References

Adams, S.O.; Kapadia, M.; Mills, B.; Daughaday, W.H. 1984. Release of insulin-like growth factors and binding protein activity into serum-free medium of cultured human fibroblasts. Endocrinology 115 (2):520-526.

Adams, S.O.; Nissley, S.P.; Handwerger, S.; Rechler, M.M. 1983. Developmental patterns of insulin-like growth factor-I and -II synthesis and regulation in rat fibroblasts. Nature 302:150-153.

Ballard, F.J.; Read; L.C.; Francis, G.L.; Bagley, C.J.; Wallace, J.C. 1986. Binding properties and biological potencies of insulin-like growth factors in L6 myoblasts. Biochem. J. 233:223-230.

Birnboim, H.C.; Doly, J. 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. 7 (6):1513-1523.

Bortz, J.D.; Rotwein, P.; DeVol, D.; Bechtel, P.J.; Hansen, V.A.; Hammerman, M.R. 1988. Focal expression of Insulin-like growth factor I in rat kidney collecting duct. J. Cell Biol. 107:811-819.

Daughaday, W.H.; Hall, K.; Raben, M.S.; Salmon, Jr., W.D.; Van den Brande, J.L.; Van Wyk, J.J. 1972. Somatomedin: proposed designation for sulphation factor. Nature 235:107.

Daughaday, W.H.; Yanow, C.E.; Kapadia, M. 1986. Insulin-like growth factors I and II in maternal and fetal guinea pig serum. Endocrinology 119 (2):490-494.

D'Ercole, A.J.; Stiles, A.D.; Underwood, L.E. 1984. Tissue concentrations of somatomedin C: further evidence for multiple sites of synthesis and paracrine or autocrine mechanisms of action. Proc. Natl. Acad. Sci. USA 81:935-939.

Dodson, M.V.; Allen, R.E.; Hossner, K.L. 1985. Ovine somatomedin, multiplication-stimulating activity, and insulin promote skeletal muscle satellite cell proliferation in vitro. Endocrinology 117 (6):2357-2363.

Ewton, D.Z.; Florini, J.R. 1980. Relative effects of the somatomedins, multiplication-stimulating activity, and growth hormone on myoblasts and myotubes in culture. Endocrinology 106 (2):577-583.

Goldberg, A.L. 1967. Work-induced growth of skeletal muscle in normal and hypophysectomized rats. Am. J. Physiol. 213 (5):1193-1198.

Hill, D.J.; Crace, C.J.; Milner, R.D.G. 1985a. Incorporation of [^{3}H] thymidine by isolated fetal myoblasts and fibroblasts in response to human placental lactogen (HPL): possible mediation of HPL action by release of immunoreactive SM-C. J. Cell. Physiol. 125:337-344.

Hill, D.J.; Crace, C.J.; Nissley, S.P.; Morrell, D.; Holder, A.T.; Milner, R.D.G. 1985b. Fetal rat myoblasts release both rat somatomedin-C (SM-C)/insulin-like growth factor I (IGF I) and multiplication-stimulating activity in vitro: partial characterization and biological activity of myoblast-derived SM-C/IGF I. Endocrinology 117 (5):2061-2072.

Isgaard, J.; Nilsson, A.; Vikman, K.; Isaksson, O.G.P. 1989. Growth hormone regulates the level of insulin-like growth factor-I mRNA in rat skeletal muscle. J. Endocrinology 120:107-112.

Jennische, E.; Hansson, H.A. 1987. Regenerating skeletal muscle cells express insulin-like growth factor I. Acta Physiol. Scand. 130:327-332.

Lund, P.K.; Moats-Staats, B.M.; Hynes, M.A.; Simmons, J.G.; Jansen, M.; D'Ercole, A.J.; Van Wyk, J.J. 1986. Somatomedin-C/insulin-like growth factor-I and Insulin-like growth factor-II mRNAs in rat fetal and adult tissues. J. Biol. Chem. 261 (31):14539-14544.

Maniatis, T.; Fritsch, E.F.; Sambrook, J. 1982. Molecular Cloning. A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Moses, A.C.; Nissley, S.P.; Short, P.A.; Rechler, M.M.; Whjite, R.M.; Knight, R.B.; Higa, O.Z. 1980. Increased levels of multiplication-stimulating activity, an insulin-like growth factor, in fetal rat serum. Proc. Natl. Acad, Sci. USA 77 (6):3649-3653.

Murphy, L.J.; Bell, G.I.; Duckworth, M.L.; Friesen, H.G. 1987a. Identification, characterization, and regulation of a rat complementary deoxyribonucleic acid which encodes insulin-like growth factor-I. Endocrinology 121 (2):684-691.

Murphy, L.J.; Bell, G.I.; Friesen, H.G. 1987b. Tissue distribution of insulin-like growth factor I and II messenger ribonucleic acid in the adult rat. Endocrinology 120 (4):1279-1282.

Poggi, C.; Marchand-Brustel, Y.L.; Zapf, J.; Froesch, E.R.; Freychet, P. 1979. Effects and binding of insulin-like growth factor I in the isolated soleus muscle of lean and obese mice: comparison with insulin. Endocrinology 105 (3):723-730.

Riss, T.L.; Novakofski, J.; Bechtel, P.J. 1986. Skeletal muscle hypertrophy in rats having growth hormone-secreting tumor. J. Appl. Physiol. 61 (5):1732-1735.

Rotwein, P.; Burgess, S.K.; Milbrandt, J.D.; Krause, J.E. 1988. Differential expression of insulin-like growth factor genes in rat central nervous system. Proc. Natl. Acad. Sci. USA 85:256-269.

Salmon, Jr., W.D.; Daughaday, W.H. 1957. A hormonally controlled serum factor which stimulates sulfate incorporation by cartilage in vitro. J. Lab. Clin. Med. 49 (6):825-836.

Schoenle, E.; Zapf, J.; Hauri, C.; Steiner, T.; Froesch, E.R. 1985. Comparison of in vivo effects of insulin-like growth factors I and II and of growth hormone in hypophysectomized rats. Acta Endocrinologica 108:167-174.

Shimatsu, A.; Rotwein, P. 1987. Mosaic evolution of the insulin-like growth factors. Organization, sequence, and expression of the rat insulin-like growth factor I gene. J. Biol. Chem. 262 (16):7894-7900.

Thomas, P.S. 1983. "Hybridization of denatured RNA transferred or dotted to nitrocellulose paper." In: R. Wu, L. Grossman, and K. Moldave (Eds.), Methods in Enzymology, Vol. 100, Academic Press, Inc., London and New York, pp. 255-266.

Towle, H.C.; Mariash, C.N.; Oppenheimer, J.H., 1980. Changes in the hepatic levels of messenger ribonucleic acid for malic enzyme during induction by thyroid hormone or diet. Biochemistry 19:579-585.

Turner, J.D.; Rotwein, P.; Novakofski, J.; Bechtel, P.J. 1988. Induction of mRNA for IGF-I and -II during growth hormone-stimulated muscle hypertrophy. Am. J. Physiol. 255 (Endocrinol. Metab. 18): E513-E517.

Yu, K.T.; Czech, M.P. 1984. The type I insulin-like growth factor receptor mediates the rapid effects of multiplication-stimulating activity on membrane transport systems in rat soleus muscle. J. Biol. Chem. 259 (5):3090-3095.

Zorzano, A.; James, D.E.; Ruderman, N.B.; Pilch, P.F. 1988. Insulin-like growth factor I binding and receptor kinase in red and white muscle. FEBS Lett. 234 (2):257-262.

Discussion

S. Mills: Relating to Terry's talk, we all recognize the effects of growth hormone (GH) on adipose tissue metabolism, specifically as it relates to the inhibition of lipogenic activity and the effect of the treatment to prevent adipose tissue accretion. Terry, I think you present some very compelling evidence that there are some direct actions, but I think that there is probably just as equally strong evidence to suggest (perhaps) the opposite. There is a model which suggests that if you feed animals inappropriately, (i.e., not enough protein of energy) that you may not get the same prevention of lipid accretion in the pST-treated animal. So my question obviously is, is the direct effect of pST on adipose tissue sufficient to explain the decrease in adipose accretion? Perhaps to carry it further and more importantly, if you

could employ a mechanism to *enhance* feed intake, what would you predict then to be the fat accretion in an animal at this point that has increased energy consumption?

T. Etherton: Scott, there are several lines of evidence that would support our contention that the effects are predominantly due to GH. If you treat pigs with GH long term, there is a suppression of feed intake that may be 10% to 15% or more (up to 22% in some studies). We have done studies with pigs that have been treated for 7 days that have been fed what I would think would be an adequate diet as far as amino acid content, and in those pigs you can still show the inhibitory effects of GH. That is, using adipose tissue explants, basal rates are lower, they are not insulin responsive.

Secondly, we've taken adipose tissue from pigs and cultured it for 2 days in defined medium and can mimic by and large all the effects that we see in vivo. That is, there is an antagonism of insulin action and GH can essentially abolish the ability of insulin to maintain lipogenic capacity. So in a defined culture medium with adipose tissue of the pig, if you include insulin and hydrocortisone you can maintain lipogenic capacity for up to 2 days, both in the pig and bovine. You put GH in and you inhibit insulin action in a very dose-dependent manner.

Thirdly, Kevin Glynn at Monsanto has shown in some 3T3-like adipocytes in culture that you see essentially a scenario where GH inhibits glucose utilization. He designed some experiments that also showed that GH antagonizes insulin action. So I would say there is enough evidence to clearly say that GH has a very direct effect metabolically, that you can segregate from the effects on feed intake. Where you treat pigs for 30-60 days in vivo and there is some suppression in feed intake, that plays a role, but I don't think it is as important as the biological effects of the hormone.

D. Boyd: I agree. What I thought I would do is deal with the last question that you posed with respect to feed intake and in that regard would use as my base models established by Colin Whitimore and also Roger Campbell with respect to the effect of energy intake on accretion of lipid and protein in the body of growing pigs. I am of the opinion that one can increase protein (accretion) in a linear manner up to some level of energy intake, after which there needs to be an endocrine stimulus or stimulus of some sort that causes a relative change in protein synthesis and degradation to cause any further increase in protein accretion.

On the other hand, as one continues to increase energy intake, you continue to see an increase in lipid accretion so that individuals selected for greater energy intake may in fact gain more but in fact would not accrue lean tissue to any greater extent, and any perceived changes in feed efficiency may be due only to an ability to dilute to some extent the daily maintenance requirement in the pig because of more rapid growth.

Etherton: Scott, there are a couple of other things that just occurred to me. Studies have been done with rats where they have taken normal rats, administered a GH antiserum and neutralized the biological effects of the circulating GH. Then they take adipose tissue and isolate the adipocytes and they can show that if you neutralize GH you get an increase in insulin sensitivity. A clinical parallel is that if you look at obese humans, obese rats, in fact any obese animal I can think of, they are relatively hypo-GH and hyperinsulimic. So a characteristic manifestation, certainly in humans that are obese, is that they have low levels of GH. So I view GH as really being a very important antagonistic or counter-regulatory hormone to insulin. I think if you add all the bits and pieces together, it's a reasonable argument.

Mills: I like your hypothesis very much. Take the scenario that you've fed your animals 10% to 12% protein, now those animals are not going to have, (correct me if I'm wrong) they won't have the same lean tissue gain as if you fed them 18% protein or whatever percent you'd want to. Now, those animals fed 12% protein are probably going to be fatter than the ones fed 18% protein. Now in that situation, given the same GH dose, that's my question directly. Is the decrease in lipogenic activity sufficient to explain all the decrease in fat accretion that occurs?

Etherton: If you look at the problem quantitatively, what we need are some estimates of the massive glucose disappearance into pig fat per day on a kinetic basis, and some estimate of fatty acid turnover. That is, a true in-vivo assessment of lipolysis, and those data don't exist. But I think that Roger Campbell has some data and Dean, I think you can add to this, which suggests that if you feed pigs an amino acid inadequate diet, you can very greatly reduce the effects of GH on protein deposition. You can still show that GH inhibits lipid deposition rates, even on an inadequate diet. So I think Roger's argument is that you can separate the lipid accretion effects on GH from both protein and muscle deposition and still show some metabolic effects independent of the dietary manipulation.

Boyd: I agree again. Roger Campbell has some good data for answering your questions. We have some data as well that indicate that they (protein and lipid accretion effects) are operating independently to some extent. By giving growth hormone to pigs fed different diets, we were able to keep lipid accretion down to a certain level across the board, irrespective of the level of protein. As we fed increments of protein, we were able to observe increased levels in protein accretion, presumably lean tissue accretion, but the fat accretion stayed pretty much constant. However, there were some differences in actual feed intake between the treatment groups. The pigs that tended to accrue more protein tended to eat a little bit more and I know, based on some work of Frank Dunshea's, that as the animal accrues more and more protein that the glucose requirement of that muscle tissue mass increases as well. On a per gram of dry tissue basis, it's about what it is to deposit fat, energetically.

J. Turner: I would like to ask about the importance of serum IGF-I levels. If you look at a variety of animal models in which you increase serum IGF-I, as with growth hormone injections or inducing growth hormone secreting tumors or in creating transgenic animals, we all see a wall of about a 3-fold increase in serum IGF-I concentration. You never seem to get past that. With regard to your comments this morning, Terry, I am intrigued by your suggestion that the IGF-I in serum may never actually get to the cells of interest. I would like to know what you think the role of IGF-I in the circulation really is. Certainly you have some good ideas of tissue levels (paracrine/autocrine). What is the significance of the circulatory level of IGF-I?

D. DeVol: I would say that at the serum level, as Terry

clearly pointed out today, there are questions as to whether IGF-I even gets off the binding protein. As far as IGF-I blood levels only going up a fraction of what growth hormone levels do when you administer growth hormone, it's important to realize that you only get a 3 to 4 fold increase in mRNA levels in the liver as a result of growth hormone administration. So it appears to be at *that* level (mRNA modulation) where regulation occurs, leading to lack of parallel or equivalent increases in IGF-I relative to growth hormone levels. Response to growth hormone at the tissue level in muscle and other non-hepatic tissues also reflects a muted or smaller increase than the elevation in circulating GH. We see a 2 to 3 fold increase in muscle IGF-I levels due to growth hormone elevation. We have found that you are able to get greater increases in IGF-I mRNA with induced compensatory muscle hypertrophy, for example, than you are by elevating growth hormone levels in the blood. So you seem to get away from that problem you have with growth hormone. This would also suggest that there are different regulatory mechanisms for GH and non-GH dependent IGF-I mRNA modulation in skeletal muscle.

Etherton: A perspective I can share is that I would say that there is some role of endocrine IGF; that is, the stuff floating around in blood. That primarily comes from the literature with hyposectomized rats where people have either infused or injected IGF-I and shown some growth response. The problem is that those rats are IGF binding protein deficient rats, or at least they are deficient in the GH-dependent binding protein. So you have a confounded experiment. If you treat normal rats with exogenous IGF-I, and infuse it, the data are sort of mixed. I am aware that Jim Florini has some data at Syracuse which suggest that you can get some, what I would call, modest growth response. That would argue that circulating IGF (in the blood) might be important, but it is not clear if that IGF is bound to the binding protein or if it is free. It's not clear whether IGF affects the binding protein concentration. What we have done, essentially, is one experiment where we treated pigs daily with IGF-I for about 3 days and gave them different doses of IGF and looked at blood parameters and what happens is the IGF disappears very quickly. We've not done a study where we have infused IGF and the reason is we'd need an inordinate quantity of IGF-I. We guesstimated it once and you're talking about significant gram quantities. So we haven't done that experiment.

L. Thiel: By the name of the IGF-I and other insulin-like growth factors, I was wondering if any of you would like to speculate on what the potential for insulin cross-over and interaction at the IGF receptor is in response to growth hormone treatment.

Etherton: I can't comment about what the cross-over would be. I can tell you that Norm Steele and I did a study about 7 years ago where we treated pigs with exogenous insulin. We did not give them glucose, so we could not give them insulin and maintain glycemia. We never published that study because basically nothing happened other than we made the pigs really hypoglycemic. The other thing that happens in the GH-treated animal is that you get this very marked insulin resistance. So I would argue that whether there is any change in binding of insulin to the IGF receptor or not, that the biological effects of insulin in vivo are really attenuated significantly. Now if you're asking "Are some of the effects of IGF mediated through the insulin receptor?" there have been a lot of studies where people have looked at that and one conclusion has been that IGF-I is a much more potent mitogen than insulin. Insulin is a much more potent metabolic hormone than IGF. But to look at that in-vivo scenario, it really doesn't matter because you're looking at the IGF's. The important effects they play are probably, at least in GH-treated pigs, the mitogenic effects and it's probable that insulin is of less importance than IGF-I.

DeVol: I would just add that in in-vitro experiments with muscle cells, it seems that one really needs pharmacological doses of IGF-I to cross over to the insulin receptor and vice-versa.

Boyd: I'll make one further comment, just as an interesting note. I was reading a review by Rob Baxter that must have come out in 1988, in which human levels of IGF's was discussed. It's kind of impressive how the body has worked to prevent some extremely bad situations from arising, but it appears that the circulating IGF-I concentrations are about 50 to 100 times greater than insulin. If they were not associated with the binding protein or cleared rapidly, then there would be some very detrimental affects because of binding. The levels would be high enough to cause a problem.

J. Novakofski: I would like to ask Terry Etherton about his comment regarding the importance of lipogenesis vs. lipolysis in response to growth hormone administration, and I ask this question from four points of view. First of all, there are the well-known effects of GH enhancing lipolysis in states of nutrient deprivation or nutrient restriction. In fact, animals with restricted nutrient intake and treated with growth hormone become leaner, not fatter. This is just the opposite of what you're hypothesizing. Secondly, experiments with beta-agonists which work through the lipolytic pathway show a marked lack in episodic secretion of growth hormone, suggesting that there is perhaps some role of growth hormone in stimulating lipolysis as well. Thirdly, with regard to your data, which shows GH inhibits lipogenesis, you mention that you don't see any affects on fatty acid levels. In the Illinois data, we see increased free fatty acid concentration in the blood and the only place that they can come from would be through lipolysis if you're inhibiting lipogenesis. Fourth, there are the well-known effects of thyroid hormone on lipolysis which infer that thyroid hormone replaces most of the effects of growth hormone, or many of the effects of growth hormone, in a critical situation. Would you comment on your thoughts about the role of lipolysis in relation to growth hormone repartitioning?

Etherton: There are studies, as you pointed out, that would suggest that blood levels of free fatty acids go up. The definitive experiment will be when somebody does the in-vivo kinetic study to look at that. Dale Bauman has done those studies with dairy cows, and I think what Dale's data indicate are 1) that in cows in early lactation and in negative energy balance, GH is lipolytic; 2) In dairy cows in later lactation that are in positive energy balance, then his interpretation of his data is that GH is not a lipolytic hormone. We have only looked at static concentrations of free fatty acids in the blood, and we have done a number of those measurements. We just haven't seen any very demonstrable trends. But even if there are, the real useful data would come from experiments where people look at turnover of free fatty acids and production rates and clearance rates. I know that Dale and Frank

Dunshea and some other people at Cornell have done those studies, but the data aren't yet out and available.

Boyd: We have actually looked at the NEFA concentration in the blood for several years using different GH preps and it's clear that some of the preps used previously are not pure. The NEFA responses that you see might not be seen if the prep was cleaned up. But after some careful measurements and looking carefully at the temporal pattern of change, I think our conclusion is that in pigs which are in a positive energy balance, there is an increase in the NEFA concentration. This would tend to imply mobilization to some extent. Terry is correct, the very careful kinetic studies need to be done and Frank Dunshea has actually done those at Cornell where he has quantitated in-vivo lipogenesis and looked at glycerol as well as NEFA kinetics. He is just now putting together material on re-esterification and mobilization in the animal using what we consider to be a very pure prep, which has been validated as such, and I can't give you the conclusion, other than to say lipolysis is occurring but we don't have any idea how turnover is affected. That will be reported in the Animal Science summer meetings.

D. Gerard: When you were looking at the message for the beta-actin, was that indicative of the message that is being called for, (the beta-actin) or was that just an indicator of what you were loading onto the gel?

Devol: It is the amount of beta-actin messenger RNA that is being expressed, so it does reflect how much, if you want to look at it that way, is needed during the hypertrophy response. We were just using it as a control because it is constitutively expressed as a given (constant) amount per microgram of total RNA. We were using it simply to show that when I said I added 12.5 micrograms of RNA into a solution hybridization reaction among the different treatments, that is indeed what I added (the amount of total RNA I had). So I was using it, in a sense, as a marker to just control quantities of RNA being put into the assay I was using.

Gerard: Would you expect the message for all structural proteins to also increase with hypertrophy? Maybe the IGF message was transcriptionally controlled by just the factor that was also controlling structural proteins.

DeVol: Well, I would expect first of all, that beta-actin mRNA as an amount or a percent of the total RNA did not increase, whereas IGF-I and IGF-II mRNA did. I would expect that during a rapid hypertrophy like that, you would detect increased levels of transcripts for myosin genes and actin genes, (that is alpha-actin, the muscle-specific actin). The beta-actin is not the muscle-specific actin. People use it in all sorts of tissues and all sorts of models as a control as it is constitutively expressed. It is not modulated by various things which you do to manipulate your model. But to answer the second part of the question, there clearly are other gene products besides IGF-I and IGF-II being modulated during hypertrophy. Muscle-specific proteins and receptors, I'm sure, are being modulated during this type of response, so I wouldn't suggest that IGF-I and II are the only proteins being modulated. Quite the contrary. However, I do not think that a lot of different genes are being turned on, just by some general regulating factor. I think the control signals are much more tight than that.