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ORIGINAL RESEARCH

Effect of dietary protein on body composition and insulin resistance using a pig model of the child and adolescent

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Abstract

There has been an increase in the incidence of obesity and related metabolic disorders in children and adolescents, so effective dietary and exercise interventions are needed. Over the past decade, there has been growing scientific evidence and public acceptance of the role that dietary protein plays in regulation of satiety, feed intake and obesity-related disorders. Dietary protein appears to suppress food intake and delay the return of hunger more than fats or carbohydrates in a manner not due to energy content alone. Some protein sources, particularly dairy, contain specific peptides or proteins that may elicit direct effects upon satiety. Therefore, the aim of the present study was to investigate the role of level and type of dietary protein using the pig to model the important growth and developmental stages of human life. Increasing dietary protein intake reduced feed intake and fat and weight gain with the response being most pronounced in pigs consuming diets containing soy protein isolate (SPI) rather than whey protein isolate (WPI). However, in female pigs consuming diets rich in SPI from infancy to adolescence, there was a catch-up growth that resulted in increased food intake and weight and fat gain. Insulin sensitivity was negatively related to the rate of fat deposition and was improved in pigs consuming WPI compared with SPI. While high-protein diets may decrease calcium balance and bone strength, it appears that these effects are attenuated by WPI. These findings suggest that high-protein diets may reduce hunger and food intake, thereby reducing fat deposition and improving insulin sensitivity.

Key words: body composition, insulin, protein, resistance.

INTRODUCTION

There has been an increase in the incidence of obesity and related metabolic disorders in children and adolescents with these problems only expected to become worse.[1] In order to combat the problem of childhood obesity and comorbidities, effective paediatric dietary and exercise interventions are essential.[2] Recent interest has focused on the role of dietary protein in weight and appetite control,[3] and in a review of the role of dairy proteins in satiety and weight control, four lines of evidence were presented to support a role for dietary proteins in the regulation of food intake and weight maintenance.[4] First, proteins suppress food intake more than fats or carbohydrates and the extent of this reduction is greater than can be accounted for by their energy content alone. Second, proteins make a stronger contribution to satiety and delay the return of hunger compared with fat and carbohydrates. Third, high-protein diets support the maintenance of lean body mass under circumstances of energy restriction, thereby promoting weight loss primarily as adipose tissue. Finally, protein digestion leads to the stimulation of many physiological and metabolic responses involved in the regulation of feed intake. The role of high protein diets in weight control have been criticised for possible adverse effects on calcium balance and renal function especially those individuals with chronic kidney disease.[5] However, the data on the effects of high-protein diets on calcium metabolism are equivocal, with negative calcium balance and bone loss implicated in some studies,[6,7] but not others.[8–10] Therefore, the following study was conducted to investigate the role of level and type of dietary protein on food intake, body composition, insulin sensitivity and bone density. The rapidly growing pig ranging in age from the neonate to post-puberty was utilised as it is an excellent model of the important growth and developmental stages of human life.[11–14]

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METHODS

Twenty-four female and 24 male cross-bred (Large White × Landrace) pigs were weaned (21 days old) and were randomly allocated to a 2 × 3 factorial design with the respective factors being source of protein whey protein isolate (WPI) (NaturaPro MG2460, MG Nutritionals, Brunswick, Vic.) versus soy protein isolate (SPI) (Profarm 974, ADM, Palm Beach, Qld) or level of dietary protein (1.0, 1.4 and 1.8 × the recommended dietary intake (RDI)). The dietary treatments were designed to be balanced for amino acids and adequate for nutrients and energy with a protein content of 1.0, 1.4 and 1.8 × RDI for pigs over the weaner (3–6 weeks), grower (6–12 weeks) and finisher (12–23 weeks) stages. These phases correspond to infancy, childhood and adolescence in humans.[11–13] The protein contents of the 1.0 × RDI diets were formulated based on requirements to maximise protein deposition,[15] and were 21%, 19% and 16% for the three phases, respectively. Body composition was determined using dual-energy X-ray absorptiometry (DXA) at 21, 63, 105, 126, 147 and 167 days of age.[16] Measurements made by DXA included total tissue mass, lean tissue mass, fat tissue mass, bone mineral content and bone mineral density. All experimental procedures used in this investigation were approved by the Victorian Department of Primary Industries Animal Ethics Committee.

At 21 weeks (147 days) of age, the female pigs were surgically prepared with two indwelling catheters prior to conducting a hyperinsulinaemic/euglycaemic/eulysinaemic clamp.[17–19] On the day of the clamp, pre-infusion blood samples (8 mL) were taken every 15 minutes for one hour prior to beginning the infusion to obtain basal glucose and plasma lysine concentrations. Glucose was measured using portable glucose meters (Glucotrend® Roche Diagnostics Australia Pty. Ltd, Castle Hill, NSW) and lysine was measured using a kinetic reaction.[20] The infusion of insulin (Actrapid® Novo Nordisk Pharmaceuticals, Baulkham Hills, NSW), glucose (50% dextrose (wt/vol), Baxter Healthcare, Old Toongabbie, NSW) and amino acids (10% (wt/vol), Synthamin Intravenous Infusion without electrolytes; Baxter Healthcare) were performed using infusion pumps (LIFECARE 5000 Plum Infusion System, Abbott Laboratories, North Ryde, NSW) and the delivery rate was set at 0.6 mU/kg/minute, 90 mL/hour and 45 mL/hour, respectively. Blood samples (3 mL) were obtained every five minutes for the initial one hour following the initial infusion and rapidly assayed for glucose levels. Glucose and lysine concentrations were analysed upon taking each blood sample throughout the infusion period to ensure that euglycaemia (constant glucose) and eulysinaemia (constant lysine) were maintained. Infusion rates of dextrose and the amino acid mix were altered until blood glucose and plasma lysine concentrations had stabilised (i.e. were clamped). After stabilising glucose and amino acid levels, 8 mL of plasma samples were collected every 15 minutes for one hour for analysis of glucose and lysine. During this period, 3 mL of samples continued to be taken at 7.5-minute intervals to ensure that the euglyacemia and eulysinia were maintained. This procedure was immediately repeated using an insulin dose of 6.0 mU/kg/minute. The clamp was only conducted in females because of the logistics of having so many animals catheterised and was only conducted at one time period because the pigs could only be catheterised once.

Food intake, growth and body composition data for various stages of growth and development were analysed by ANOVA suitable for a 2 × 2 × 3 × 3 factorial design to determine the effects of protein type, sex, protein level and developmental phase with pig as a blocking factor. The plasma data from the hyperinsulinaemic/euglycaemic/eulysinaemic clamps conducted in female pigs were analysed using a residual maximal likelihood model with protein type, protein level and insulin dose as fixed effects. A generalised linear model was used to relate dextrose and amino acid infusion rates during the hyperinsulinaemic/euglycaemic/eulysinaemic clamps to fat deposition over the adolescent phase and to account for the effects of protein type and insulin dose. All data were analysed using Genstat 8th Edition.[21]

RESULTS

Overall, feed intake was higher in pigs consuming diets containing WPI rather than SPI (1733 vs 1546 g/day, $P < 0.001$). There was also a linear reduction in feed intake ($P = 0.009$) with increasing level of dietary protein, this being most pronounced in pigs consuming diets containing SPI. Weight gain was greater in pigs consuming diets containing WPI than SPI (778 vs 711 g/day, $P = 0.007$) over the entire study. There was also a reduction in average weight gain ($P = 0.005$) at the highest level of dietary protein, this being most pronounced in pigs consuming diets containing SPI (756, 758 and 623 g/day and 795, 799 and 750 g/day for pigs consuming 1.0, 1.4 and 1.8 × RDI SPI and WPI diets, respectively). However, there was a significant interaction ($P = 0.018$) between protein type and stage of growth such that pigs fed WPI gained more weight than those fed SPI during infancy and early childhood but not during adolescence, perhaps as a result of 'catch up' growth in those consuming SPI.[22]

Lean tissue gain was greater (456 vs 370 g/day, $P < 0.001$) in males than in females especially during adolescence as indicated by an interaction ($P = 0.06$) between sex and growth phase. There was no main effect of dietary protein type on lean tissue gain ($P = 0.42$), whereas lean tissue gain was greatest at the intermediate dietary protein level (407, 437 and 396 g/day for 1.0, 1.4 and 1.8 × RDI, respectively, $P = 0.038$).

Fat gain was not different ($P = 0.23$) between the sexes and was lower in pigs consuming SPI (219 vs 272 g/day, $P < 0.001$). There was a linear reduction in fat deposition with increasing level of dietary protein (271, 252 and 215 g/day for 1.0, 1.4 and 1.8 × RDI, respectively, $P = 0.001$). However, there were a number of interactions between sex, protein level, protein type and stage of development that occurred because there was a different dose–response to protein type between the sexes during adolescence. Thus, fat

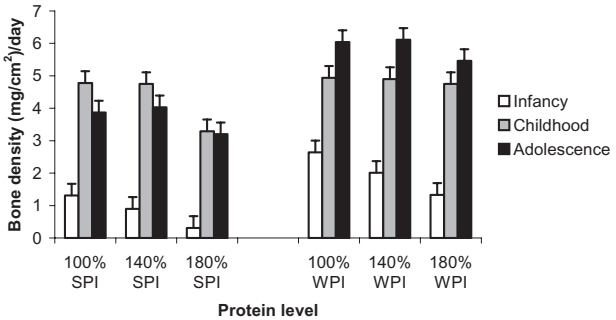

Figure 1 Effect of stage of growth and amount and type of dietary protein on change in bone density.[23]

deposition increased with increasing dietary protein during adolescence in females consuming SPI whereas for WPI there was a decrease in fat deposition. On the other hand, during the other phases of development, there was generally a decrease in fat deposition with increasing level of protein.

Males had higher rates of bone mineral growth than females (22.1 vs 20.5 g/day, $P < 0.001$) while pigs consuming WPI had higher rates of bone mineral deposition growth than those consuming diets containing SPI (23.0 vs 20.5 g/day, $P < 0.001$). Similarly, males had a greater change in bone density than females (3.73 vs 4.32 mg/cm^2/day, $P = 0.009$) and the change in bone density was greater in pigs consuming WPI than those consuming SPI (4.69 vs 3.36 mg/cm^2/day, $P < 0.001$, Figure 1). There was a linear decrease in both the rate of bone mineral deposition growth and change in bone density with increasing dietary protein content, although the interaction between stage of growth and dietary protein type and level meant that this was less pronounced in pigs consuming WPI during middle childhood and adolescence.

The hyperinsulinaemic/euglycaemic/eulysinic clamps were only conducted in females during late adolescence. Dietary protein source had no main effect on the amount of 50% dextrose required to be infused to maintain euglycaemia (168 vs 151 mL/hour, $P = 0.59$), nor was there a main effect of level of dietary protein (157 vs 163 and 158 mL/hour, $P = 0.88$). As expected, the amount of dextrose required to maintain glycemia was higher at the higher dose of insulin (104 vs 215 mL/hour, $P < 0.001$). However, there was an intriguing interaction between dietary protein source and level such that the amount of dextrose required was highest in pigs fed the lowest level of SPI before declining, whereas the converse was true for the pigs fed the WPI. Dietary protein source had no effect on the amount of 10% amino acid solution required to be infused in an effort to maintain plasma lysine concentrations (100 vs 94 mL/hour, $P = 0.69$), nor was there a main effect of level of dietary protein (94 vs 100 and 98 mL/hour, $P = 0.88$). As expected, the amount of amino acids required to maintain basal lysine concentrations was higher at the higher dose of insulin (64 vs 130 mL/hour, $P < 0.001$). However, as for dextrose, there was an interaction between dietary protein source and level such that the amount of amino acids required was highest in pigs fed the lowest level of SPI before declining, whereas the converse was true for the pigs fed the WPI.

At first thought, these data may appear to be somewhat perplexing. However, when the dextrose and amino acid infusion rates were correlated against the rates of fat deposition over the adolescent period, there are strong relationships such that with increasing rate of fat deposition, there was a decrease in the amount of dextrose and amino acid mix to maintain euglycaemia or eulysinaemia, suggesting increased insulin resistance (Figure 2). Also, regardless of the rate of fat deposition, pigs consuming WPI required more dextrose and amino acid mix to maintain euglycaemia or eulysinia, suggesting improved insulin sensitivity.

DISCUSSION

These data show that increasing the protein content of the diet above that required to maximise lean tissue deposition decreases feed intake and fat deposition during childhood and adolescence. However, there were differences between the different protein types with pigs consuming the SPI eating less and depositing less fat than those consuming diets containing WPI, at least during infancy and childhood. However, during adolescence there was greater food intake and fat deposition in pigs consuming diets with high levels of SPI and this was perhaps as a result of compensatory or catch-up growth.[22] There is some evidence that different types of proteins may have differing effects on satiety. For example, anecdotal observations indicate that different types of meat protein affect satiety and weight loss to varying degrees, with beef, lamb and pork being more filling and resulting in greater weight loss than chicken and fish. However, Melanson *et al*. reported similar weight and fat loss in subjects fed beef or chicken meals.[24] Similarly, Uhe *et al*. reported no difference in satiety between beef and chicken, but found that satiety was greater following the consumption of a meal containing fish compared with the beef or chicken meals.[25]

Food intake was suppressed to a greater extent in rats gavaged with whey protein as compared with egg albumen or soy protein.[26] However, others have found that the source of protein had no effect upon food intake suppression. Lang *et al*. compared six different protein sources including casein, gelatin, egg albumen, gluten, soy protein and pea protein, and found no difference in energy intake at the next buffet meal eight hours later.[27] In a subsequent study, these same workers found that appetite was reduced to a greater extent after consumption of a high-protein meal containing gelatin than after a meal containing casein.[27] In a more recent study with individuals on a weight-reducing caloric intake, it was found that there was no difference in chronic feed intake or weight and fat loss in individuals consuming either a high-dairy-protein, high-calcium diet (low-fat cheese and yoghurt as major protein sources) or a mixed-protein, low-calcium diet (lean ham, eggs as major protein sources).[28] While insulin resistance was improved over the duration of the weight loss program, there were no differences between

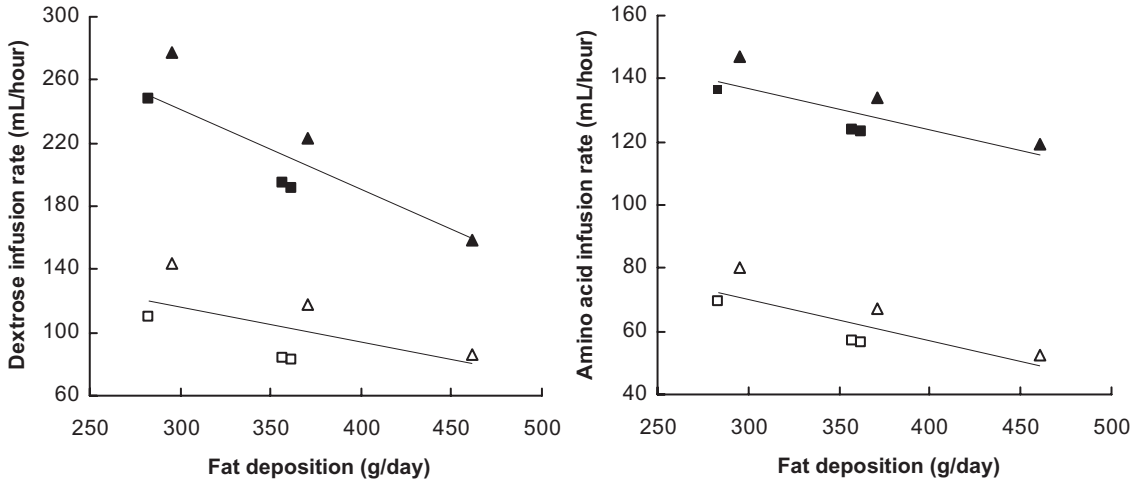

Figure 2 Relationships between dextrose infusion rate or amino acid infusion rate required to maintain euglyaemica and eulysinia, respectively, during infusion of 0.6 (dotted line, hollow symbols) or 6.0 (solid line, filled symbols) mU insulin/kg·minute in adolescent female pigs consuming diets containing WPI (triangles) or SPI (squares). The equations were Y = 451 − 0.72 * X − 242 (Low insulin) + 0.37 * (X * Low insulin) + 38 (WPI), S.E. = 18.7, R^2 = 0.926 and Y = 184 − 0.17 * X − 67 (Low insulin) + 12.7 (WPI), s.e. = 6.67, R^2 = 0.967 for dextrose and amino acid infusion rates, respectively.

the diets in any metabolic parameters normally associated with insulin resistance. Similarly, Bowen *et al*. found that although consumption of a high-protein diet reduced energy intake and plasma gherelin (a stimulator of hunger) and increased plasma glucagon-like peptide-1 and cholecystokinin (inhibitors of hunger) compared with a glucose meal, there was no difference between protein sources (WPI, SPI or gluten).[29] On the other hand, we have shown that high-protein diets, particularly those containing WPI enriched with glycomacropeptide (GMP), decreased feed intake and body and fat weight gain and improved insulin sensitivity in mature obese minipigs.[3] With respect to metabolic parameters of insulin resistance, the source of dietary protein did not appear to be important.

These data show that insulin resistance is related to the rate of fat deposition in adolescent female pigs, even in pigs that were not obese. Also, the regression analysis suggests that insulin sensitivity with respect to both glucose and amino acid metabolism is greater in adolescent pigs consuming WPI as compared with those consuming SPI regardless of the rate of fat deposition. Recently, McIntosh *et al*. demonstrated that supplementing diets containing WPI with GMP reduced weight gain, abdominal fat, plasma insulin and lipid status in growing rats compared with diets containing WPI alone as a protein source.[30] Therefore, there may be a role for high-protein diets enriched with WPI in reducing insulin resistance in adolescents.

The growing pig is considered an excellent model for childhood nutrition and adolescent Type II diabetes. For example, the ontogeny of gastrointestinal tract development during the post-weaning period from three to six weeks in the pig is very similar to what occurs around the introduction of solid food to human infants around six months.[13] Sebert and colleagues have studied the effect of obesity during sexual maturation in miniature pigs to model lipoprotein metabolism and insulin resistance during the progression from childhood to adult.[11,12] These and other studies have clearly indicate that data obtained in young pigs can judiciously be used to model intermediate metabolism in young humans. Also, the young pig has been used to model bone turnover and bone mineral metabolism.[31]

Although the role of high-protein diets in weight loss have been criticised for possible adverse effects on calcium balance, the data on the effects of high-protein diets on calcium metabolism are equivocal. These differences may be in part due to differences in calcium levels and amino acid composition between the experimental treatments. One epidemiological study associated long-term consumption of diets high in animal protein with increased hip fracture rates in an elderly population.[5] These authors attributed this to increased glomerular filtration rate and decreased fractional renal reabsorption which in turn may be mediated by changes in acid load or increased circulating insulin concentrations.[32] However, it is unclear whether this is true for both animal and plant protein sources. For example, it may be that dairy proteins may prevent osteoporosis by providing key nutrients important to bone development and maintenance, by enhancing calcium absorption or retention, by building peak bone mass or by suppressing bone turnover, and therefore bone loss. Bowen *et al*. looked at indices of bone turnover and calcium metabolism in human subjects consuming high-protein, energy-restricted diets formulated around either dairy or mixed proteins.[33] Calcium excretion decreased during both interventions perhaps as a result of the reduction in energy intake. By week 16, the subjects consuming the mixed protein diet had a 40% larger increase in deoxypyridinoline (a bone turnover marker) compared

with those consuming the dairy protein diet. Osteocalcin (a marker of bone formation) increased in subjects consuming the mixed-protein diet only. Overall, the dairy protein conferred a modest advantage over the mixed-protein diet by reducing the accelerated bone turnover associated with weight loss.

The mechanism by which dietary protein intake may strengthen bone is still unclear, but an effect on the structure and function of bone-related proteins is plausible. Differences in protein quality and availability between dairy and vegetable sources related to amino acid distribution or associated dietary constituents with effects on digestibility, absorption and metabolism of amino acids, may underlie the different associations between dairy and vegetable protein intake and bone development.

In conclusion, these data show that increasing the protein content of the diet decreases feed intake and fat deposition during childhood and adolescence. However, there were differences between the different protein types with pigs consuming the SPI eating less and depositing less fat than those consuming diets containing WPI, at least during infancy and childhood. Insulin sensitivity was negatively related to the rate of fat deposition and was improved in pigs consuming WPI compared with SPI. While high-protein diets may decrease calcium balance and bone strength, it appears that these effects are attenuated by WPI. These findings suggest that high-protein diets may reduce hunger and food intake, thereby reducing fat deposition and improving insulin sensitivity.

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CONFLICT OF INTEREST

No conflict of interest has been declared by F.R. Dunshea or M.L. Cox.

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