measuring today than we did 15 years ago or earlier. Now we can do a better job of estimating. You can try to hypothesize that hormone levels were higher 15 years ago, but it's very difficult to prove that.

DR. POLLAK: And no one has a milk bank?

DR. RICH-EDWARDS: But what about the Minnesota herd?

DR. THATCHER: If you look at the plasma levels in the Minnesota herd, they show lower progesterone in the selected animals than they do in the control. That's in the plasma. The comment about the stage of pregnancy was really related to the dynamics of estrogen and progesterone, whether the levels are going to be elevated. I would suspect that if you went into any carton of milk anywhere in the United States you'd always find progesterone greater than 1 nanogram per milliliter. It's just a

very short window where cows are cycling, maybe 20% of the cycle when progesterone is low. So you have 80% of the cycle when progesterone is elevated and you've got elevated progesterone during pregnancy. Also my old biology indicated that you needed estrogen to get duct growth in the mammary gland.

DR. NEVILLE: That's for duct growth, but once the ducts are grown, it's not clear to me. There's a fair amount of work from Barbara Vonderhaar's lab that suggests you don't get much added bang with estrogen. We have a lot of ideas from the uterus that estrogen is necessary for progesterone receptor expression, but I'm not sure that data applies to the mammary gland.

DR. COLLIER: We do have some seasonal dairy industries in the world. One of them is New Zealand where virtually all the cows are dried off at the same time and that means that that particular industry would have a slightly different hormonal pattern in milk because all the cows there are synchronized to the same state of pregnancy and lactation.

DR. THATCHER: There, compared to here, you'd have a seasonal period of anestrous when you'd have a high probability of getting low progesterone.

MS. DEMKO: I think I understood from the literature that multi-parous cows have higher levels of IGF-I, and I believe I read that younger cows generate higher levels of steroid hormones. Compared to dairy herds 30 or 50 years ago, today's herds have a higher concentration of younger cows. Has the change in the makeup of the dairy herd in favor of younger cows and fewer lactation cycles per cow affected hormone levels in milk?

DR. THATCHER: The herd turns over about 30% per year. So once the cows initially lactate you're talking about 3 years and then you've essentially regenerated the herd.

DR. ROGERS: I can't speak to how it affects the hormone levels in milk as far as age is concerned but the point Bill (Thatcher) is making is true. On average the cow will have 3 different parturitions. Of course many will have only one and some will have as many as 7 or 8. So the distribution has changed over the past 10 or 15 years due to the reproductive and health aspects related to genetic selection. But we still calve about the same percentage of first parity animals every year because essentially all the females that get born on a farm become replacements. And since we only calve them once every 16 months that doesn't allow us to have more than about 35 per 100 cows per year.

MS. DEMKO: Are the milk levels of steroid hormones higher from the 1st and 2nd lactation cows in comparison to 7th lactation cows?

DR. ROGERS: I don't know.

DR. THATCHER: I'm pretty confident that the 1st lactation progesterone levels would be higher than the 2nd or 3rd just because the amount of milk produced is less in the first lactation and there would be less hormone metabolism, less clearance by the liver. So they'd have a higher level than a multi-parous cow that is producing a lot more milk and has a higher dry matter intake. Their lactation curves are completely different.

DR. DANBY: There is another influence on hormone levels in what we consume that we haven't discussed. The fact of the matter is that fluid milk consumption has dropped over the last 35 years by almost 40%, maybe 50%. At the same time cheese consumption has gone up about 2 1/2 times. Cheese goes through a fermentation process, which seems to add even more hormones to the end product. So we're looking at an increased intake of hormones that to my knowledge has never even been measured. Now if the industry knows of some estimates on the reproductive hormones in cheese I would love to see it. But it's just not been done. It is one of the major defects in our knowledge.

DR. COLLIER: Just add one more variable. 85% of the milk consumed is skim. You're removing progesterone and estradiol when you take the fat out.

DR. ROGERS: No, that would be skim and 2% milk or less. In other words it's not all whole milk and a lot of the data we're seeing on steroid levels is based on whole milk.

DR. DANBY: That's right. The steroid hormones travel in the fat.

DR. NEVILLE: Not estrone sulfate.

DR. DANBY: No, I'm talking about progesterone.

DR. WILLETT: We haven't heard much about androgens and from the standpoint of breast cancer; androgens seem to be about as important as estrogens. If we measure them in blood and then look prospectively, there is about the same relative risk for testosterone and breast cancer as there is for estrogen and breast cancer. As mentioned, it is progesterone that really is greatly accelerating breast carcinogenesis when we take it in the form of HRT. But we also have recent data that taking androgens, which more women are doing now, also really quickly increases the risk of breast cancer. So that is just one more piece we need to keep our eye on here. Michael (Pollak) you're probably going to be talking tomorrow about where we go from here. But it does seem that getting those measurements is important. There is a lab that is very well equipped to do that in Holland. Maybe it would be good to see around the table if people connected with the dairy industry would be willing to pay for a series of analyses that would look at milk from different points in the reproductive cycle for an expanded list of hormones that are measured and in several other forms of dairy products such as cheese and yogurt as well. Just something we might want to be thinking about.

DR. POLLAK: Sure, I think that is the kind of thing that will come out of this meeting, hopefully. Like any scientific meeting we know that we're going to conclude that more studies are needed. The question though is if we can be a little bit more specific and actually act as catalysts to get those extra studies to happen. I can't think of any more obvious or important than the ones that Walter (Willett) just mentioned. To use the most modern analytical techniques to plug some very embarrassing gaps in knowledge.

DR. HAN: If I can just add another comment on the origin of cancer or any other adult disease in infants and children. Either they are affected in utero or immediately after birth during the formative stages. One needs to look not only at the levels in the blood but I think it will be important to look at the tissue susceptibility. Because nutrition will change the levels of different hormone receptors in different tissues you're looking at as well. So it's a matter of not just looking at the changes in levels of IGF or any other growth factors you want to measure in the blood but also looking at the receptors and binding proteins and tissue susceptibility. I think that some of the animal models will be able to address that.