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SERUM IGFBP-2 LEVELS ARE ASSOCIATED WITH REDUCED INSULIN SENSITIVITY IN OBESE CHILDREN

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Abbreviations: IGFBP-2, Insulin-like growth factor binding protein 2 (IGFBP-2); COBRA, Childhood Overweight Biorepository of Australia; RCH, The Royal Childrens Hospital; OGTT, oral glucose tolerance test; ALT, alanine aminotransferase; GGT, gamma-glutamyl trans peptidase; ALP, alkaline phosphatase; FFQ, food frequency questionnaire; SD, standard

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deviation.

1. 'What is already known about this subject?'

- The GH/IGF-1 axis plays a central role in childhood growth and development.
- IGFBP-2 appears to be associated with insulin sensitivity.
- IGFBP-2 levels are reduced in obesity and Type 2 diabetes.

'What this study adds?'

- IGFBP-2 levels are negatively correlated with age, obesity and fasting plasma insulin levels in children.
- IGFBP-2 associates positively with insulin sensitivity, and negatively with triglycerides and blood pressure in obese children after correction for weight status.
- IGFBP-2 levels in obese children do not appear to be associated with diet or physical activity levels.

2. Abstract

Background: Insulin-like growth factor binding protein 2 (IGFBP-2) may represent a critical link between body composition and insulin sensitivity. We investigated the relationship between circulating IGFBP-2 levels, body composition, insulin sensitivity, energy intake and physical activity in children with obesity.

Methods: Children were recruited via the Weight Management Service at the Royal Children's Hospital, Melbourne, as part of the Childhood Overweight BioRepository of Australia (COBRA). Comprehensive anthropometric, biochemical and environmental data were collected and compared to serum IGFBP-2 levels (measured by ELISA). Multiple regression modelling was used to assess the influence of circulating IGFBP-2 levels on anthropometric and biochemical measures.

Results: 194 children were included in this study (46% male). Circulating IGFBP-2 negatively correlated with age, anthropometric measures, blood pressure and insulin concentration. Positive associations were observed between insulin sensitivity index (ISI-HOMA) and serum IGFBP-2. In multiple regression modelling, IGFBP-2 significantly contributes to variance in systolic blood pressure (-19%, $p < 0.05$), circulating triglycerides (-16%, $p < 0.05$) and ISI-HOMA (18%, $p < 0.05$). No associations were observed between dietary energy intake or physical activity and IGFBP-2 levels.

Conclusions: Circulating IGFBP-2 levels in children with obesity correlate inversely with body mass and markers of metabolic dysfunction, and positively with insulin sensitivity. These findings suggest that reduced levels of IGFBP-2 may play an important role in the pathogenesis of obesity complications in early life.

3. Introduction

In Australia, approximately one third of children are overweight or obese and are at high risk of and metabolic complications including cardiovascular disease and cancers in adult life¹. Similar to insulin, though with less specificity, insulin-like growth factor-1 (IGF-1) has been shown to regulate blood glucose levels through its insulin-like activity on metabolically-active cells. The IGF-axis is essential for key biological functions including growth, development and metabolism. The bioavailability of IGFs are modulated by six high affinity IGF-binding proteins (IGFBPs). Insulin has been shown to regulate IGFBP-1, thus modifying IGF-I levels and preventing hypoglycaemia². As a result, IGFBP-1 has been used as a marker of insulin sensitivity². IGFBP-3 has been demonstrated to inhibit adipocyte differentiation³ and stimulate beta-cell replication *in vitro*⁴. Recently, IGFBP-4 was shown to inhibit adipose tissue expansion⁵. IGFBP-5 is important in postnatal growth regulation and affects pancreatic beta-cell growth and metabolism, while IGFBP-6 levels are increased in patients with type 1 diabetes⁶. However, the strongest involvement of IGFBPs in regulating adiposity and glucose metabolism is seen with IGFBP-2^{7,8}.

IGFBP-2 is the primary circulating IGFBP in infancy⁹ and is produced by numerous tissues including liver, skeletal muscle and white adipocytes¹⁰. IGFBP-2 levels are reduced in obese adults and children and these reductions are more prominent in visceral than subcutaneous adipose tissue¹¹. Studies in relatively small cohorts of children reported lower circulating IGFBP-2 concentrations in children with obesity¹² and that circulating levels of IGFBP-2 were positively associated with insulin sensitivity in prepubertal obese children¹³.

The aim of this study was to comprehensively investigate the relationships between circulating IGFBP-2, anthropometric measures, insulin sensitivity, dietary macro and micronutrient intake and physical activity parameters in a large clinical cohort of overweight and obese Australian children. We hypothesised that decreased IGFBP-2 levels would associate with lower levels of insulin sensitivity, and that IGFBP-2 concentrations would further associate with diet and physical activity parameters.

4. Materials and Methods

Study Design

The study population was recruited via the Weight Management Service at The Royal Children's Hospital (RCH) Melbourne, as part of the 'Childhood Overweight BioRepository of Australia (COBRA)' study. Recruitment and sample collection protocols have been previously described¹⁴. In brief, data collection included anthropometric, clinical, and environmental measurements, collected at the initial clinical appointment and via self-administered questionnaires completed by the child's primary caregiver. A fasting venipuncture sample was collected for analysis of biochemical parameters including serum analysis of IGFBP-2 for this study. The study protocol was approved by the RCH Human Research Ethics Committee, RCH, Melbourne, Australia (HREC Ref. No: 28081).

Assessment of Pre-diabetes, Diabetes and Insulin Sensitivity

Fasting insulin and glucose were obtained in children <10 years old and an oral glucose tolerance test (OGTT) was performed in children ≥10 years old. Serum glucose was quantified by the Beckman Glucose oxidase method using O₂ electrode (Beckman Coulter Pty Ltd, NSW, Australia). Serum insulin was quantified by the Roche Cobas® 6000 analyzer (Roche, Switzerland). A specialist paediatric endocrinologist or a consultant general paediatrician assessed the patient's pubertal stage by Tanner staging. The threshold cut-points utilised for diagnosis of insulin resistance were fasting insulin levels of; >15 mU/l 'pre-pubertal', >30 mU/l 'peri-pubertal' and >20 mU/l 'post-pubertal'¹⁵. 'Pre-diabetes' included both impaired fasting glucose=5.6mmol/L to <7 mmol/L, and impaired glucose tolerance=7.8 mmol/L to <11.1 mmol/L at 2 hour of OGTT¹⁶. Insulin sensitivity index was calculated by homeostasis model assessment (HOMA) [ISI-HOMA= 405/(glucose (milligrams per decilitre) x insulin (microunits per millilitre))]. Insulin resistance was calculated by HOMA-IR= (glucose (milligrams per decilitre) x insulin (microunits per millilitre))/405, and classified according to pubertal status.

Biochemistry, Nutrition and Physical Activity Analysis

Circulating serum alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), triglyceride, cholesterol levels (total, HDL and LDL), calcium and phosphate levels were measured using standard laboratory methods (Ortho Clinical Diagnostics Vitros® 5600, Johnson and Johnson, New Brunswick, NJ, USA and Beckman Coulter UniCel® DxC 800, Beckman Coulter, Brea, CA USA). Serum IGFBP-2 protein was quantified using an enzyme-linked immunosorbent assay (ELISA) in accordance with the manufacturer's instructions (IGFBP-2 DuoSet ELISA DY674, R&D Systems, Inc. Minneapolis, MN, USA, intra-assay CV%; 4.5% and inter-assay CV%; 5.5%).

Nutritional intake was assessed via the self-administered food frequency questionnaire (FFQ, Newcastle Innovation, Newcastle NSW, Australia). Assessment included dietary analysis of proportion of energy derived from; proteins, fats (saturated, monounsaturated and polyunsaturated), carbohydrates, sugars and fibre. Data on micronutrient intake was also collected including vitamins (folate) and minerals (calcium, phosphate and magnesium). Physical activity was assessed by continuous wear of a wrist Actical accelerometer for nine days. This allowed for analysis of; total physical activity, moderate-vigorous physical activity and total sedentary time using accompanying software (Actical, Mini Mitter Co. inc, Bend, OR, USA).

Statistics

Data sets are presented as mean \pm standard deviation (SD). Each variable was tested for normal distribution and variables that had a skewed distribution were log-transformed before analyses. These included data for, serum IGFBP-2, blood pressure, lipids, glucose, insulin, HOMA-IR, and ISI-HOMA. Associations between IGFBP-2 and other variables were calculated by Pearson's correlation coefficients. Means were compared for continuous data using Student's t-test or one-way ANOVA with Tukey's post-hoc analysis. Associations of IGFBP-2 with metabolic risk factors were investigated by multiple regression analyses adjusted for age, sex and with/without BMI. Statistical analyses were performed using SPSS (SPSS version 20.0; SPSS, Inc., Chicago, IL). Values of $p < 0.05$ were considered statistically significant.

5. Results

A total of 194 patients (46% male) were included in this study. As demonstrated by the anthropometric characteristics, this population is characterised as overweight and obese with BMI z-scores ranging from 1.33-4.35 (Table 1). Circulating levels of IGFBP-2 were higher in children with greater insulin sensitivity, and lower in those children with greater body mass, greater waist circumference/ height ratio and higher percentage of total body fat mass and truncal fat mass (Table 1). Additionally, we observed that lower IGFBP-2 concentrations in children are associated with increases in both systolic and diastolic blood pressure (SBP and DBP, Table 1). The mean serum IGFBP-2 concentration in this population was 40.8(\pm 25.7SD ng/ml), and ranged from 11.7-176.6 ng/ml. Serum concentrations of IGFBP-2 were higher in male than female study participants (36.9(\pm 26.5SD) pg/ml v 36.9(\pm 23.0SD) pg/ml; $p=0.023$). Sex specific differences were not observed in other parameters; BMI z-score, total body fat percentage, truncal body fat percentage ($p>0.05$).

Serum IGFBP-2 concentrations were higher in pre-pubertal compared to peri- and post-pubertal subgroups (Figure 1A). Overweight and obese children with insulin resistance had significantly lower serum IGFBP-2 concentrations than their non-insulin resistant peers (Figure 1B). Patients with impaired glucose tolerance also exhibited lower levels of IGFBP-2 than patients with normal glucose tolerance (Figure 1C).

A total of 133 patients completed the food frequency questionnaire. No significant relationships were observed between nutritional macro and micro-nutrient parameters and serum IGFBP-2 concentrations (Supplemental Table 1). A total of 40% ($n=78$) of the study population consented to wearing wrist actical accelerometers and had valid results for analysis. Pearson's correlations were performed to assess the relationship between total physical activity time, physical activity levels and total sedentary time with serum IGFBP-2 concentrations. No significant associations were observed between these parameters with serum IGFBP-2 levels (results not shown).

The influence of circulating IGFBP-2 levels on anthropometric and biochemical factors was then considered via multiple regression modelling. Preliminary analysis ensured that no violation of the assumption of normality, linearity, multicollinearity or homoscedasticity had occurred in the models. Each model was determined to be statistically significant via ANOVA

(Table 2). In the model controlling for age and sex, circulating IGFBP-2 concentrations independently contributed to SBP, DBP, circulating triglyceride concentrations and insulin sensitivity (Table 2). Secondary adjustment of the model to include BMI, age and sex, demonstrated that IGFBP-2 concentrations independently contribute to SBP, circulating triglycerides and insulin sensitivity index (Table 2).

6. Discussion

Decreased circulating IGFBP-2 levels aligned with markers of metabolic dysfunction in our study of a paediatric population with overweight and obesity. Further, our findings support a strong clinical link between IGFBP-2 and insulin sensitivity, as indicated by low circulating IGFBP-2 associating with higher fasting insulin levels and reduced insulin sensitivity. In line with previous reports, we have demonstrated that circulating IGFBP-2 concentrations are significantly reduced in children with increased body mass^{12,13}, but expanded this to include peri and post pubertal adolescents. Furthermore, we have demonstrated that IGFBP-2 levels are not associated with diet or physical activity levels in obese children.

Studies in adults have shown that IGFBP-2 is associated with insulin resistance, as reductions in IGFBP-2 are seen at early stages of insulin resistance development¹⁷. Here we demonstrate that children with insulin resistance or impaired glucose tolerance have significantly lower levels of IGFBP-2. Previous studies have shown that low levels of circulating IGFBP-2 are independently associated with the development of T2DM and the metabolic syndrome¹⁸. IGFBP-2 concentrations observed in the serum of our population were significantly lower than previous studies in both healthy and overweight adolescents, though these populations were not enriched to analyse obesity complications such as insulin resistance and impaired glucose tolerance¹⁹. Another possible explanation is that IGFbps are thought to act synergistically to bind IGFs, therefore levels of these other IGFbps may be compensatory in our population. IGFBP-1 has previously been shown to strongly associate with insulin resistance in children with obesity²⁰. Whilst IGFBP-3 is the major circulating IGFBP and binds up to 75% of circulating IGFs in large complexes, it acts predominantly in a growth-hormone dependent manner and was not related to insulin resistance HOMA in a cross-sectional or longitudinal analysis of overweight youth²⁰. Genetic variations of IGFBP-5 have been linked with metabolic syndrome in studies of adults, but less is known about its potential contribution to metabolic dysfunction in children²¹. We have further demonstrated that serum IGFBP-2 concentrations not only correlate positively with HOMA insulin sensitivity index, and negatively with fasting insulin concentrations and HOMA insulin resistance index, but that IGFBP-2 concentrations independently contribute to insulin sensitivity.

In accordance with previous findings, in this cohort of overweight and obese children, subjects with reduced circulating IGFBP-2 weighed more and had increased total body fat mass¹². *In vitro* studies have shown that IGFBP-2 appears capable of directly inhibiting adipogenesis, whilst *in vivo* preclinical models show that IGFBP-2 overexpression protects against obesity and diabetes²². Recently, our laboratory demonstrated a depot-specific effect of IGFBP-2 inhibiting adipogenesis and lipogenesis in visceral adipocytes, but not subcutaneous adipocytes¹¹, suggesting that IGFBP-2 may have an adipose-specific role in the regulation of body composition. Our findings also indicate an inverse relationship between visceral adiposity in children and circulating IGFBP-2, as indicated by the relationship between increased truncal obesity and decreased IGFBP-2 levels. Additional modulation of the IGF-axis has previously been described in adolescent females, whereby weight loss led to increased levels of IGFBP-2 and improved cardiovascular risk factors²³.

Furthermore, we demonstrate that circulating IGFBP-2 levels decrease with age. This finding is in accordance with previous reports that IGFBP-2 levels declined from infancy to adolescence²⁴. Puberty is associated with a reduction in insulin sensitivity, however this is modulated by a 2-fold increase in insulin production independent to changes in percentage fat mass²⁵. Here, we demonstrated that IGFBP-2 levels varied depending on pubertal stage, with levels of IGFBP-2 higher in pre-pubertal than in peri- and post-pubertal obese children. Others have demonstrated that when insulin resistance was classified according to pubertal stages, IGFBP-2 levels were higher in more insulin sensitive subjects than less insulin sensitive subjects¹⁵. Furthermore, individuals with impaired glucose tolerance had lower levels of IGFBP-2 than those without impaired glucose tolerance. Together, these findings demonstrate that IGFBP-2 negatively correlated with pubertal development, insulin resistance and impaired glucose tolerance.

In accordance with previous findings in rodent models and in human studies, we have demonstrated an association between systolic and diastolic blood pressure and IGFBP-2 levels^{10, 18}. Circulating IGFBP-2 has previously been highlighted for its potential to serve as a biomarker for cardiovascular disease¹⁸. We modelled these findings and additionally demonstrated that decreases in IGFBP-2 circulating concentrations independently attribute to both systolic and diastolic blood pressure. Future development of therapeutics carefully

targeted to the metabolic actions of IGFBP-2, which act to reinstate IGFBP-2 serum concentrations, and not the cellular proliferative actions noted in various carcinomas, in obesity may protect against the onset of hypertension in this susceptible population, as this has previously been demonstrated, albeit in pre-clinical models¹⁰.

Interestingly, serum IGFBP-2 levels in our cohort did not correlate with our analysis of nutrition intake or physical activity. IGFBP-2 was previously found to have minimal relationship with dietary intake compared to other IGF axis proteins IGFBP-1 and IGFBP-3, which are significantly modulated by changes in macro and micronutrient consumption in a similarly obese population²⁶. In a large population study, overall consumption of dairy and calcium was associated with lower concentrations of IGFBP-2, whilst increased dietary monounsaturated fats were associated with increased IGFBP-2 concentrations²⁷. Similarly, in a large animal model, our group previously demonstrated the association between monounsaturated fatty acid ingestion, reduction in IGFBP-2, promotion of visceral adiposity and decline in insulin sensitivity²⁸.

Whilst we observed associations between IGFBP-2 levels, body composition and insulin sensitivity, these observations were not reflected by changes in physical activity levels in our study. Again this may be a reflection of the cross-sectional design of our study which investigated a disease-only population, as others have previously demonstrated the effect of exercise on modulation of IGFBP-2, though not in obese populations. Increases in circulating IGFBP-2 levels are consistently seen in moderate intensity/long duration²⁹ or acute/heavy-resistance training³⁰, in healthy adolescents³¹, adults³² and elderly individuals³³. The effect of training on circulating levels of IGFBP-2 in children, lean or obese, remains unknown.

Our study has some potential limitations. Its cross-sectional design does not allow for us to establish a causal relationship between IGFBP-2 and anthropometric or metabolic measurement. Analysis of the relationship between IGFBP-2 and physical activity was limited, as only 40% of the study population consented to wearing an Actical device, and this may have introduced bias through excluding children that were less active.

In summary, our data demonstrate that circulating IGFBP-2 levels in children and adolescents with obesity inversely correlate with obesity and its metabolic markers, while positively correlating with insulin sensitivity. When adjusted for age, sex and BMI, we show

that circulating IGFBP-2 levels independently influence insulin sensitivity, blood pressure and triglycerides but not fat mass. Furthermore, serum IGFBP-2 levels in our obese paediatric cohort did not correlate with a survey of dietary intake or physical activity, whilst these results require further confirmation in an intervention study, it is possible that circulating IGFBP-2 levels in children with obesity, may be more regulated through hormonal or other intrinsic factors, rather than nutritional changes.

7. Conflicts of interest statement

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

8. Acknowledgements

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Author contributions: SWY analysed IGFBP-2 concentration in serum samples, conducted preliminary analysis of the results and drafted the manuscript. BEH oversaw and conducted analysis of results, drafted and edited the manuscript. KTK collected clinical data and edited the manuscript. EJA recruited patients to the COBRA study and collected clinical data. VCR supervised the analysis of IGFBP-2 and edited the manuscript. GAW supervised the project and edited the manuscript. MAS conceptualised and oversaw the project, collected clinical data and edited the manuscript. All authors approve of the final version of the manuscript.

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10. Figure Legends

Figure 1: (A) Pubertal stage; 'pre-pubertal' (n=78), 'peri-pubertal' (n=42), 'post-pubertal' (n=51), (B) insulin resistance; No (n=99), Yes (n=81) and (C) Impaired Glucose Tolerance; No (n=56), Yes (n=40). Serum IGFBP-2 and fasting insulin were log-transformed for analysis.

11. Tables

Table 1. Anthropometric and biochemical characteristics and correlations between serum IGFBP-2 levels and anthropometric/biochemical measurements.

	Number of patients*	Mean(\pm SD)	r	p value
Sex	194			
(male/female)	90/104			
Age (years)	194	11.0(\pm 3.6)	-0.206	0.004
Height (cm)	194	150.2(\pm 19.3)	-0.216	0.002
Weight (kg)	194	75.5(\pm 31.6)	-0.202	0.005
BMI (kg/m ²)	194	31.8(\pm 7.1)	-0.203	0.004
BMI z-score	194	2.4(\pm 0.4)	-0.055	0.450
Waist circ. (cm)	179	96.2(\pm 26.9)	-0.069	0.371
SBP (mmHG)	179	113.1(\pm 15.9)	-0.201	0.007
DBP (mmHG)	179	66.3(\pm 8.9)	-0.171	0.022
% Total fat mass	168	40.2(\pm 11.8)	-0.171	0.027
% Truncal fat mass	161	34.7(\pm 11.7)	-0.166	0.036
Triglyceride	185	1.2(\pm 0.5)	-0.241	0.003
Cholesterol	189	4.5(\pm 0.7)	-0.100	0.173
HDL	112	1.3(\pm 0.2)	-0.149	0.119
LDL	112	2.7(\pm 0.6)	-0.069	0.474
ALT	189	37.3(\pm 25.7)	0.046	0.529
GGT	189	22.3(\pm 13.9)	-0.061	0.404
ALP	189	212.0(\pm 83.3)	-0.021	0.779
Fasting glucose	190	4.6(\pm 0.5)	-0.046	0.553
Fasting insulin	181	22.5(\pm 17.3)	-0.197	0.008
HOMA-IR	180	4.5(\pm 3.9)	-0.165	0.022
ISI (HOMA)	180	8.0(\pm 17.8)	0.229	0.002

* Not all measurements were available for each study participant.

Abbreviations: ALT, alanine aminotransferase; ALP, alkaline phosphatase; BMI, body mass index; BMI z-score; age sex adjusted BMI (CDC); GGT, gamma-glutamyl transferase; HDL, High density lipoprotein cholesterol; HOMA, homeostasis model of assessment; IGFBP-2, insulin-like growth factor binding protein-2; IR, insulin resistance; ISI, Insulin sensitivity Index; LDL, Low density lipoprotein cholesterol; r, Pearson's correlation coefficient.
Bold values statistically significant, $p < 0.05$

Table 2. Multiple regression analysis for the independent influence of IGFBP-2 on metabolic risk factors after adjusting for **i)** age and sex, **ii)** age, sex and BMI.

	i) Age, Sex		Model ANOVA	ii) Age, Sex, BMI		Model ANOVA
	β	p	F (df: Regression, Residual) p value	β	p	F (df: Regression, Residual) p value
Waist Circ.	0.009	0.837	3.15 (3,166) 0.027	0.085	0.413	2.88 (4, 165) 0.024
SBP	-0.225	0.011	6.59 (3,175) <0.001	-0.194	0.032	5.30 (4,174) <0.001
DBP	-0.163	0.044	5.72 (3,175) 0.001	-0.149	0.069	4.61 (4,174) 0.001
% Total fat mass	-0.129	0.133	4.15 (3,163) 0.007	-0.099	0.326	3.187 (4,162) 0.015
% Truncal fat mass	-0.118	0.203	3.97 (3,156) 0.009	-0.135	0.088	3.05 (4,155) 0.019
Triglycerides	-0.170	0.020	5.99 (3,181) 0.001	-0.162	0.028	4.74 (4,180) 0.001
Glucose	-0.026	0.722	4.82 (3,186) 0.003	-0.030	0.642	3.99 (4,185) 0.004
Insulin	-0.093	0.311	4.33 (3,177) 0.006	-0.113	0.210	3.66 (4,176) 0.007
HOMA IR	-0.086	0.291	4.36 (3,176) 0.006	0.048	0.408	4.11 (4,188) 0.003
ISI (HOMA)	0.182	0.014	6.52 (3,176) <0.001	0.180	0.015	5.27 (4,175) <0.001

Abbreviations: IGFBP-2, insulin-like growth factor binding protein-2; Waist circ., waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA, homeostasis model of assessment; IR, insulin resistance; ISI, Insulin sensitivity Index;

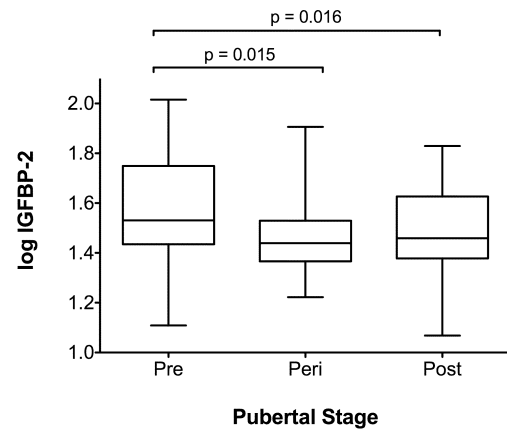
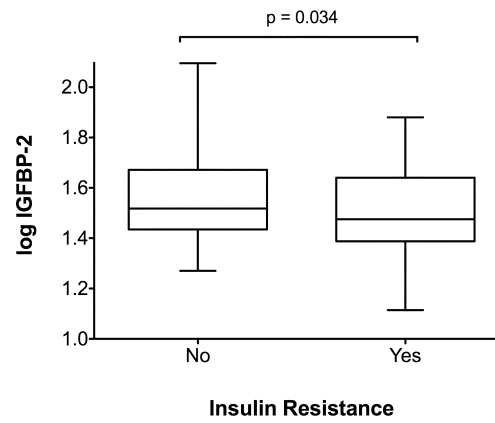
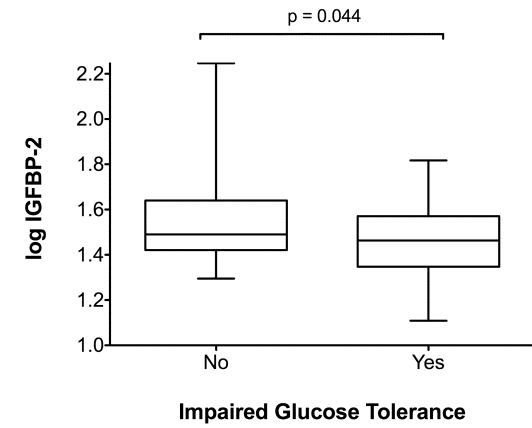
Supplementary Table 1. Correlations between serum IGFBP-2 levels and dietary energy intake.

	Number of patients	r	p value
Energy	133	0.016	0.854
Protein	133	0.001	0.992
Fats	133	0.038	0.663
Saturated Fats	133	0.084	0.335
Monounsaturated Fats	133	0.011	0.896
Polyunsaturated Fats	133	-0.054	0.540
Cholesterol	133	-0.021	0.809
Carbohydrate	133	0.011	0.896
Sugars	133	0.021	0.811
Water	133	0.017	0.842
Fibre	133	-0.078	0.370
Thiamine	133	-0.016	0.857
Riboflavin	133	0.011	0.896
Niacin	133	-0.026	0.764
Folate	133	-0.078	0.370
Vitamin A	133	-0.094	0.280
Vitamin C	133	-0.077	0.380
Retinol	133	-0.033	0.709
Beta Carotene	133	-0.124	0.155
Sodium	133	-0.002	0.978
Potassium	133	-0.014	0.871
Magnesium	133	-0.010	0.908
Calcium	133	0.077	0.378
Phosphorus	133	0.025	0.772
Iron	133	-0.098	0.260
Zinc	133	-0.055	0.530

Abbreviations: IGFBP-2, insulin-like growth factor binding protein-2; r, Pearson's correlation coefficient.

12. Figures

Figure 1

A**B****C**

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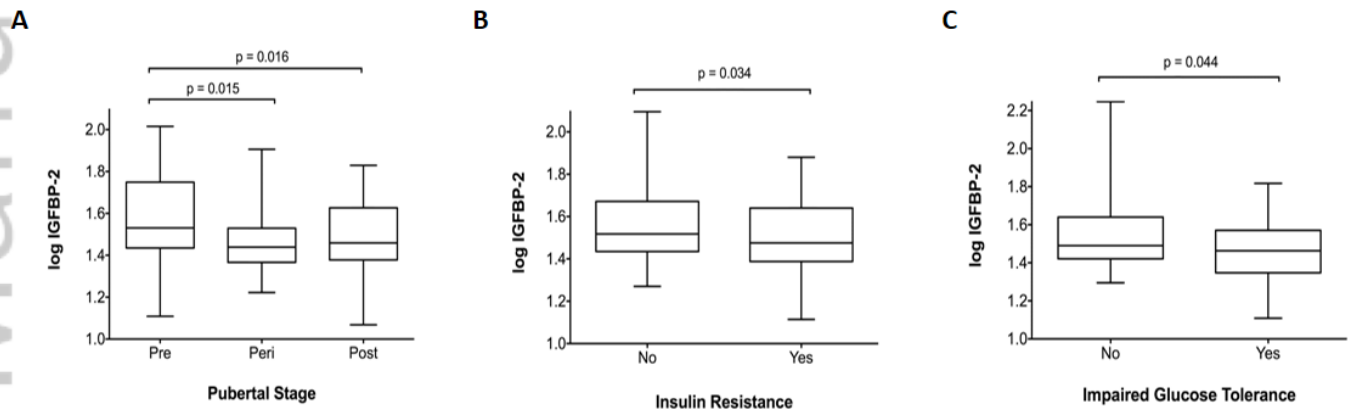


Figure1.tif

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