Special Considerations in the Design of Trials Involving Children

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There are important differences from adults that must be taken into account in considering children for clinical trials and clinical research. The two most important of these are that children are minors who are not entirely able or qualified to make decisions for themselves. Secondly, we expect that children will grow and develop physically. The latter, therefore, provides tools for the assessment of effects of experimental therapy that are not available in adults. This presentation will consider ethical concerns regarding participation of children in clinical trials, and will address some of the questions that need to be considered before involving children as research subjects. Additionally, I will summarize the value of assessing growth and physical development in determining the effects of experimental therapies. Finally, I will examine the value of measurement of peptides in the insulin-like growth factor (IGF) system in assessing experimental therapies for catabolic conditions.

ETHICAL CONCERNS REGARDING PARTICIPATION OF CHILDREN IN RESEARCH

The interest of the clinical researcher may conflict with the interest of the research subject. Informed consent, therefore, is essential. In the case of children, this informed consent must come from an appropriate, competent adult. The perceived benefit of a clinical trial or research study involving children should exceed the risk, and the benefit/risk ratio should be at least as favorable as established alternative measures. In general the benefit/risk ratio should be higher in children than in adult research subjects. While adults have the prerogative of choosing to participate or not to participate based on certain personal beliefs, it is important that the selection of children for participation in a project meet a communal standard of benefit/risk. In other words, although the prerogative of an adult may be relatively broad for himself, it must be limited in children to fit a standard that is acceptable to the wider community. Assent of the child should be secured, where appropriate.

Several questions need to be considered before involving children as research subjects (Grodin and Gantz 1994). They include the following:

1. Is the use of children in the research study justified? Has the animal research been completed and are the results sufficiently promising to warrant studies in humans? Can the research be done in adults, thereby sparing children from involvement? Has prior research in adults produced a favorable benefit/risk ratio?

2. Is the proposed number of child subjects the fewest needed to draw meaningful conclusions? Conversely, is the study design sufficient to permit meaningful conclusions?

3. Are the proposed techniques the least intrusive possible with regard to both psychological and physical intrusion?

4. Will the research benefit the child directly? If this is not the case, are the manipulations to which the child is to be subjected minimally invasive?

5. Are the methods for obtaining assent clear and fair? Is a simplified, fair presentation made to obtain the child’s assent? Is the parent present during this presentation and able to assist the investigator in interpreting to the child the demands of the studies, the anticipated benefits, and the possible risks?

6. Has every effort been made to have the parent present when the interventions occur? This often greatly reduces the burden of the research intervention upon the child.

7. Is the child with a rare disease at risk for being “over-studied”? Investigators sometimes undertake extensive investigation of children who have relatively rare diseases in order to better understand the pathophysiology of the patient’s disorder. Such investigations may impose an excessive burden on the affected child.

8. Is money or other rewards being provided to the child? It is appropriate to cover the expenses of the parents in enabling a child to participate in a research study. However, significant rewards for parents or for...
The growth spurt in boys is late relative to that of girls and male genital and pubic hair development is nearly complete by the time of the maximal pubertal growth rate.

The assessment of statural growth, weight gain, head circumference (in the first 1–2 years), and onset and rate of pubertal development are among the most sensitive means of evaluating the overall well-being of the child. These indices of growth and development provide net expressions of genetic makeup, adequacy of nutrition and environment, and residual effects of previous disease. Therefore, when a child has a catabolic condition, weight may decline and statural growth may be impaired. With the institution of appropriate therapeutic measures, there may be a resumption of weight gain and the occurrence of “catch up” of statural growth.

MEASUREMENTS OF INSULIN-LIKE GROWTH FACTOR 1 (IGF-I) AND IGF BINDING PROTEINS (IGFBPs) IN THE ASSESSMENT OF CATABOLIC STATES AND THEIR RESPONSE TO THERAPY

IGF-I is a peptide that is structurally related to insulin. (Daughaday and Rotwein 1989, Underwood and Van Wyk 1991). It is one of the principal mediators of statural growth, cellular proliferation and tissue repair. Human IGF-I has 70 amino acid residues and is a single chain peptide of 7.5 kilodaltons (kDa). In contrast to most hormonal peptides, IGF-I is secreted as it is produced. Consequently there are no organs in which it is concentrated. The liver is believed to be the principle source of the circulating hormone, but the highest concentrations are observed in blood. IGF-I is produced by most organs and exerts biological effects on most cell types. The ubiquity of sites of production and action has lead to the concept that this peptide acts by autocrine and paracrine mechanisms, as well as by classical endocrine mechanisms. IGF-I in serum is complexed with high affinity binding proteins (IGFBPs) (Clemmons 1991). There are now at least 6 distinct IGFBPs (IGFBP-1 to 6). Less than 5% of the IGF-I in the circulation is in a free form, and most (greater than 90%) is bound to a 150 kDa complex which consists of IGF-I, IGFBP-3, and an acid-labile protein subunit. This complex is believed to be the principle carrier form of IGFs in serum. The remainder of the IGF in the circulation is bound into smaller, 30–40 kDa, molecular weight complexes which consists of IGFBP-1, 2, 4, etc... IGF-I is bound primarily to the type 1 IGF receptor. This receptor has approximately a 40% homology with the insulin receptor, and is present on virtually all cell types.

IGF-I is produced under the stimulation of growth hormone and nutrients (Fig. 2). In states of growth hormone deficiency, IGF-I is low, and when growth hormone is present in excess, IGF-I is increased. Nutritional status is also a critical modulator of the level of IGF-I and of some of the IGF binding proteins (Thissen et al. 1994, Underwood 1996). It is important to understand the role of nutrition in the regulation of IGF-I and IGFBPs, because intake, and utilization of nutrients is perhaps the most critical determinant of the anabolic/catabolic status of the patient. Fasting decreases serum immunoreactive IGF-I in adult humans over a period of several days. We have observed that 10 days of fasting in minimally overweight subjects was sufficient to decrease serum IGF-I into a range observed for growth hormone deficient patients (from 0.83 units/ml pre-fasting to 0.21 units/ml at the end of fasting) (Fig. 3) (Clemmons et al. 1981). The decrease in serum IGF-I observed during fasting correlated with the decrease in excretion of urinary urea (R = 0.74; P < 0.001) suggesting that serum IGF-I measurements can serve as an indicator of nitrogen loss.
Experiments in human volunteers suggest that both protein and energy are needed to restore IGF-I after fasting. To discriminate between the relative importance of protein intake versus energy intake in the regulation of serum IGF-I, we fasted 5 normal weight subjects for 5 days on 3 occasions and refed 3 diets of different composition in 5 post-fast days (Isley et al. 1983) (Fig. 4). Fasting reduced serum IGF-I to 36% of pre-fast values. Refeeding a normal diet of 35 kcal/kg/day containing 1.35 grams of protein/kg/day raised the IGF-I to nearly 70% of basal pre-fast values by the fifth day. On the other hand, refeeding a protein deficient isocaloric diet (0.43 grams/kg/day) resulted in a 2 day delay in the upward inflection of IGF-I, and increased IGF-I to only 50% of control pre-fast values by the fifth refeeding day. Refeeding a diet deficient in both protein (0.40 grams/kg/day) and in energy (11 kcal/kg/day) caused a further decrease in IGF-I to 17% of control values. We found a highly significant correlation in this study between changes in IGF-I and change in the nitrogen balance during fasting and refeeding (r = 0.90) suggesting that the changes in serum IGF-I reflect changes in protein metabolism. Such results in studies utilizing diet restriction, suggest that IGF-I may be useful for assessing the catabolic state of a given individual. Other studies confirm these results indicating that a minimal requirement of calories in the range of 12 kcal/kg ideal body weight/day is necessary to maintain IGF-I. Below this intake, IGF-I declines or, if it occurs after fasting, will not return to normal. On the other hand an incremental change in IGF-I is seen in states of protein restriction. (Isley et al. 1984).

IGF-I can serve as a useful tool for monitoring an individual’s catabolic state and for assessing the adequacy of nutritional support. We have reported that nutritional rehabilitation for 10-16 days of chronically malnourished patients who are more than 10% below ideal body weight was associated with a prompt and marked increase in serum IGF-I, such that the mean value at day 10 was 2.7 fold higher than the basal value (Clemmons et al. 1985) (Fig. 5). The superiority of serum IGF-I is an index of nutritional repletion, by comparison with other serum proteins was supported by the minimal change in these subjects of transferrin, prealbumin, and reti...
nol-binding protein (<1.5 fold). Each patient also entered positive nitrogen balance during nutritional repletion.

Others have shown that serum IGF-I in malnourished patients is responsive to nutritional support (Donahue and Phillips 1989, Unterman et al. 1985). Donahue and Phillips (1989) observed a 246% increase in IGF-I, (p < 0.001) compared to transferrin (59 ± 16%, p < 0.005) or albumin (9 ± 6%, p > 0.05). Changes in serum IGF-I also correlated with changes in nitrogen balance. It is generally agreed therefore, that in studies involving nutritional repletion and, presumably in studies involving drug trials that diminish the catabolic state, relative changes in IGF-I are more useful than absolute values.

The IGF binding proteins are also tightly regulated by nutrients. (Underwood et al. 1994) (Table 1) Serum concentrations of IGFBP-3 are decreased in chronic malnutrition and in short term restriction of calories in protein. IGFBP-3 seems to be less dependent on protein supply than IGF-I. On the other hand, IGFBP-2 serum concentrations are increased in chronic malnutrition and in short term dietary restriction. IGFBP-2 is exquisitely sensitive to the availability of dietary protein. IGFBP-1 is regulated primarily by glucose and insulin. The value of this peptide rises sharply over a few hours when glucose supply or insulin secretion is diminished.

Along with assessment of IGF-I we have studied the changes in IGFBPs in a group of 22 chronically undernourished children who had sustained the superimposed insult of acute Shigella dysentery. (Kabir et al. 1992, Pucilowska et al. 1993). Assessments were carried out during a period of refeeding. Two groups were studied during 21 days of refeeding. One group received a 150 k/cal/kg/day diet with a normal (6%) protein content. Another group received a high protein diet (15% protein) of equal caloric value. At the end of 21 days of refeeding, the children fed high protein had a better weight gain, greater increase in mid-upper arm circumference and triceps skin fold thickness, and had greater total serum protein and IGF-I values. In the control and protein supplemented groups before treatment, the IGF-I values were 31.9 ± 19.6 ng/dl (1SD) and 24.0 ± 26.3 ng/dl, respectively. After 21 days of refeeding, these values had risen to 128 ± 70 and 295 ± 124, ng/ml respectively (P < 0.01 for controls vs. protein rich diet). We analyzed the GHBP's by ligand blotting and immunoblotting and observed that the IGFBP-3 was almost absent before refeeding, during the catabolic stage. Levels of IGFBP-3, however, increased during ingestion of each of the 2 refeeding diets to values comparable to those of normal American children. (Fig. 6, top panel). Concurrently, IGFBP-2 values which were higher than controls before refeeding declined sharply in children fed the high protein diet. (Fig. 6, bottom panel). The results of this study indicate that the directional changes in IGFBP-3 during nutrient manipulation are similar to those observed for IGF-I. We also observed that there is increased proteolytic activity in serum to bring about the degradation of IGFBP-3. We conclude that the IGFBP-3 in chronically undernourished and acutely insulted, catabolic children contributes significantly to their low serum IGF-I.

The finding of increased proteolytic activity provides at least one mechanism for the decreased IGFBP-3. The study also illustrates the exquisite sensitivity of these binding proteins to

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**TABLE 1**

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<th>IGFBP</th>
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<tr>
<td>IGFBP-3</td>
<td>Decreased in short-term restriction of calories and proteins</td>
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<td>IGFBP-2</td>
<td>Increased in chronic malnutrition</td>
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<td></td>
<td>Increased in short-term diet restriction</td>
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<td>IGFBP-1</td>
<td>Rises sharply when glucose supply and insulin secretion are diminished</td>
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**FIGURE 4** Serum somatomedin-C during periods of fasting and refeeding. Each point represents the mean ± 1 SD for the five study subjects. Normal diets (f); low protein, isocaloric diet (h); low protein, low energy diet (n). From Isley et al. 1983.

**FIGURE 5** Changes in plasma Sm-C/IGF-I and cumulative nitrogen balance in response to nutritional therapy. Nutritional support was instituted following the control day. Total urinary and stool nitrogen losses were obtained for periods of 48 hours and the results are expressed as the mean grams of nitrogen ± SE/24 hours. Six subjects were studies up to 10 days, 5 subjects up to day 12 and 4 subjects up to 16 days. Plasma SM-C/IGF-I was obtained every other day at 0800 and the values are expressed as the mean ± SE. From Clemmons et al. 1985.
nutrient manipulation and, in the case of IGFBP-2 the marked sensitivity to the concentration of protein available to the study subject.

We have made similar observations in another study in which normal children and adults were made mildly catabolic by feeding restricted diets (Smith et al. 1995). Normal adult and child volunteers were subjected to either caloric restriction (½ normal intake) or dietary protein restriction (⅔ normal intake) for 1 week. As expected, IGFBP-I increased quickly with caloric restriction and returned to normal with refeeding. IGFBP-2 concentrations did not change significantly in adults or children during caloric restriction and IGFBP-3 values declined. On the other hand, with protein restriction, IGFBP-I did not change in children or adults. IGFBP-2 however, rose in adults and children within 24 hours (Fig. 7).

The time course of rise in IGFBP-2 during protein restriction was similar to that seen in fasting, suggesting that the change that occurs in IGFBP-2 with fasting may be due to restriction of protein. In other studies in humans, IGFBP-2 has been shown to be elevated in underweight patients with anorexia nervosa (Counts et al. 1992), individuals with chronic protein malnutrition (Pucilowska et al. 1993), and in volunteers fasted for 9 days (Clemmons et al. 1991).

CONCLUSION

Aside from the considerable ethical restraints on the use of children in clinical trials, the fact that children are in a state of life when growth and development are occurring provides the investigator with tools that are not available for the study of adults. Movement toward a disease state in children results not only in weight loss, but in attenuation of statural growth and a slowing of the process of physical maturation. Conversely, improvement related to recovery leads to rapid weight gain, growth rates that are sometimes in excess of normal and a more rapid progression of the overall process of physical development. In both children and adults, measurement of the peptide components of the IGF system provide a means of assessing an individual's state of catabolism/anabolism. Measurement of these peptides is particularly useful in following short term changes in the metabolic state of a given individual. Therefore, the measurement of IGF-I, IGFBP-3, and IGFBP-2 provides a means for assessing the overall catabolic state of an individual and for determining in a relatively short interval time, whether therapeutic interventions are useful.

LITERATURE CITED


