Article

Profiling of Insulin-like growth factor binding proteins (IGFBPs) in obesity and their association with Ox-LDL and Hs-CRP in adolescents

Abdur Rahman [1,#], Maha M Hammad [2,#], Irina Al Khairi [2], Preethi Cherian [2], Reem Al-Sabah [3], Fahd Al-Mulla [4], Mohamed Abu-Farha [2,*] and Jehad Abubaker [2*]

[1] Department of Food Science and Nutrition, College of Life Sciences, Kuwait University, Kuwait
[2] Biochemistry and Molecular Biology Department, Dasman Diabetes Institute, Kuwait
[3] Department of Community Medicine and Behavioural Sciences, Faculty of Medicine, Kuwait University, Kuwait
[4] Genetics and Bioinformatics Department, Dasman Diabetes Institute, Kuwait
#   These two authors contributed equally.
*   Correspondence: jehad.abubakr@dasmaninstitute.org (J.A.); mohamed.abufarha@dasmaninstitute.org (M.A.-F.); Tel.: +965 2224 2999 Ext. 3563 (J.A.); +965 2224 2999 Ext. 3010 (M.A.-F.)

Abstract: Insulin-like growth factor binding proteins (IGFBPs) are critical modulators of the metabolism. In adults, IGFBPs are associated with obesity and insulin resistance but the association of IGFBPs with metabolic homeostasis in children and adolescents is not fully characterized. In this study we investigated the association of plasma IGFBPs (IGFBP-1, 3 and 7) with weight status, central adiposity and cardiovascular disease markers Hs-CRP and Ox-LDL. A total of 420 adolescents (age 11-14 years) were randomly recruited from public middle schools in Kuwait. IGFBPs were measured using bead-based multiplexing while Hs-CRP and Ox-LDL were measured using ELISA. IGFBP-1 levels were significantly lower in obese and overweight participants compared to normal weight children. Only IGFBP-1 was negatively associated with waist circumference to height (WC/Ht) ratio. IGFBP-1 was negatively correlated with Hs-CRP while IGFBP-3 and IGFBP-7 were negatively correlated with Ox-LDL. These data demonstrate a robust negative association of IGFBP-1, but not IGFBP-3 or -7, with overweight and obesity, and the inflammation marker Hs-CRP. Central adiposity (WC/Ht ratio) was a stronger predictor of IGFBP-1 than BMI-for-age z-score. IGFBP-1 could thus be used as a sensitive predictive diagnostic tool for obesity and its subsequent effects in screening and monitoring of obesity-related metabolic complications in adolescents.

Keywords: Adolescents; high sensitivity C-reactive protein; Insulin-like growth factor binding proteins; Obesity; Oxidized Low-Density Lipoprotein; Predictive diagnostics

1. Introduction

Childhood obesity is a public health pandemic, and the Arabian Gulf region is one of the areas with the highest reported rates of childhood obesity [1]. Kuwait in particular has been reporting very alarming rates, with surveillance data showing 45% of school children to be overweight or obese [2,3]. Many factors are causing this epidemic but lifestyle choices including poor dietary habits and lack of physical activity are among the major contributing factors as well as genetic background [4]. Children and adolescents with obesity are likely to become obese adults. Obesity is associated with many complications including dyslipidemia, hypertension, heart failure, and atherosclerotic cardiovascular diseases [5-7]. Because of the increased health care cost to deal with these chronic diseases and the poor quality of life, tackling obesity in early life should be a public health priority.

The need for a shift from reactive to predictive, preventive and personalized medicine (PPPM) is eminent [8,9]. While precision/ personalized medicine still has limited applications due to the many challenges it faces, tremendous efforts are dedicated for its advancements. One of these is screening for novel and easy-to-measure molecular biomarkers. The metabolic syndrome and its related pathologies, especially obesity, are in the center of this shift for being strong economic burden on the healthcare systems [10]. Identifying diagnostic biomarkers that could be used in detecting metabolic complications associated with obesity is important for risk stratification and intervention program monitoring and evaluation The individual variability in genes, environment and lifestyle, which are key contributors to the obesity epidemic, are taken into account when a precision medicine approach is used [8].

Similar to insulin, insulin-like growth factors (IGFs) regulate diverse physiological functions related to growth, development and, in particular, glucose homeostasis through common signaling pathways [11]. The transport, metabolism and signaling by the IGFs are modulated by a family of binding proteins which is comprised of six IGF binding proteins (IGFBP-1 – 6) as well as the IGFBP-related protein (IGFBP-rP1, designated as IGFBP-7) [11-13]. Some of the IGFBPs specifically bind to their own receptors or translocate to various cellular compartments to mediate IGF-independent actions [13]. Different tissues produce different IGFBPs and their functions vary according to the metabolic conditions surrounding them [14]. IGFBPs have been implicated in the development and pathogenesis of obesity and its related comorbidities like diabetes, metabolic syndrome and cardiovascular diseases (CVDs) through IGF-dependent as well as IGF-independent roles [11,12].

The association of IGFBPs with obesity, metabolic syndrome and diabetes has been the subject of many investigations. Of these, IGFBP-1 has been consistently shown to be inversely associated with overweight and obesity [15-17], plasma insulin and glucose levels [18,19], as well as fasting plasma leptin levels [20]. Low serum IGFBP-1 levels have been reported to be predictive of the development of diabetes [21-23]. The association of IGFBP-1 with metabolic homeostasis has been consistent across gender, different age groups and across various ethnicities [20,24].

IGFBP-3 is the most abundant among all IGFBPs and transports more than 90% of IGF-I and IGF-II in circulation as a ternary complex with IGFs [11]. However, its association with weight status, metabolic syndrome and glucose homeostasis has not been consistent across studies. Some studies reported increased levels of IGFBP-3 in overweight and obese subjects [15], while others reported no association with the weight status [25]. In a prospective case-control study conducted on female nurses, a positive correlation of IGFBP-3 with the development of diabetes, BMI and waist circumference was reported [26].

IGFBP-7 is the most recent addition to the IGFBPs family. It has a similar amino acid sequence and structure to other human IGFBPs and can specifically bind to IGF-I and IGF-II. It is the least studied member of the IGFBP family in the context of its association with metabolic homeostasis. The available literature suggests that its serum levels are positively associated with BMI [27] and type 2 diabetes [28], fasting glucose levels [29], insulin resistance and metabolic syndrome [27,30]. A recent study reported higher levels of IGFBP-7 in coronary artery disease (CAD) patients compared to healthy subjects [31]. In addition, IGFBP-3 was reported to be negatively associated with the levels of some inflammatory markers including C-reactive protein and interleukin-6 [32], whereas high levels of serum IGFBP-7 were associated with increased CRP levels [27].

The aim of this study was thus to investigate the association of IGFBP-1, 3 and 7 with weight status, waist circumference and well-established CVD risk factors, specifically Hs-CRP and Oxidized Low-Density Lipoprotein (Ox-LDL) in a group of healthy adolescents from Kuwait. Such data is essential for establishing the predictive utility of these biomarkers as early diagnostics, as well as tools in targeted prevention of metabolic disease programs.

2. Results

2.1. Study population characteristics

Table 1 summarizes the characteristics of the participants that were involved in the study. Data were analyzed for 420 participants of whom 192 (45.7%) were male. Mean (SD) age was 12.41 (1.53) years. Of the total sample, 47% adolescents were normal weight, 21% were overweight and 32% were obese. Median (IQR) for IGFBP-1 was 5.7 (3.5, 10.0) ng/mL, while median (IQR) for IGFBP-3 and IGFBP-7 were 835.0 (688.5, 1007.8) and 17.7 (15.2, 20.0) ng/mL, respectively. Female adolescents had significantly lower levels of IGFBP-1 compared to males (p<0.001) (Figure 1A), whereas the differences in plasma levels of IGFBP-3 and IGFBP-7 between male and female subjects were non-significant (data not shown). IGFBP-1 levels were significantly higher in the 10-<12 year age group compared to 12-<13 years (p<0.01) and 13+ years group (p<0.001) (Figure 1B), whereas, there were no significant differences among different age groups in IGFBP-3 and IGFBP-7 levels (data not shown).

Table 1. Characteristics of the study population.

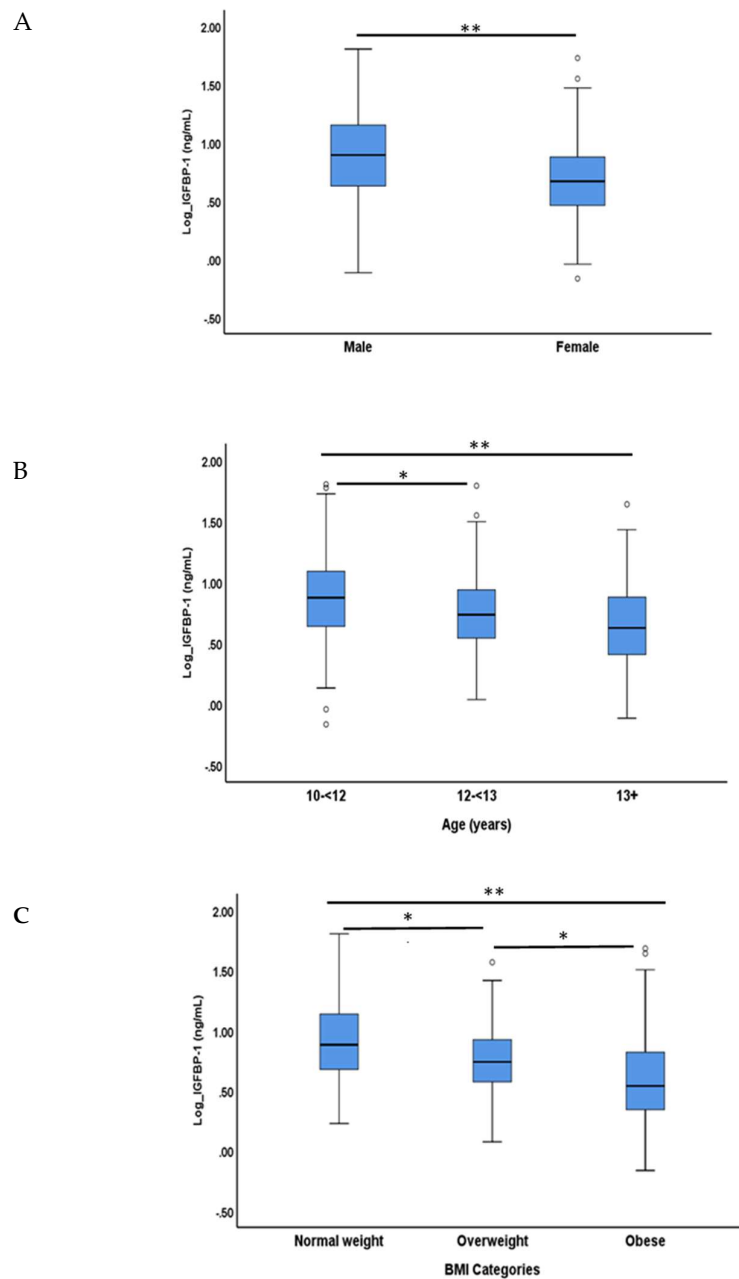|		N	%-age
Gender	Male	192	45.7
	Female	228	54.3
Nationality	Kuwaiti	303	71.93
	Non-Kuwaiti	117	28.07
Age groups	10 - <12 years	179	42.5
	12 - <13 years	143	34.1
	13+ years	98	23.4
Weight Status	Normal weight	198	47.1
	Overweight	88	21.0
	Obese	134	31.9

Figure 1. Differences in IGFBP-1 levels based on (A) sex, (B) age and (C) weight status.

N=191 male, 228 female; t=6.18, p<0.001; N=178 (10-<12), 143 (12-<13) and 98 (13+); N= 198 normal weight, 88 overweight, 135 obese. Males and females were compared by t-test for independent samples, whereas, age groups and weight status groups were compared with one way ANOVA. *, p<0.01; **, p<0.001.

2.2 Association of IGFBPs and weight status

IGFBP-1 levels were significantly lower in obese and overweight participants compared to normal weight children (p<0.01) (Figure 1C). On the other hand, no significant differences were observed in the levels of IGFBP-3 and IGFBP-7 in different BMI categories (data not shown). Parallel to these results, IGFBP-1 was negatively associated with weight status (BMI categories) in univariable regression analysis [β (95% CI) = -0.16 (-0.20, -0.13)], and this association remained significant in the multivariable regression when adjusted for age and sex [β (95% CI) = -0.17 (-0.20, -0.13)]. The association of weight status with either IGFBP-3 or IGFBP-7 was not significant either in multivariable regression, or in the adjusted multivariable regression (Table 2). In binary logistic regression, the odds of being overweight/obese (combined category) were significantly higher in the lower tertile of IGFBP-1 compared to the upper tertile (reference) [(OR (95% CI) = 6.82 (2.29, 20.30)] in the unadjusted model and this association sustained in the model adjusted for age and sex [(OR (95% CI) = 7.22 (3.92, 13.32)] (Table 3). IGFBP-3 and IGFBP-7 tertiles did not show significant association with overweight/obesity in binary logistic regression models, either unadjusted or adjusted.

Table 2. Linear regression showing association of IGF binding proteins with weight status in adolescents.

	β	95% CI	P-value
Univariable			
IGFBP-1 (log)	-0.16	-0.20, -0.13	<0.001
IGFBP-3	-0.18	-35.40, 36.04	0.99
IGFPB-7	0.02	-0.47, 0.51	0.93
Multivariable*			
IGFBP-1 (log)	-0.17	-0.20, -0.13	<0.001
IGFBP-3	-0.18	-35.53, 35,18	0.78
IGFPB-7	0.02	-0.47, 0.50	0.78

* Adjusted for age categories and sex. IGF binding protein (IGFBP) were used as continuous variable (independent variable) and weight status (dependent variable) was categorized as normal weight, overweight or obese based on the WHO cutoffs of the BMI z-scores. N= 419 (IGFBP-1), 332 (IGFBP-3) and 428 (IGFBP-7). IGFBP-1 was log-transformed for normality.

Table 3. Odds of overweight/obesity in different tertiles of IGF binding proteins in adolescents.

	Unadjusted		Adjusted*	
	OR	95% CI	OR	95% CI
IGFBP-1				
Lower Tertile	6.82	2.29, 20.30	7.22	3.92, 13.32
Middle Tertile	1.79	0.51, 6.26	1.53	0.83, 2.79
Upper Tertile	1.00	Ref	1.00	Ref
IGFBP-3				
Lower Tertile	1.56	0.58, 4.18	1.57	0.58, 4.22
Middle Tertile	0.82	0.27, 2.51	0.83	0.27, 2.57
Upper Tertile	1.00	Ref	1.00	Ref
IGFBP-7				
Lower Tertile	0.81	0.34, 1.94	0.80	0.33, 1.94
Middle Tertile	0.96	0.42, 2.22	0.98	0.42, 2.27
Upper Tertile	1.00	Ref	1.00	Ref

* Adjusted for age categories and sex. Odds ratios were calculated using binary logistic regression in which the response variable (weight status) was categorized into normal weight or overweight/obese, with the normal weight as the reference category.

2.3 Association of IGFBPs with waist circumference

In the univariable regression analysis, IGFBP-1 was negatively associated with WC/Ht ratio [β (95% CI) = -1.72 (-2.11, -1.33); p<0.001], and this association remained significant after adjusting for age and sex [β (95% CI) = -1.72 (-2.07, -1.37), p<0.001]. IGFBP-3 and IGFBP-7 did not show significant association with WC/Ht ratio either in the univariable or multivariable regression (Table 4). In the binary logistic regression the odds of obesogenic waist, defined as a WC/Ht ratio of >0.5, was significantly higher in the lower tertile of IGFBP-1 compared to the higher tertile [OR (95% CI) = 4.55 (2.74, 7.56)] in the unadjusted model and this remained significant in the model adjusted for age and sex [OR (95% CI) = 5.70 (3.26, 9.96)]. IGFBP-3 and IGFBP-7 tertiles did not show significant association with obesogenic waist in binary logistic regression models, either unadjusted or adjusted (Table 5). When adjusted for age and sex, the negative association of IGFBP-1 (log-transformed) with WC/Ht ratio [β (95% CI)= -1.73 (-2.08, -1.38)] was stronger than its association with the BMI-for-age z-scores [β (95% CI)= -0.11 (-0.13, -0.09)].

Table 4. Linear regression showing association of IGF binding proteins with waist-to-height ratio in adolescents.

	β	95% CI	P-value
Univariable			
IGFBP-1 (log)	-1.72	-2.11, -1.33	<0.001
IGFBP-3	86.13	-301.12, 473.37	0.66
IGFPB-7	1.24	-4.03, 6.50	0.65
Multivariable*			
IGFBP-1 (log)	-1.72	-2.07, -1.37	<0.001
IGFBP-3	89.21	-299.35, 477.78	0.65
IGFPB-7	1.19	-4.08, 6.47	0.66

* Adjusted for age categories and sex. IGF binding protein (IGFBP) and WC/Ht ratio were used as continuous variables N= 419 (IGFBP-1), 332 (IGFBP-3) and 428 (IGFBP-7). IGFBP-1 was log-transformed for normality.

Table 5. Odds of Obesogenic waist in different tertiles of IGF binding proteins in adolescents.

	Unadjusted		Adjusted*	
	OR	95% CI	OR	95% CI
IGFBP-1				
Lower Tertile	4.55	2.74, 7.56	5.70	3.26, 9.96
Middle Tertile	1.26	0.78, 2.03	1.44	0.87, 2.39
Upper Tertile	1.00	Ref	1.00	Ref
IGFBP-3				
Lower Tertile	1.05	0.62, 1.80	1.06	0.62, 1.80
Middle Tertile	1.06	0.62, 1.81	1.05	0.62, 1.80
Upper Tertile	1.00	Ref	1.00	Ref
IGFBP-7				
Lower Tertile	0.77	0.48, 1.23	0.77	0.48, 1.24
Middle Tertile	1.10	0.69, 1.76	1.11	0.69, 1.77
Upper Tertile	1.00	Ref	1.00	Ref

* Adjusted for age categories and sex. Odds ratios were calculated using binary logistic regression in which the response variable (WC/Ht ratio) was categorized into non-obesogenic waist (WC/Ht ratio ≤0.5 and obesogenic waist (WC/Ht ratio >0.5) , with the normal non-obesogenic waist as the reference category.

2.4 Association of IGFBPs with markers of oxidative stress

In linear regression analysis, Ox-LDL was not associated with IGFBP-1 ($\beta=0.06$; $p=0.40$), whereas, it was negatively associated with IGFBP-3 ($\beta=-0.18$; $p=0.001$) and IGFBP-7 ($\beta=-0.02$; $p<0.001$) (Figure 2, A-C). On the other hand, Hs-CRP was negatively associated with IGFBP-1 ($\beta=-0.30$; $p=0.001$), but no significant association of Hs-CRP was found with either IGFBP-3 or IGFBP-7 (Figure 2, D-F). We further analyzed mean differences of Ox-LDL and Hs-CRP across different tertiles of the IGFBPs. The results are shown in Figure 3. The levels of Ox-LDL were significantly higher in the lower teritles of IGFBP-3 and IGFBP-7 compared to the middle and higher tertiles, whereas no differences were observed between Ox-LDL levels in the middle and upper tertiles (Figure 3, B & C). Levels of Ox-LDL across the three tertiles of IGFBP-1 were not significantly different (Figure 3A). On the other hand, levels of Hs-CRP across the three tertiles of IGFBP-3 and IGFBP-7 were not significantly different (Figure 3E, 3F), whereas, it was significantly higher in the lower tertile of IGFBP-1 compared to the middle and upper tertiles. Levels of Hs-CRP were not different between the middle and upper tertiles of IGFBP-1 (Figure 3D).
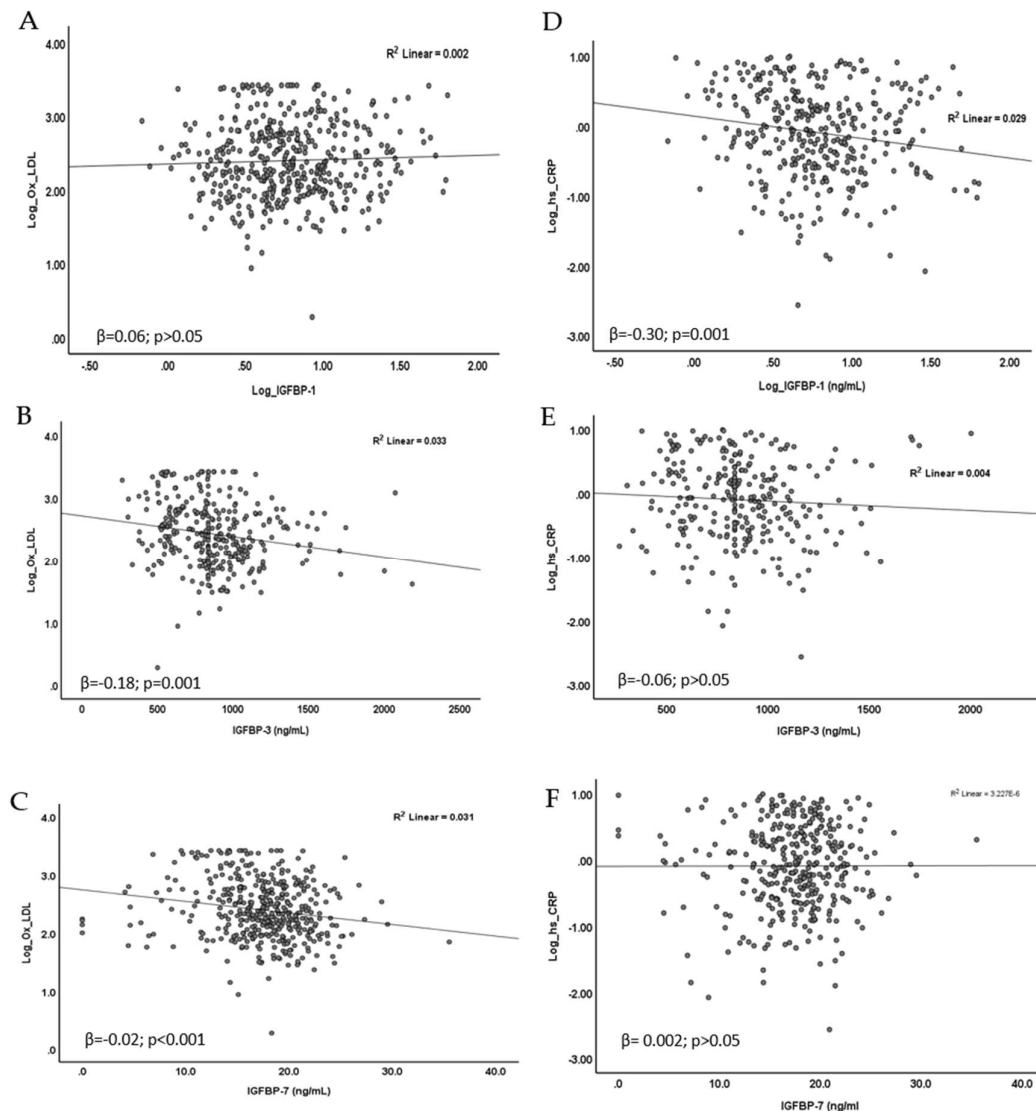

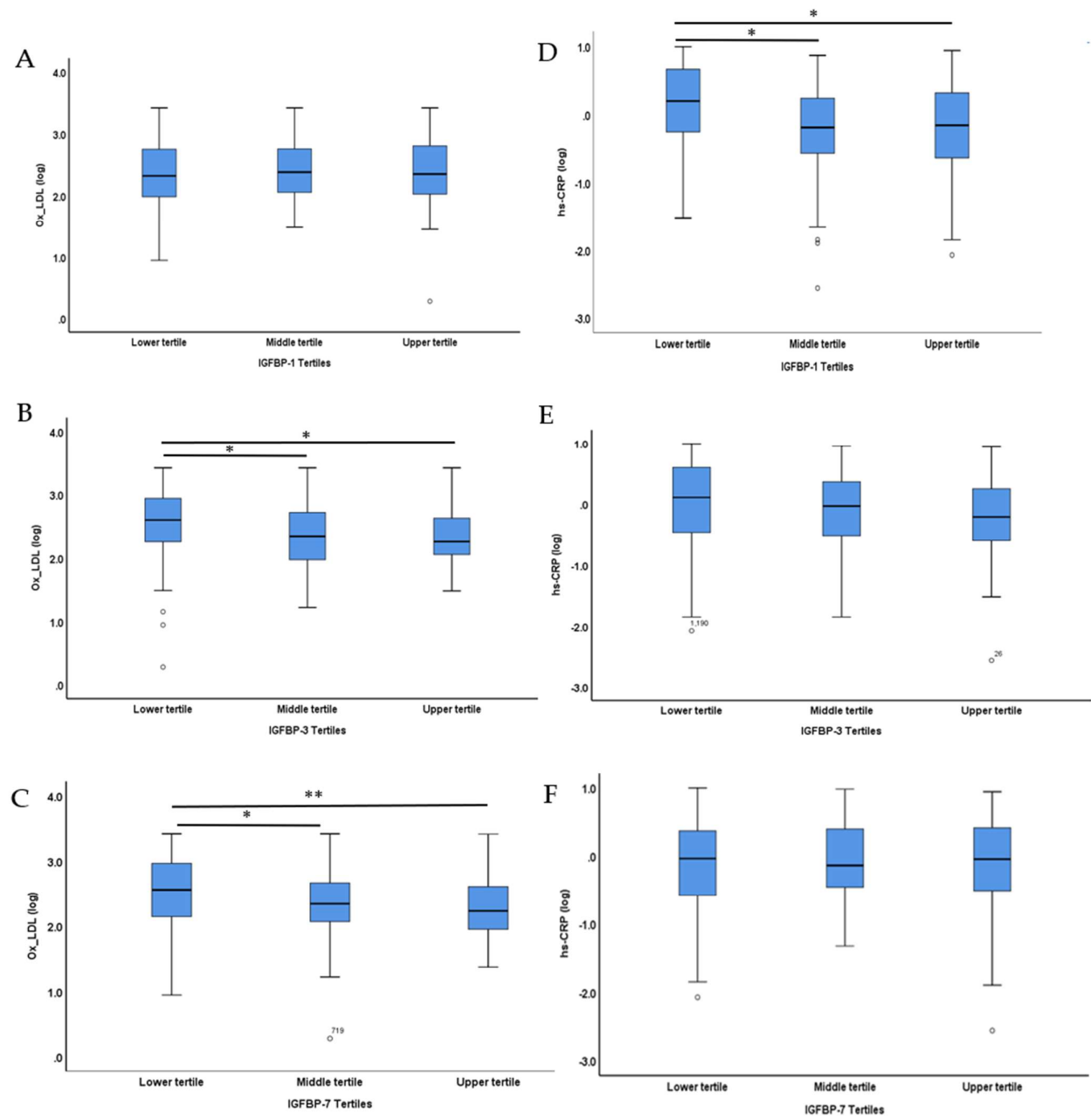**Figure 2.** Association of Ox-LDL (A-C) and Hs-CRP (D-F) with IGFBPs.

Figure 3. Distribution of Ox-LDL (A-C) and Hs-CRP (D-F) in different tertiles of IGFBPs. N=144 in each group.

3. Discussion

This study investigated the association of obesity with IGFBP-1, 3 and 7 in a group of Kuwaiti adolescents and investigated their utility in predictive, preventive and personalized medicine. Our findings show that IGFBP-1 was decreased in overweight and obese children while IGFBP-3 and IGFBP-7 were not affected. Similarly, IGFBP-1, but not IGFBP-3 and IGFBP-7, was negatively associated with WC/Ht ratio. WC/Ht ratio was a better predictor of IGFBP-1 compared to the BMI-for-age z-scores. Additionally, IGFBP-1 was a better predictor of Hs-CRP.

Childhood obesity is becoming increasingly high, especially in the Gulf region. Early predictors of obesity complications can contribute to reduction in obesity-related metabolic complications. Identification of specific biomarkers facilitates the advancement of predictive, preventive and personalized medicine. Upper body obesity (truncal obesity) has been consistently shown to be more strongly associated with obesity-related comorbidities like diabetes and CVD. We thus investigated the association of truncal obesity, measured by WC/Ht ratio, with IGFBPs. Only IGFBP-1 was lower in the children with obesogenic WC/Ht ratio and the other two IGFBPs were not different in the two categories of obesogenic WC/Ht ratio. To our knowledge, this is the first report to specifically correlate central obesity with IGFBP-1. Whereas the overall pattern of association was similar whether we used the weight status categories based on the BMI or based on WC/Ht ratio, we found that WC/Ht ratio was a better predictor of the IGFBP-1 levels compared to the BMI-for-age z-scores, suggesting that central body adiposity has a stronger influence on IGFBP-1 than the overall body adiposity. In linear regression, the effect size of WC/Ht ratio on IGFBP-1 level was more pronounced than the effect size of the BMI-for-age z-scores. Several other studies has supported this notion that central adiposity is more strongly associated with the adverse health consequences of obesity [33-36] and that WC/Ht ratio is a better indicator of adiposity than the BMI [37-39].

Hs-CRP and Ox-LDL are well established cardiometabolic risk factors. In this study we found a differential association pattern of the different IGFBPs with markers of oxidative stress and cardiovascular diseases. Whereas, IGFBP-1 was a significant predictor of Hs-CRP but not Ox-LDL, IGFBP-3 and 7 were significant predictors of Ox-LDL but not Hs-CRP. Obesity is a state of low-level chronic inflammation. High levels of Hs-CRP in overweight and obese individuals thus are consistent with this notion. However, the temporal relationship between IGFBP-1 and Hs-CRP could not be deduced from this study. A prospective study will help delineate if the increased levels of Hs-CRP are the cause or the consequence of lower levels of IGFBP-1 in children with obesity.

A study conducted in Kuwait reported significantly lower levels of IGF-1 and IGFBP-3 in patients with coronary heart disease (CHD) and significant correlation between IGFBP-3 and some metabolic markers including cholesterol, TG and HDL [40]. Recently, a cross-sectional observational study conducted on 84 children under 10 years of age from two schools in Colombia demonstrated an inverse correlation of both IGFBP-1 and IGFBP-2 with triglyceride levels, as well as a direct correlation with HDL levels [41]. The study also reported lower levels of IGFBP-1 with obesity. However, and to the best of our knowledge, this is the first study to report an association of IGFBPs with Hs-CRP and Ox-LDL in adolescents. This is significant because both of these markers are essential for detecting low inflammatory processes. It was interesting to find that while IGFBP-3 and -7 levels were not affected by obesity, they correlated negatively with Ox-LDL levels. This suggests that the role of some IGFBPs in mediating inflammation and CVD can be independent of weight status and body fat composition. Further investigations are needed to delineate the mechanism of action for IGFBPs specifically IGFBP-3 and 7 in the development of CVDs. The present data suggest that the different IGFBPs have non-redundant functions and can be utilized for predictive diagnostics, targeted prevention and personalized medicine.

In this study IGFBP-1 was negatively associated with age but not IGFBP-3 and 7. Although the age range of the study participants was very narrow (11-14) years, significant differences in various age groups, which were only one year apart, were observed in IGFBP-1 levels. This suggests that IGFBP-1 is a sensitive biomarker, compared to the other IGFBPs. The age-dependent changes in IGFBP-1 levels could be explained by changes in either body fat level or sex hormones level. As the sex hormone levels are also affected by the body fat percentage, body fat content appears to be the major common determinant of IGFBP-1 levels. The lower levels of IGFBP-1 in girls compared to boys can also be explained by body fat content, as girls generally have higher body fat percentage compared to boys at this age. However, the effect of changes in hormones could not be ruled out. Our results appear to be in contrast with a study conducted on Turkish prepubertal children in which the serum levels of IGFBP-3 increased steadily with age [42]. However, as we do not have information of the pubertal stage of the subjects in our study, a direct comparison cannot be made. Nonetheless, these discrepancies emphasize the importance of individualized medicine and group-specific interventions since what would work with Turkish children might not necessarily work in their Kuwaiti counterparts. Further studies that cover a larger population with a wider age range are needed to determine the exact interplay between growth and sex hormones, body fat and the levels of IGFBPs.

To our knowledge, this is the first study that investigated the association of IGFBPs with markers of inflammation and oxidative stress (Hs-CRP and Ox-LDL). In addition, we for the first time, investigated the association of IGFBPs with a measure of central body adiposity. Most studies in this field have used BMI as a measure of adiposity. While BMI is generally a good indicator of overall body fatness, it does not discriminate between upper and lower body fatness. Upper body fatness (central adiposity) is more closely associated with obesity-related comorbidities. The fact that IGFBP-1 was more strongly associated with WC/Ht ratio than the BMI z-scores, makes IGFBP-1 a sensitive biomarker for obesity-related metabolic abnormalities. Other strengths of this study include a large sample size, which was representative of the adolescent population in Kuwait, using several statistical approaches to minimize the bias in the association, and the narrow age range of the study subjects. The latter is particularly important, as it will minimize the age-dependent influence on the association between obesity and IGFBPs. Furthermore, this study is based on healthy subjects without any overt obesity-related complications, and thus it highlights the use of IGFBP-1 as a screening tool. There are however, a few limitations in this study. First, the cross-sectional design of the study does not allow us to establish causality. Second, the narrow age of the study subject, which has been mentioned as a strength of the study, could also be a limitation, as the association between IGFBP-1 and obesity cannot be extrapolated to other age groups.

4. Materials and Methods

4.1 Study participants

This is a cross-sectional study that was conducted in selected public middle schools from the State of Kuwait as previously described [43-45]. Study participants were adolescents in the age range of 11–14 years. Data on socioeconomic status and other covariates were collected from the parents through a self-administered questionnaire, and from the adolescents using face-to-face interview.

4.2 Ethics statement

The study was approved by The Ethics Committee at Ministry of Health, Kuwait (No: 2015/248), the Ethics Committee of the Health Sciences Centre, Kuwait University (No: DR/EC/2338) and the Ethical Review Committee at Dasman Diabetes Institute (RA2017-026). Written informed consent was obtained from the parents and verbal ascent was obtained from all the study subjects. We certify that the work conducted in this research complies with the ethical standards recommended by the Helsinki Declaration.

4.3 Blood collection and biochemical analyses

A sample of venous blood (5 mL) was collected from each child. Plasma was separated and stored at −80 °C till analysis. IGFBPs levels were assessed using multiplexing immunobead array platform according to the manufacturer's instructions (R & D Systems). Median fluorescence intensities were collected on a Bioplex-200 system and data were processed using the Bio-Plex Manager Software version 6 (Bio-Rad), with five-parametric curve fitting. Hs-CRP concentrations were determined using ELISA (Hycult Biotech, Cat. # HK369) following manufacturer's instructions. Optimal dilution was found to be 1:1000. Ox-LDL concentrations were determined using ELISA (Immundiagnostik AG, Germany, Cat. # K 7810) following manufacturer's instructions. Optimal dilution was found to be 1:10.

4.4 Anthropometric measurements and other covariates

Standing height and bodyweight of the study participants were measured in a standardized manner, using digital weight and height scale (Detecto, Webb City, MO, USA) with the participants standing erect without shoes and wearing light clothes. BMI-for-age z-scores were calculated using WHO growth charts. Obesity was defined as BMI-for-age ≥ +3 Standard Deviation (SD), while overweight was defined as BMI-for-age > +2 SD and < +3 SD. Waist circumference (WC) was measured in the horizontal plane at the superior border of the right iliac crest to the nearest 0.1 cm with a non-stretchable tape by a trained data collector. Measurements were taken at the end of normal expiration; three readings were taken, and the average of the three was recorded. Care was taken to ensure that the tape was horizontal to the floor and touched the skin without compressing it. The ratio of waist circumference (cm) to height in centimeters (WC/Ht ratio) was calculated and the obesogenic waist was defined as a WC/Ht ratio of >0.5 [46].

4.5 Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) for Windows version 26 (IBM Corp., Armonk, N.Y., USA). IGFBP-1 was log-transformed for normality. Data for the IGFBP in different weight status groups and in male and female were presented as box plots showing median with inter-quartile range (IQR). Association between IGFBPs and weight status categories was assessed by both univariable and multivariable linear regression adjusting for age and sex. The association of IGFBPs with Hs-CRP and Ox-LDL (both log-transformed) was also assessed by linear regression analysis. Levels of IGFBPs were categorized into tertiles and the odds of overweight/obesity (combined) were determined in various tertiles of IGFBPs using binary logistic regression without and with adjusting for age and sex. Mean differences in the IGFBPs across weight status groups, age groups and gender were assessed by one way ANOVA and t-test for independent samples. A p-value of <0.05 was considered as statistically significant.

5. Conclusions

In conclusion, the data presented demonstrate a robust negative association of IGFBP-1, but not other IGFBPs, with overweight and obesity in adolescents. This negative association was more robust with central adiposity compared to overall increased body weight. Furthermore, IGFBP-1 was negatively associated with Hs-CRP, a marker of inflammation, whereas IGFBP-3 and 7, which were not associated with body weight, were negatively associated with Ox-LDL. Together these data illustrate the importance of IGFBPs in childhood obesity and highlight the distinct functions for the different members in the family of IGFBPs. Whereas, IGFBP-1 could be used as a sensitive marker for obesity-related inflammation and its subsequent effects. The identification of such markers, especially in younger subjects, is a step forward towards the advancement of the field of Predictive, Preventive and Personalized Medicine. Screening for, and monitoring obesity-related metabolic complications is an issue of public health importance.

References

1. Al Hammadi, H.; Reilly, J. Prevalence of obesity among school-age children and adolescents in the Gulf cooperation council (GCC) states: a systematic review. *BMC Obes* **2019**, *6*, 3, doi:10.1186/s40608-018-0221-5.

2. Weiderpass, E.; Botteri, E.; Longenecker, J.C.; Alkandari, A.; Al-Wotayan, R.; Al Duwairi, Q.; Tuomilehto, J. The Prevalence of Overweight and Obesity in an Adult Kuwaiti Population in 2014. *Front Endocrinol (Lausanne)* **2019**, *10*, 449, doi:10.3389/fendo.2019.00449.

3. Ministry of Health, K. Kuwait_Nutrition_Surveillance_System: 2017 Annual Report. Kuwait, Ministry of Health, 2017. Accessed 2 Jul 2019. 2017; pp https //www.moh.gov.kw/Renderers/ShowPdf.ashx?Id=e63e21b64-78db-46d61-69c64f-d3793c005064.

4. Alrashidi, M.; Shahwan-Akl, L.; James, J.; Jones, L. *Contributing Factors to Childhood Overweight and Obesity in Kuwait*; 2015; Vol. 3.

5. Carr, M.C.; Brunzell, J.D. Abdominal Obesity and Dyslipidemia in the Metabolic Syndrome: Importance of Type 2 Diabetes and Familial Combined Hyperlipidemia in Coronary Artery Disease Risk. *The Journal of Clinical Endocrinology & Metabolism* **2004**, *89*, 2601-2607, doi:10.1210/jc.2004-0432.

6. Poirier, P.; Giles, T.D.; Bray, G.A.; Hong, Y.; Stern, J.S.; Pi-Sunyer, F.X.; Eckel, R.H.; American Heart, A.; Obesity Committee of the Council on Nutrition, P.A.; Metabolism. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease from the Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. *Circulation* **2006**, *113*, 898-918, doi:10.1161/CIRCULATIONAHA.106.171016.

7. Weiss, R.; Kaufman, F.R. Metabolic complications of childhood obesity: identifying and mitigating the risk. *Diabetes Care* **2008**, *31 Suppl 2*, S310-316, doi:10.2337/dc08-s273.

8. Golubnitschaja, O.; Baban, B.; Boniolo, G.; Wang, W.; Bubnov, R.; Kapalla, M.; Krapfenbauer, K.; Mozaffari, M.S.; Costigliola, V. Medicine in the early twenty-first century: paradigm and anticipation - EPMA position paper 2016.

9. Golubnitschaja, O.; Kinkorova J Fau - Costigliola, V.; Costigliola, V. Predictive, Preventive and Personalised Medicine as the hardcore of 'Horizon 2020': EPMA position paper.

10. Bubnov, R.A.-O.; Babenko, L.; Lazarenko, L.; Kryvtsova, M.; Shcherbakov, O.; Zholobak, N.; Golubnitschaja, O.; Spivak, M. Can tailored nanoceria act as a prebiotic? Report on improved lipid profile and gut microbiota in obese mice.

11.   Haywood, N.J.; Slater, T.A.; Matthews, C.J.; Wheatcroft, S.B. The insulin like growth factor and binding protein family: Novel therapeutic targets in obesity & diabetes. *Mol Metab* **2019**, *19*, 86-96, doi:10.1016/j.molmet.2018.10.008.

12.   Firth, S.M.; Baxter, R.C. Cellular actions of the insulin-like growth factor binding proteins. *Endocr Rev* **2002**, *23*, 824-854, doi:10.1210/er.2001-0033.

13.   Allard, J.B.; Duan, C. IGF-Binding Proteins: Why Do They Exist and Why Are There So Many? *Front Endocrinol (Lausanne)* **2018**, *9*, 117, doi:10.3389/fendo.2018.00117.

14.   Holly, J.; Perks, C. The role of insulin-like growth factor binding proteins. *Neuroendocrinology* **2006**, *83*, 154-160, doi:10.1159/000095523.

15.   Frystyk, J.; Vestbo E Fau - Skjaerbaek, C.; Skjaerbaek C Fau - Mogensen, C.E.; Mogensen Ce Fau - Orskov, H.; Orskov, H. Free insulin-like growth factors in human obesity.

16.   Fontana, L.; Villareal, D.T.; Das, S.K.; Smith, S.R.; Meydani, S.N.; Pittas, A.G.; Klein, S.; Bhapkar, M.; Rochon, J.; Ravussin, E., et al. Effects of 2-year calorie restriction on circulating levels of IGF-1, IGF-binding proteins and cortisol in nonobese men and women: a randomized clinical trial.

17.   Song, Z.; Dai, X.; Yu, H.; Luo, Q.; Zhang, H.; Wu, L.A.-O. Increased Serum IGFBP-1 and Reduced Insulin Resistance After Roux-En-Y Gastric Bypass in Chinese Patients with Type 2 Diabetes: a 6-Month Follow-Up.

18.   Heald, A.H.; Cruickshank, J.K.; Riste, L.K.; Cade, J.E.; Anderson, S.; Greenhalgh, A.; Sampayo, J.; Taylor, W.; Fraser, W.; White, A., et al. Close relation of fasting insulin-like growth factor binding protein-1 (IGFBP-1) with glucose tolerance and cardiovascular risk in two populations. *Diabetologia* **2001**, *44*, 333-339, doi:10.1007/s001250051623.

19.   Gokulakrishnan, K.; Velmurugan, K.; Ganesan, S.; Mohan, V. Circulating levels of insulin-like growth factor binding protein-1 in relation to insulin resistance, type 2 diabetes mellitus, and metabolic syndrome (Chennai Urban Rural Epidemiology Study 118). *Metabolism* **2012**, *61*, 43-46, doi:10.1016/j.metabol.2011.05.014.

20.   Liew, C.F.; Wise, S.D.; Yeo, K.P.; Lee, K.O. Insulin-like growth factor binding protein-1 is independently affected by ethnicity, insulin sensitivity, and leptin in healthy, glucose-tolerant young men. *J Clin Endocrinol Metab* **2005**, *90*, 1483-1488, doi:10.1210/jc.2004-1501.

21.   Lewitt, M.S.; Hilding, A.; Ostenson, C.G.; Efendic, S.; Brismar, K.; Hall, K. Insulin-like growth factor-binding protein-1 in the prediction and development of type 2 diabetes in middle-aged Swedish men. *Diabetologia* **2008**, *51*, 1135-1145, doi:10.1007/s00125-008-1016-x.

22.   Petersson, U.; Ostgren, C.J.; Brudin, L.; Brismar, K.; Nilsson, P.M. Low levels of insulin-like growth-factor-binding protein-1 (IGFBP-1) are prospectively associated with the incidence of type 2 diabetes and impaired glucose tolerance (IGT): the Soderakra Cardiovascular Risk Factor Study. *Diabetes Metab* **2009**, *35*, 198-205, doi:10.1016/j.diabet.2008.11.003.

23.   Lewitt, M.S.; Hilding, A.; Brismar, K.; Efendic, S.; Ostenson, C.G.; Hall, K. IGF-binding protein 1 and abdominal obesity in the development of type 2 diabetes in women. *Eur J Endocrinol* **2010**, *163*, 233-242, doi:10.1530/EJE-10-0301.

24.   Rajpathak, S.N.; McGinn, A.P.; Strickler, H.D.; Rohan, T.E.; Pollak, M.; Cappola, A.R.; Kuller, L.; Xue, X.; Newman, A.B.; Strotmeyer, E.S., et al. Insulin-like growth factor-(IGF)-axis, inflammation, and glucose intolerance among older adults. *Growth Horm IGF Res* **2008**, *18*, 166-173, doi:10.1016/j.ghir.2007.08.004.

25.   Frystyk, J.; Brick, D.J.; Gerweck, A.V.; Utz, A.L.; Miller, K.K. Bioactive insulin-like growth factor-I in obesity. *J.Clin.Endocrinol.Metab.* **2009**, *94*, 3093-3097, doi:10.1210/jc.2009-0614.

26.   Rajpathak, S.N.; He, M.; Sun, Q.; Kaplan, R.C.; Muzumdar, R.; Rohan, T.E.; Gunter, M.J.; Pollak, M.; Kim, M.; Pessin, J.E., et al. Insulin-like growth factor axis and risk of type 2 diabetes in women. *Diabetes* **2012**, *61*, 2248-2254, doi:10.2337/db11-1488.

27.   Lopez-Bermejo, A.; Khosravi, J.; Fernandez-Real, J.M.; Hwa, V.; Pratt, K.L.; Casamitjana, R.; Garcia-Gil, M.M.; Rosenfeld, R.G.; Ricart, W. Insulin resistance is associated with increased serum concentration of IGF-binding protein-related protein 1 (IGFBP-rP1/MAC25). *Diabetes* **2006**, *55*, 2333-2339, doi:10.2337/db05-1627.

28.   Gu, H.F.; Gu, T.; Hilding, A.; Zhu, Y.; Karvestedt, L.; Ostenson, C.G.; Lai, M.; Kutsukake, M.; Frystyk, J.; Tamura, K., et al. Evaluation of IGFBP-7 DNA methylation changes and serum protein variation in Swedish subjects with and without type 2 diabetes. *Clinical epigenetics* **2013**, *5*, 20, doi:10.1186/1868-7083-5-20.

29.   Shao, L.; Huang, Q.; Luo, M.; Lai, M. Detection of the differentially expressed gene IGF-binding protein-related protein-1 and analysis of its relationship to fasting glucose in Chinese colorectal cancer patients. *Endocrine-related cancer* **2004**, *11*, 141-148, doi:10.1677/erc.0.0110141.

30.   Liu, Y.; Wu, M.; Ling, J.; Cai, L.; Zhang, D.; Gu, H.F.; Wang, H.; Zhu, Y.; Lai, M. Serum IGFBP7 levels associate with insulin resistance and the risk of metabolic syndrome in a Chinese population. *Sci Rep* **2015**, *5*, 10227, doi:10.1038/srep10227.

31.   Lisowska, A.; Swiecki, P.; Knapp, M.; Gil, M.; Musial, W.J.; Kaminski, K.; Hirnle, T.; Tycinska, A. Insulin-like growth factor-binding protein 7 (IGFBP 7) as a new biomarker in coronary heart disease. *Advances in medical sciences* **2019**, *64*, 195-201, doi:10.1016/j.advms.2018.08.017.

32.   Mogul, H.R.; Marshall, M.; Frey, M.; Burke, H.B.; Wynn, P.S.; Wilker, S.; Southern, A.L.; Gambert, S.R. Insulin like growth factor-binding protein-1 as a marker for hyperinsulinemia in obese menopausal women. *J Clin Endocrinol Metab* **1996**, *81*, 4492-4495, doi:10.1210/jcem.81.12.8954066.

33.   Cox, B.D.; Whichelow, M. Ratio of waist circumference to height is better predictor of death than body mass index. *Bmj* **1996**, *313*, 1487, doi:10.1136/bmj.313.7070.1487.

34.   Thomas, G.N.; McGhee, S.M.; Schooling, M.; Ho, S.Y.; Lam, K.S.L.; Janus, E.D.; Lam, T.H. Impact of sex-specific body composition on cardiovascular risk factors: the Hong Kong Cardiovascular Risk Factor Study. *Metabolism* **2006**, *55*, 563-569, doi:https://doi.org/10.1016/j.metabol.2005.08.004.

35.   Pischon, T.; Boeing, H.; Hoffmann, K.; Bergmann, M.; Schulze, M.B.; Overvad, K.; van der Schouw, Y.T.; Spencer, E.; Moons, K.G.; Tjonneland, A., et al. General and abdominal adiposity and risk of death in Europe. *N Engl J Med* **2008**, *359*, 2105-2120, doi:10.1056/NEJMoa0801891.

36.   Zhang, C.; Rexrode, K.M.; van Dam, R.M.; Li, T.Y.; Hu, F.B. Abdominal obesity and the risk of all-cause, cardiovascular, and cancer mortality: sixteen years of follow-up in US women. *Circulation* **2008**, *117*, 1658-1667, doi:10.1161/CIRCULATIONAHA.107.739714.

37.   Schneider, H.J.; Friedrich, N.; Klotsche, J.; Pieper, L.; Nauck, M.; John, U.; Dörr, M.; Felix, S.; Lehnert, H.; Pittrow, D., et al. The Predictive Value of Different Measures of Obesity for Incident Cardiovascular Events and Mortality. *The Journal of Clinical Endocrinology & Metabolism* **2010**, *95*, 1777-1785, doi:10.1210/jc.2009-1584.

38.   Ashwell, M.; Lejeune, S.; McPherson, K. Ratio of waist circumference to height may be better indicator of need for weight management. *Bmj* **1996**, *312*, 377, doi:10.1136/bmj.312.7027.377.

39.   Hsieh, S.D.; Yoshinaga, H.; Muto, T. Waist-to-height ratio, a simple and practical index for assessing central fat distribution and metabolic risk in Japanese men and women. *International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity* **2003**, *27*, 610-616, doi:10.1038/sj.ijo.0802259.

40.   Akanji, A.O.; Suresh, C.G.; Al-Radwan, R.; Fatania, H.R. Insulin-like growth factor (IGF)-I, IGF-II and IGF-binding protein (IGFBP)-3 levels in Arab subjects with coronary heart disease. *Scandinavian journal of clinical and laboratory investigation* **2007**, *67*, 553-559, doi:10.1080/00365510601173153.

41.   Vera, S.; Figueroa, T.; Aranzalez, L.H.; Mockus, I. Cardiovascular disease risk markers in children under 10 years of age and their relationship with serum concentrations of IGF-1, IGFBP-1, IGFBP-2 and IGFBP-3. *Revista de la Facultad de Medicina* **2020**, *68*, 51-58, doi:10.15446/revfacmed.v68n1.69979.

42.   Bereket, A.; Turan, S.; Omar, A.; Berber, M.; Ozen, A.; Akbenlioglu, C.; Haklar, G. Serum IGF-I and IGFBP-3 Levels of Turkish Children during Childhood and Adolescence: Establishment of Reference Ranges with Emphasis on Puberty. *Hormone Research in Paediatrics* **2006**, *65*, 96-105, doi:10.1159/000091301.

43. Al-Taiar, A.; Rahman, A.; Al-Sabah, R.; Shaban, L.; Al-Harbi, A. Vitamin D status among adolescents in Kuwait: a cross-sectional study. *BMJ Open* **2018**, *8*, e021401, doi:10.1136/bmjopen-2017-021401.

44. Hammad, M.M.; Abu-Farha, M.; Al-Taiar, A.; Alam-Eldin, N.; Al-Sabah, R.; Shaban, L.; Al-Mulla, F.; Abubaker, J.; Rahman, A. Correlation of circulating ANGPTL5 levels with obesity, high sensitivity C-reactive protein and oxidized low-density lipoprotein in adolescents. *Sci Rep* **2020**, *10*, 6330, doi:10.1038/s41598-020-63076-7.

45. Rahman, A.; Al-Taiar, A.; Shaban, L.; Al-Sabah, R.; Al-Harbi, A.; Mojiminiyi, O. Plasma 25-Hydroxy Vitamin D Is Not Associated with Either Cognitive Function or Academic Performance in Adolescents. *Nutrients* **2018**, *10*, doi:10.3390/nu10091197.

46. Gibson, S.; Ashwell, M. A simple cut-off for waist-to-height ratio (0.5) can act as an indicator for cardiometabolic risk: recent data from adults in the Health Survey for England. *The British journal of nutrition* **2020**, *123*, 681-690, doi:10.1017/S0007114519003301.