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Discussion

Prof. Henning Beck-Nielsen (Odense, Denmark): I have heard from epidemiologists that 2-hour plasma glucose levels correlate better with diabetic complications than fasting glucose levels. Can you comment on this, Professor Skyler?

Prof. Jay Skyler (Miami, Florida, USA): If you divide by deciles, you find that there are no differences between fasting or 2-hour glucose levels or predominant glycosylated haemoglobin (HbA_{1c}). The curves are almost superimposable for all 3 populations studied. I think that argument is spurious.

Dr Markolf Hanefeld (Dresden, Germany): Are there targets for postprandial plasma glucose?

Prof. Skyler: The American Diabetes Association (ADA) has not widely promoted target levels of postprandial glucose. I'm not sure why that is, but they quote 180 mg/dl, or 10 mmol/L.

Prof. Eveline Eschwège (Villejuif, France): We have found that plasma glucose levels correlating with increased cardiovascular risk in middle-aged French males are in accordance with the indicative levels set by the ADA for increased risk of microvascular complications.

Dr Jörg-Rudolf Finn (Berlin, Germany): What are the recommended levels for fasting venous or capillary glucose levels?

Prof. Skyler: I've only talked about plasma glucose levels, but blood glucose levels are mentioned in the WHO report. I think they're about 15% lower.

Prof. Ralph DeFronzo (San Antonio, Texas, USA): We have looked at about 500 members of Hispanic families, and have found hepatic insulin resistance in some families and muscular insulin resistance in others. We have also identified families with insulin resistance in both the liver and muscle. Obviously, the only way to detect this is through insulin clamp studies. This is also important in genetic studies: there are several types of insulin resistance, and if one is trying to match a phenotype termed simply 'diabetes' or 'insulin resistance' with a specific abnormal segment of DNA, it may be difficult to define this linkage in a statistically significant manner; I think Dr Del Prato made a very cogent point in his presentation.

Prof. Beck-Nielsen: You didn't mention insulin resistance in adipose tissue.

Prof. DeFronzo: That's a separate problem. Last year, at the ADA, we defined a method whereby one could look at insulin resistance in adipose tissue. There is severe insulin resistance in adipose tissue in diabetes, but the problem is that insulin dose-response curves have to be plotted at a very low level because adipose tissue is so sensitive to insulin. Most insulin clamp studies are not sensitive enough to show major differences between individuals in this respect, but the defect in the adipose tissue is linked to the sensitivity of muscle to insulin and the ability of the liver to handle glucose. It's possible to set up the insulin clamp in different ways, depending on what is being examined.

Prof. Beck-Nielsen: So, what type of insulin resistance really matters in the metabolic syndrome?

Dr Stefano Del Prato (Padova, Italy): We still need to examine our hepatic glucose production data to see whether there is a specific relationship between differing degrees of insulin resistance and other abnormalities that make up the insulin resistance syndrome, or syndrome X. My point is that it is possible that there are various pathogenetic mechanisms involved; here we are looking at the phenotype, not specific defects that account for this syndrome.

Prof. Beck-Nielsen: But isn't it the effect of insulin on peripheral glucose uptake that correlates best with the components of the syndrome?

Dr Del Prato: Yes, definitely: I think this underlines the need for a measure of insulin action or sensitivity to insulin in preference to the use of surrogate markers.

Prof. Per Björntorp (Göteborg, Sweden): I have a couple of comments on the definition of the metabolic syndrome. I think the limit of 0.95 for the waist : hip ratio is too low: we set a limit of 1.0 in our work, because that's where there is a marked increase in the risk of diabetes and cardiovascular disease. I'm also reluctant to include microalbuminuria. The strongest association we see is between the waist : hip ratio and insulin resistance, and we find that the full syndrome is very rare. You identified 6% inheritance in terms of the waist : hip ratio, but there are Canadian data derived from CT scans that show considerably higher values. Of course, the measurement techniques are different, and there could also be ethnic differences. My guess is that 6% is too low, but that does leave ample space for an environmental effect.

Prof. Beck-Nielsen: I agree with you that the waist : hip ratio provides a rough estimate. It may be better to do CT scans; we have done this, but only in a small number of patients.

Prof. Leif Groop (Malmö, Sweden): Microalbuminuria was added to identify individuals at a higher risk of cardiovascular disease. That doesn't mean that it has, by itself, to be associated with insulin resistance.

Question: Professor Beck-Nielsen, I don't think the cut-off limits you use are very well balanced. You use 1.7 mmol/L for triglycerides – I think that's fine – but you use 165/95mm Hg for blood pressure. I think that is much too high. If the aim is to prevent coronary heart disease, I think we should reduce this to at least 140/90mm Hg in view of everything we know about albuminuria, kidney disease and cardiovascular disease in type 2 diabetes.

Prof. DeFronzo: I think the blood pressure is ridiculously high: every epidemiological study in the world shows that if you want to die of a heart attack or stroke, those are good levels at which to keep your blood pressure. JNC-V recommends that diastolic blood pressure should be 90mm Hg and systolic in the range of 130 to 140mm Hg. The reason you exclude everybody in that correlation is that nobody meets the blood pressure requirement; I think that if you reduced blood pressures to more reasonable levels, you would find that more people would have all the components of the syndrome.

Prof. Groop: I agree, Professor DeFronzo. I think that, for patients without diabetes, the values are too high; however, in manifest diabetes, even at these levels, about 50% of the patients would have hypertension.

Prof. Hans Häring (Tübingen, Germany): Professor Groop, you mentioned IRS-1 polymorphism. Did you see any differences related to this in insulin secretion?

Prof. Groop: This wasn't our study: I was referring to Olof Pedersen's more recent work. I don't know about insulin secretion. Did you see any difference?

Prof. Häring: Yes, we did. We didn't see any difference in insulin sensitivity, only in secretion.

Prof. Groop: That's interesting. In our work on the β3 mutation, we found differences in insulin secretion and action, so the situation may be more complex than we thought.

Prof. Beck-Nielsen: You surprised me a little when you were talking about stress and low cortisol levels. I always link stress with high levels of cortisol. Can you expand on this?

Prof. Björntorp: Yes, we were also very surprised, but chronic stress develops like this in animal models – you eventually get a flat, low curve with low early morning cortisol levels. It has caused some controversy among researchers, and we will be discussing this further in New York in December. On the other hand, in stress, cortisol levels are usually high: it's only when you have a 'burnt-out' hypothalamopituitary-adrenal (HPA) axis that you see these low levels.

Prof. Groop: I have a problem with the idea of growth hormone deficiency as a cause of metabolic syndrome. It's clear that patients with this deficiency have more intra-abdominal fat; however, there are a lot of differences between growth hormone deficiency and the metabolic syndrome: patients with growth hormone deficiency have low levels of insulin and free fatty acids compared with people with true metabolic syndrome.

Prof. Björntorp: No, they don't. If you give cortisol and steroids without growth hormone to

hypophysectomised individuals, they are actually insulin resistant.

Prof. Groop: Yes, they certainly are insulin resistant, but there are also clear differences: in cases of *isolated* growth hormone deficiency, you should see low insulin and free fatty acid levels.

Prof. Björntorp: I think I see what you mean, Professor Groop. The dogma is that growth hormone confers insulin resistance, and we were very surprised by these data. However, most of the literature on this subject deals with large injected doses of growth hormone; perhaps the situation is different at normal physiological levels.

Prof. Beck-Nielsen: How can you be sure that what you call the stress syndrome, with low cortisol levels and other changes, are not secondary to obesity itself?

Prof. Björntorp: This is not necessarily an obesity-related syndrome. I see it like this: obesity entails increased total body fat, and is caused by appetite and decreased activity, but there is also something else that determines where this excess goes. I think that this other factor is the HPA axis.

Prof. Björntorp: Professor Häring, do you think that leptin receptors in blood cells assist glucose transport?

Prof. Häring: These results were unexpected. C2 and C12 myotubules have very low levels of insulin receptors, and we think therefore that this effect may be related to the muscle cell line that we were using. I don't think that the insulin-like effect is seen in normal skeletal muscle, but it could play a physiological role in muscle types in which the stimulation of glucose transport is required across the entire 24-hour period rather than in the postprandial period only. We are therefore investigating this in tissues such as the Langendorff heart and the diaphragm. Nobody knows whether this effect is seen in erythrocytes: we can only suggest physiological functions that appear to make sense on the basis of studies of mechanisms in cell models. I think that, in a normal insulin-sensitive cell that expresses a large number of insulin receptors, the insulin receptor system dominates and excludes other signalling mechanisms.

Prof. Paul Zimmet (Melbourne, Australia): Some epidemiological data suggest that your *in vitro* and animal findings apply in humans. We have shown an inverse correlation between leptin levels and insulin sensitivity that is independent of obesity, similar results obtained with the euglycaemic clamp technique have been published by Haffner in the *International Journal of Obesity*, and we have another paper in progress with Richard Donahue and Jay Skyler to show the same in an epidemiologically selected group. So, I believe your theory.

Dr Jean-Paul Riou (Lyon, France): We have shown that it is possible to activate the gene for PI3 kinase with insulin in human muscle, although it has recently been demonstrated in Paris that PI3 kinase cannot be activated with insulin in type 2 diabetes. You have provided interesting data on insulin action in muscle, mainly through PI3 kinase. Do you have any data to show insulin resistance in conjunction with a lack of action of leptin?

Prof. Häring: No, we don't have any human data. Ten years ago, I measured insulin receptor kinase activity in skeletal muscle, but I stopped these experiments because it is very difficult to measure PI3 kinase activity in a biopsy sample of human skeletal muscle. I think we need to await the development of laboratory techniques that will give us conclusive answers before we can justify the taking of biopsy samples from patients for this purpose.

Dr Riou: I agree with you; that's why we measure RNA. It is much easier than the enzyme assay.

Prof. Philippe Vague: Professor Zimmet, do you think that the effect of metformin on triglyceride levels in type 2 diabetes is related to the decrease in blood glucose or is specifically directed against synthases of very low density lipoproteins, triglycerides, etc.?

Prof. Zimmet: I suspect it is a liver-mediated mechanism, but Professor Reaven may be in a better position to comment on this.

Prof. Gerald Reaven: It's not completely clear, but there is almost always a decrease in ambient free fatty acid levels, and free fatty acids contribute substantially to the production of very low density lipoprotein triglycerides. It's safe to assume that

any fall in triglyceride levels would be partly secondary to a decrease in lipolysis that is secondary to a decrease in ambient free fatty acid levels.

Question: I haven't followed this field in detail for some years, but the mechanism of action of metformin at the cellular level was not known some years ago. Is it known now?

Prof. Zimmet: I understand that it is not known. Dr Bailey may like to comment on this, as it is his territory.

Dr Clifford J. Bailey (Birmingham, England): Dr Wiernsperger and I will comment on this later in the discussion.

Prof. Vague: You have included hyperleptinaemia in the cluster of abnormalities that make up the insulin resistance syndrome. Is there a direct relationship between leptin and insulin resistance, or is this secondary to excess body mass index (BMI)? Do you adjust for BMI?

Prof. Zimmet: This is like the debate over whether sugar causes diabetes: fifty percent say it does and fifty percent say it doesn't. The studies need to be examined very carefully. We have been working on models that will remove obesity from the equation and can show that it is independent of BMI. Hans Häring's work shows the cross-talk beautifully, and that it can change according to the level of insulinaemia. Two papers presented at the International Obesity Congress showed that leptin directly causes angiogenesis. There is still much to be learned about the peripheral effects of leptin.

Prof. Reaven: There is *in vitro* and *in vivo* evidence that insulin itself enhances messenger RNA for leptin and leptin secretion. The relationships are thus very complicated.

Question: Professor Zimmet, you have shown that polymorphisms relating to obesity are common in Nauruans. Are these and other polymorphisms that could result in a propensity to develop type 2 diabetes more frequent in this population than in others?

Prof. Zimmet: We are currently investigating a number of other populations to see whether polymorphisms (not just those relating to obesity, but others too: there are about 2000) could lead to type 2 diabetes. These data are due to be published in the *International Journal of Obesity* within about 2 months.

Dr Hans Ulrich Iselin (Rheinfelden, Germany): Professor DeFronzo, you demonstrated the effect of metformin on insulin sensitivity in muscle. Do you have any evidence that lean muscle mass might be increased by such an effect?

Prof. DeFronzo: We have examined lean body mass and found that it's not affected when individuals are treated with metformin. On the other hand, we and other investigators have seen a decrease in fat mass. In about 60% of the studies, individuals have a documented weight loss at 6 months of about 7 to 8 pounds. This hasn't been explained to my knowledge, but is not attributable to a decrease in nutrient absorption from the gastrointestinal tract or from a decrease in appetite. I do not know of any studies that have investigated the stimulation of protein metabolism by examining amino acid incorporation into protein. Dr Reaven, of course, has reviewed a large number of studies of effects on lipid metabolism.

Dr Riou: You have clearly shown the acute *in vitro* effect of metformin on GLUT-4, the insulin receptor and PI3 kinase. What is your explanation for the lack of an acute effect of metformin in patients with diabetes? You need a month of treatment to see an effect.

Prof. DeFronzo: Professor Groop has shown an acute effect after giving metformin for 2 days. There are other studies: an Italian group has shown an effect within 24 hours; but you are absolutely correct in that the *maximum* effect is seen after 3 to 4 weeks, which suggests to me that metformin is working on some gene product to change a variety of intracellular processes, which then results in improvements in metabolism. I think that, although these are nice descriptive studies, the basic receptor through which metformin's effects are mediated remains to be clarified.

Question: There are a large number of studies showing increased numbers of insulin receptors and increased binding to these receptors with metformin, but there are also studies that don't show this. Can you comment on these varying findings?

Prof. DeFronzo: Yes, as you say, the studies are variable. Vigneri's Italian group was among the first to show increased insulin binding in erythrocytes and monocytes. We have seen a slight improvement in muscle, but we do not think that this can account for the large improvement in insulin sensitivity, and believe that this is primarily a post-binding defect. On the other hand, the improvement in insulin receptor tyrosine kinase is rather large, and one could argue that this alone could activate the entire cascade of effects of metformin.

Prof. Vague: Dr Wiernsperger, you showed that metformin increases glucose transporter activity through a membrane effect. Do you think that the increase in tyrosine kinase activity is mediated through a membrane effect, or after the intracellular transport of metformin?

Dr Nicolas Wiernsperger (Lyon, France): The effect on tyrosine kinase is present where there is no metformin in the cell. We also have indirect evidence that the site of action is what I would call the 'internal leaflet' of the cell membrane.

Prof. Vague: Did you inject metformin into the oocyte in the *Xenopus* experiments, or did you add it to the medium?

Dr Wiernsperger: All the data I have shown refer to incubation with therapeutic levels of metformin in the cell medium. We also performed experiments (not shown here) in which we injected metformin into the oocyte with no metformin in the medium. We still found an effect, but it was weaker and took longer to become apparent. It is likely that under these conditions metformin is pushed out of the cell and subsequently acts upon the membrane.

Question: In your model involving hyperglycaemia plus insulin plus metformin, with the translocation of glucose transport from microsome to cell membrane, do you think that metformin promotes membrane transport only?

Dr Wiernsperger: No, I think it does both. At therapeutic concentrations, metformin is unable to translocate GLUT-4 without the presence of insulin. Thus, in the presence of insulin *in vivo*, more translocation takes place because of an increased insulin signal. When the transporter is inserted into the membrane, metformin changes the configuration to allow the transportation of more glucose. It's a double effect: one direct effect of the drug and the other through amplification of the action of insulin.

Prof. Reaven: I have what is probably an unfair question for Dr Bailey: we have heard about the many activities of metformin, and I wonder if you can formulate a ranking of the importance of the multiple effects of this agent?

Dr Bailey: If we consider the time period after consumption of a metformin tablet, we first get a high pulse of drug in the portal vein, with a primary effect on the liver. There is rapid uptake by the liver, and I think this is the initial key. About 4 hours after metformin administration, a large amount appears in the muscle, where it remains for a couple of hours before it starts to diminish. Thus, I would think that this is a second level of effect. There is also a large amount of metformin present within the intestinal tissue at all times. There is therefore a sequence in which each of these is important and, thus, if you ask which is most important at the cellular level, I would say that's for Dr Wiernsperger to tell us.

Question: We can see from the general pharmacology of metformin that the dose needed for an effect on insulin resistance appears much lower than that needed for activation of glucose transport or reduction of gluconeogenesis, which shows that very low drug concentrations will suffice to reduce insulin resistance. The liver is involved in the reduction of hyperglycaemia and, as has been shown for gluconeogenesis, relatively high drug concentrations are needed. The liver is the first organ (after the intestine) to be exposed to metformin, and we know from radiolabelling studies that high concentrations are found there. There is a 10-fold difference between the liver and muscle in drug concentrations: the biological effects in each are clearly not the same, but they are both what would be expected in a patient with diabetes.

Prof. Groop: In support of the last statement, in a study we carried out in patients with impaired glucose tolerance we saw a clear improvement in insulin-stimulated glucose uptake with a small dose

of metformin (500mg twice daily), with suppression of lipid oxidation and increased glucose oxidation.

Question: We have been observing a small group of patients with polycystic ovaries plus insulin resistance and a degree of obesity: some with a BMI of 25 to 30 kg/m^2, and a few with one greater than 30 kg/m^2. We have had to stop metformin in 2 of our group of 12 patients because of pregnancy.

Prof. Michel Pugeat (Lyon, France): This supports published findings on the benefit of metformin in anovulation.

Question: I have reviewed the work with metformin in experimental models, and this drug is most effective in corticosteroid-induced insulin resistance in animals.

Prof. Pugeat: This is quite interesting, because we used to treat polycystic ovarian syndrome with dexamethasone. We stopped this because we were inducing insulin resistance and weight gain with no discernible benefit in terms of hyperandrogenism in these patients.

Prof. Reaven: Is insulin resistance and/or hyperinsulinaemia linked to increased 17α-hydroxyprogesterone, or is some other intrinsic ovarian factor needed? Put another way, if you take a series of women with insulin resistance, do they all have these problems or are there some who do and some who don't?

Prof. Pugeat: There are patients with no insulin resistance but with overexpression of this particular hormone. There is some evidence that the level of expression can change, and that these patients do not need insulin resistance or hyperinsulinaemia to be hyperandrogenic and to have polycystic ovarian syndrome. Most patients have insulin resistance, hyperinsulinaemia and some evidence of increased androgen expression and, if the insulin resistance is treated, improvements in the hyperandrogenism can be gained. However, treatment of ovarian androgen production with progestogen only does not alleviate insulin resistance. This has been clearly shown in the literature.

Prof. Alan Garber (Houston, Texas): Professor Taskinen, in one of your slides you showed survival data after acute infarction. Were those corrected for failure to reach the hospital, i.e. for sudden death?

Prof. Marja-Riitta Taskinen (Helsinki, Finland): Yes, they were corrected because there is a high frequency of death before reaching hospital: this is higher for individuals with than without diabetes.

Question: Do you know anything about the effect of metformin on the production or secretion of factors affecting angiogenesis, such as VHEF, FGF and angiostatin? In addition, angiogenesis is important in areas such as menstruation and wound healing, so how would you control undesirable effects of metformin on the beneficial effects of angiogenesis?

Dr Wiernsperger: We have not specifically studied either of these areas. However, the second part of your question does refer to a key question in angiogenesis research: what differentiates the desirable and undesirable effects?

Prof. Taskinen: Can you speculate on the mechanisms underlying the action of metformin on vasomotion?

Dr Wiernsperger: Not really, because nobody knows how these cells work. So-called 'pacemaker' cells are supposed to be involved, which suggests ionic exchange (possibly potassium, or EDHF or the hyperpolarising factor). This is my favoured explanation, but it has yet to be demonstrated.

Dr H. Laube (Giessen, Germany): Professor Yki-Järvinen, can you speculate on why patients on metformin eat less?

Prof. Hannele Yki-Järvinen (Helsinki, Finland): Maybe patients have a bad taste in the mouth, or maybe they have abdominal symptoms; I don't know, but it works.

Dr Del Prato: I think you have demonstrated nicely the advantages of combining metformin with insulin. However, I wonder if we should try to mimic the natural dynamics of insulin secretion better than is possible with 2 daily injections of long-acting insulin.

Prof. Yki-Järvinen: The only way to mimic normal physiology is to use multiple insulin injections. Patients receiving the multiple dose regimen were receiving 3 daily shots of regular insulin and one of long-acting insulin in the evening: that may be theoretically preferable, but it is very difficult

to get patients to adjust their doses correctly with this many injections. They then don't achieve their glycaemic targets, their weight increases, and after a year the doctor withdraws insulin because of disappointing results. We use this bedtime regimen because it is so simple for the patients: they just take a fasting glucose level and adjust the dose in the evening. I certainly admit that, under ideal conditions with ideal insulins and well trained patients, one could aim for near-ideal results with a regimen designed to mimic endogenous insulin. However, in practice this is very difficult.

Dr Agostino Consoli (Francavilla al Mare, Italy): You said that patients on metformin gain less weight because they eat less, which makes perfect sense. But in your previous work, when you treated patients with metformin plus insulin, they gained less weight than patients on metformin plus sulphonylurea.

Prof. Yki-Järvinen: In the previous study, we compared 2 combination regimens: one with bedtime and one with morning long-acting insulin. Both groups were using sulphonylurea plus metformin, but we found that the bedtime insulin group gained less weight than the morning group. I think that this may be because patients are more likely to eat and perhaps even become hypoglycaemic with insulin and a sulphonylurea during the daytime, as with the morning regimen. I can only say on the basis of the surprisingly high frequency of hypoglycaemia that I can no longer recommend the combination of long-acting insulin with sulphonylurea because of the problem with hypoglycaemia. If metformin is added under these circumstances, it doesn't prevent weight gain.

Dr Consoli: Which sulphonylurea were you using?

Prof. Yki-Järvinen: Glibenclamide.

Prof. Taskinen: Can you comment on cardiovascular risk factors?

Prof. Yki-Järvinen: Triglyceride levels decreased from a mean of 2.5 mmol/L to about 1.7 mmol/L, although half the patients still had levels that were too high. However, we didn't see any differences between metformin users and the other patients. Perhaps the study wasn't powerful enough, as insulin itself is a very powerful regulator of triglyceride.

Dr Michel Harter (Nice, France): Professor Lalau, I would like to congratulate you on your presentation, and hope that your work will be disseminated and widely recognised among diabetologists. Just one short question: is the assay of blood levels of metformin very difficult?

Prof. Jean-Daniel Lalau (Amiens, France): Not nowadays. It is a straightforward HPLC method.

Question: I think that there is evidence that gluconeogenesis in the kidney might be more important than we had anticipated. Can you say whether impairment of this mechanism might make patients more prone to acidosis?

Prof. Reaven: Are you saying that this is related to poor lactate removal?

Unidentified speaker: Yes.

Prof. Lalau: I don't think that the kidney is that important in terms of lactate removal; hepatic function is more important in this respect.

Prof. Garber: I think the point of the discussion is that gluconeogenesis by the kidney tends to be favoured under acidotic conditions. Perhaps part of the problem in patients with renal insufficiency is that they don't remove lactate, apart from the effect of metformin itself.

Question: What is the mechanism behind the increase in lactate when one takes metformin (without lactic acidosis)?

Prof. Lalau: I think that metformin counteracts extraction of lactate by the liver, but not to the extent that lactic acidosis is precipitated.

Question: In view of the different and additive effects of sulphonylureas and metformin, can we justify monotherapy from the beginning of treatment or should we advocate combined therapy, perhaps at lower dosages?

Prof. Garber: That's an excellent question, and I think we're going to get the answer in Barcelona. The early use of combination therapy may result in less deterioration in what appears to be pancreatic secretory reserve and stabilisation, whereas monotherapy may not achieve this. I would refer this to Professor Turner and his group of investigators.

Prof. Vague: I'd like to return to the prevention of type 2 diabetes by metformin. If we want to know whether an intervention can prevent diabetes, the best way is to see whether overt diabetes develops more frequently in the treatment group than in the placebo group. In the BIGPRO (Biguanides and the Prevention of the Risk of Obesity) trial, participants were at risk of diabetes and, if I remember correctly, the first analysis showed that diabetes was seen less frequently in the metformin group than in the placebo group. What happened in subsequent analyses?

Dr Aline Charles (Villejuif, France): Five patients in the placebo group developed diabetes, compared with none in the metformin group, but the BIGPRO trial was not designed to investigate the development of diabetes, so this was not checked carefully during the course of the trial. That's why I didn't include these data in the presentation.