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Original Article

Omega-3 polyunsaturated fatty acids reduce insulin resistance and triglycerides in obese children and adolescents

Juárez-López C, Klünder-Klünder M, Madrigal-Azcárate A, Flores-Huerta S. Omega-3 polyunsaturated fatty acids reduce insulin resistance and triglycerides in obese children and adolescents.
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Background: Approximately 50% of obese children are insulin resistant. It has been suggested that pharmacological and nutritional options should be considered to improve the management of insulin resistance (IR).
Objective: To assess the effect of metformin (Met) or omega-3 (ω-3) polyunsaturated fatty acids (PUFA) on the homeostasis model assessment-estimated insulin resistance (HOMA-IR) index, lipid profile, and body mass index (BMI) of obese children.
Methods: We included 201 obese and insulin-resistant children and adolescents. Ninety-eight of them received 500 mg of Met, and 103 received 1.8 g of ω-3 PUFA for 12 wk. This was an open-label study with assignment of treatment based on which school the child attended. At the baseline and at the end of study, the following parameters were measured: weight, height, waist circumference, blood pressure, insulin, glucose, lipid profile, and HOMA-IR index. There were no lifestyle interventions.
Results: At baseline, the age, BMI, and IR in children of both groups were comparable. The treatment assigned for each group was well tolerated. Metabolic changes were adjusted for age, sex, and change in BMI. Concerning the IR profile at the end of intervention, ω-3 significantly decreased the concentrations of glucose and insulin while reducing HOMA-IR values; meanwhile, Met negligibly affected insulin levels. Regarding lipids, Met increased high density lipoprotein cholesterol (HDL-C) and decreased low density lipoprotein cholesterol (LDL-C), but triglycerides were not affected; in contrast, triglycerides were decreased significantly by ω-3. The effects on BMI were marginal under Met but were significant with ω-3.
Conclusion: The results of this work suggest that ω-3 may be useful as an adjuvant therapy in obese children and adolescents with IR.

Carlos Juárez-López[a], Miguel Klünder-Klünder[b], Adrián Madrigal-Azcárate[a] and Samuel Flores-Huerta[b]

[a]Department for Innovation and Quality, Institute for Decentralized Public Health Services (INDESALUD), Campeche, Campeche, 24040, Mexico; and [b]Community Health Research Department, Federico Gomez Pediatric Hospital of Mexico, Ministry of Health (SSA) Mexico, Mexico, District Federal, 06720, Mexico

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Corresponding author: Samuel Flores-Huerta MD,
Dr. Márquez 162,
México, D.F. 06720, México.
Tel: +52-55-52289917; Fax: +52 555 761 8974;
e-mail: floreshuertamd@gmail.com

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As people's lifestyles change to affect even children, obesity is emerging as a major public health challenge. Epidemiologic data indicate that school-age Mexican children have an increased rate of 1.1 percentage points per year for general and abdominal obesity, one of the highest in the world (1–3). Morphological and functional changes in the adipose tissue after weight gain lead to chronic low-intensity inflammation, which is accepted as a pathway for the development of insulin resistance (IR), metabolic syndrome (MS), and other comorbidities (4). At least half of obese children and teenagers present IR (5, 6) and other alterations, such as dyslipidemia (7) and high blood pressure (8).

The recommended first-line treatment for obese children and teenagers with IR is a change in lifestyle that promotes healthy nutritional habits and physical activity, along with complementary treatments, such as metformin (Met) therapy or omega-3 (ω-3) polyunsaturated fatty acids (PUFA), that help to improve or reverse IR (9, 10). Studies in obese

children and teenagers with IR in which Met and placebos have been compared indicate that this drug reduces fasting insulin levels and has a modest but significant effect on body mass index (BMI) reduction (11–14). However, the observed weight loss appears to be independent of glucose homeostasis changes. Because there is no consensus on the use of Met in the treatment of IR in obese children and adolescents, it is a priority to further explore its benefits.

Regarding ω-3, studies in animal models for obesity have shown that fatty acids improve IR and hyperlipidemia (15). These effects have also been observed in epidemiologic studies in which triglyceride reduction in adults constituted an important effect (16, 17), suggesting that ω-3 may reduce inflammatory processes in obese patients (18).

Lifestyle changes in obese people are gradual and usually refractory processes. In light of evidence suggesting that either Met or ω-3 can improve the metabolic states of obese children, our work focused on administering these drugs to IR obese children to assess their effects on fasting glucose, insulin, homeostasis model assessment-estimated insulin resistance (HOMA-IR) index, lipid profile, and BMI.

Materials and methods

The study was performed in 15 public elementary schools in Campeche, México with children attending the 5th and 6th grades. Prior to the study, ethical clearance was obtained from Campeche State research ethics committees and school authorities in accordance with the Declaration of Helsinki. The protocol was implemented in three stages (Fig. 1) between February 2007 and January 2008, as previously reported (6). Briefly, the first stage consisted of identifying obese children (BMI $\geq$ 95th percentile by age and sex) (19); in the next stage, we identified IR obese children (HOMA-IR values >3.4) [HOMA-IR = fasting glucose (mg/dL) $\times$ (fasting insulin (μU/mL))/405] corresponding to the 90th percentile of a healthy child population (20). In the third stage, we obtained informed consent from the children and their parents to perform an open-label trial in which the assignment of treatment was based on the school the child attended. Using a simple randomized method, eight schools were allocated to Met and seven to ω-3. Children and adolescents with type 2 diabetes who were attending a formal weight loss program or who had identified syndromes that predisposed them to obesity were excluded. Previously trained nurses used internationally accepted procedures to obtain the weights, heights, and waist circumferences (WCs) of the participants. Weight and height were measured without shoes but with light clothing. Weight was taken using a digital scale (Seca 884, Hamburg, Germany) to the nearest 0.1 kg; height was measured using a Seca 225 stadiometer. WC was measured in a standing position at the end of an exhalation with an inelastic, flexible tape (Seca 200) at the midpoint between the lower costal border and the iliac crest, according to the World Health Organization protocol (21). To decrease parallax error, subjects were asked to step on an anthropometric box especially designed for this purpose. Blood pressure was measured on one visit using a sphygmomanometer (ALPK2, Tokyo, Japan) with an appropriate cuff size for the arm length, following the North American guidelines issued in 2004 (22).

Venous blood was sampled after 12 h of fasting and was used to measure glucose, insulin, triglycerides, total cholesterol, and HDL-C. The children were treated for 12 consecutive weeks. In the Met-assigned schools, the dose was 250 mg for the first 2 wk (one-half of a 500-mg tablet; Dabex Merck Metformin, 500 mg, lot M64547), and from week 3 to the end of the study, they received 500 mg daily (one tablet). The ingestion of metformin was indicated with breakfast. In the ω3-assigned schools, the capsules used contained 600 mg of PUFA ω-3, [(360 mg of eicosapentaenoic acid (20:5 n-3) and 240 mg of docosahexaenoic acid (22:6 n-3)] (Omega-3 Prime Fitness®, Lot 1205051). Each participant received three capsules daily (1.8 g/d), ingesting one with each meal of the day. The schools were visited every week to deliver the treatments, to evaluate the study adherence, and to assess possible side effects. After 12 wk, we obtained the anthropometric, clinical, and metabolic measurements under the same conditions as the beginning of the study. The Campeche State Public Health Laboratory performed all of the analyses. The methods used included chemiluminescence for insulin (Access, Beckman Coulter Instruments, Brea, CA, USA) and an analyzer for glucose, triglycerides, and HDL-C (Synchron Cx® Beckman Coulter Instruments). For LDL-C, we utilized DeLong's modified Friedwald formula (23).

The anthropometric, clinical, and metabolic data were tested for normal distributions using skewness and kurtosis. Continuous variables and groups were compared with the Student's t test or the Mann–Whitney U test according to the distribution of the data. For categorical data, the chi-squared test was used. To evaluate the changes between baseline and the end of the intervention, we computed adjusted coefficients for each dependent variable with a linear regression model adjusted for age, sex, BMI change, and treatment given. After these estimations were calculated, we obtained the mean adjusted change for each dependent variable. Additionally, we explored the relationship between HOMA-IR (log transformed) and BMI percentile at the baseline and end treatments

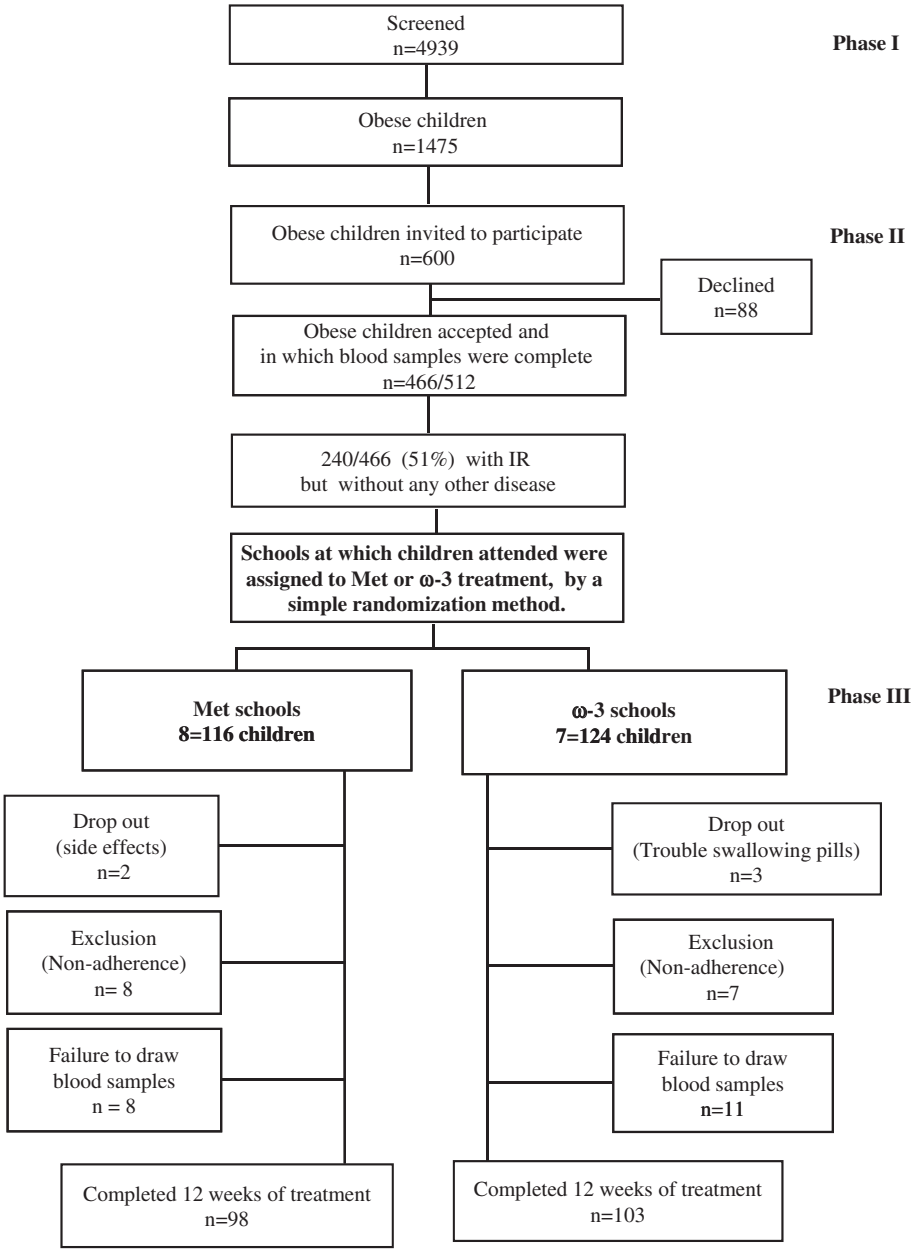

Fig. 1. Flow diagram of patient enrollment and study completion.

with linear regression and plotted the resulting lines for each group, reporting the R-square. We used STATA SE v.11.0 (Stata Corp, College Station, TX, USA) to perform all analyses.

Results

In the Met group, 116 children were initially included, and 98 completed the study; in the ω-3 group, 124 children were enrolled, and 103 completed the study. The reasons for dropout are described in Fig. 1. Analysis using demographic, anthropometric, and metabolic data between those who dropped out and those who remained in the study did not reveal significant differences (data not shown). No serious adverse effects were observed in the Met-treated children; 3% reported meteorism, and two children experienced abdominal pain that subsided when the dose was divided into two doses. In the ω-3 group, 80% reported fish-smelling burps, which did not affect their adherence to the study. Overall, both treatments were well tolerated. At the baseline of the intervention, children from both groups had comparable anthropometric and metabolic characteristics, including age, BMI, metabolic parameters, and blood pressure. The only exception was LDL-C, which was significantly higher in the ω-3 group ($p < 0.001$; Table 1).

Table 1. Anthropometric and metabolic baseline characteristics of obese children with insulin resistance according to treatment group

	Met n = 98 Mean ± SD	ω-3 n = 103 Mean ± SD	p
Sex (female) %	59	48	0.100
Age (yr)	11.4 ± 0.8	11.6 ± 0.7	0.062
Weight (kg)	64.4 ± 10.4	64.6 ± 11.5	0.904
Height (m)	1.5 ± 0.1	1.5 ± 0.1	0.696
SBP (mmHg)	107.6 ± 12.5	107.7 ± 10.3	0.957
DBP (mmHg)	67.6 ± 9.8	66.8 ± 8.9	0.559
BMI (kg/m^2)	28 ± 3.5	27.9 ± 3.4	0.714
BMI (percentile)	97.7 ± 1.3	97.8 ± 1.2	0.712
WC (cm)	87.9 ± 8.9	88.2 ± 8.5	0.784
Glucose (mg/dL)*	90 (85–94)	92 (88–95)	0.052
Insulin (μU/mL)*	23.7 (19.5–31.4)	22.1 (18.4–28.0)	0.147
HOMA-IR*	5.2 (4.3–6.7)	4.9 (4.1–6.4)	0.300
Total-C (mg/dL)	164.9 ± 25.9	163.6 ± 33.1	0.747
LDL-C (mg/dL)	86.7 ± 20.3	101.2 ± 33.4	< 0.001
HDL-C (mg/dL)	36.2 ± 8.4	36.9 ± 8.3	0.560
Triglycerides (mg/dL)*	137 (107–183)	132 (92–192)	0.367

BMI, body mass index; C, cholesterol; DBP, diastolic blood pressure; HOMA-IR, homeostasis model to assess the insulin resistance index; SBP, systolic blood pressure; WC, waist circumference.
*Median (interquartile range).

Table 2 shows the differences between the basal and end values of the anthropometric, clinical, and metabolic measurements for each intervention, adjusted by age, sex, and change in BMI. Significant reductions in BMI, systolic blood pressure, total cholesterol, and LDL-C were observed in participants treated with Met, along with an increase HDL-C. There were no changes in the rest of the parameters. In the ω-3 group, BMI, glucose, fasting insulin HOMA-IR, and triglycerides decreased, while HDL-C increased. There were no changes in the rest of the parameters. Comparing the interventions, Metformin reduced LDL-C, but ω-3 reduced fasting glucose and triglycerides.

Figure 2 shows the correlation between BMI as percentile and HOMA-IR in each of the Met and ω-3 groups. Panel A shows the baseline conditions, which did not differ between the groups, as indicated by the slopes of the lines. However, panel B shows that at the end of the intervention, the fitted regression lines for the ω-3 and Met groups had statistically significant changes in their slopes ($R^2 = 0.11$, $p < 0.001$ and $R^2 = 0.05$, $p = 0.022$, respectively).

Discussion

Our work explored two independent alternatives that could be used as adjuvant therapies to changes in diet and physical activities for obese children and adolescents who have already developed IR.

Met effects

In this study, the effect of Met on weight reduction and changes in cholesterol metabolites was observed. Regarding weight loss, BMI reduction was less than 0.5 kg/m^2, which was statistically significant but not clinically significant. The dose of 500 mg/d used in this study was rather low, and the 12 wk of intervention could have been too short a time to observe significant changes. Other studies used dosages of 1–2 g/d taken for 6 months in conjunction with modified dietary and exercise habits to show an improvement in BMI of 1.42 kg/m^2 (24). Alternatively, some extended the Met treatment for 1 yr and observed a reduction in BMI of 1.1 kg/m^2 compared with placebo (11). In our results, Met had an inverse association with total cholesterol and LDL-C and a direct association with HDL-C. No effect on triglyceride concentration was observed, as has been reported in other studies (25, 26). There was no effect on either fasting glucose and insulin or HOMA-IR, contrary to findings in other studies (11, 24, 27). Met's lack of effect on metabolic variables is likely due to the insufficient time and dose used in this study. However, although this drug is prescribed to improve the metabolic states of patients afflicted with DT2 or polycystic ovary syndrome (28–30), a higher dose was not used because it has not been sufficiently studied in obese individuals <12 yr of age with IR (31). On the basis of the results of previous studies, we speculate that a higher dose of Met could have improved IR (32).

ω-3 Effects

Participants treated with ω-3 experienced weight reduction and effects on fasting glucose and lipid profiles. Regarding weight loss, the BMI reduction was greater than 0.5 kg/m^2, which was statistically and clinically significant. Those individuals supplemented with ω-3 presented with significantly improved measurements for glucose, insulin, and HOMA-IR clusters. Figure 2 depicts how ω-3 modified the slope of the line correlating HOMA-IR and BMI at the end of the intervention, while the Met group line remained unchanged. This partially supports the effect of ω-3 on both BMI and IR. In terms of the lipid profile, ω-3

Table 2. Changes in anthropometric and metabolic variables at end of the study

	Metn = 98		ω-3 n = 103		Met vs. ω-3		
	n = 98		n = 103				
	Diff*	95% CI	Diff*	95% CI	Diff	95% CI	p†
Anthropometric							
BMI (kg/m^2)	−0.27	−0.53 to −0.01	−0.55	−0.81 to −0.30	−0.28	−0.65 to 0.08	0.127
BMI (percentile)	−0.42	−0.63 to −0.21	−0.64	−0.85 to −0.44	−0.22	−0.52 to 0.08	0.147
WC (cm)	−0.07	−1.16 to 1.02	−0.03	−1.07 to 1.01	0.04	−1.48 to 1.56	0.957
Blood pressure							
SBP (mmHg)	−2.58	−5.10 to −0.07	−0.91	−3.24 to 1.43	1.68	−1.80 to 5.15	0.950
DBP (mmHg)	−1.54	−3.90 to 0.83	0.95	−1.25 to 3.15	2.48	−0.78 to 5.75	0.135
Metabolic							
Glucose (mg/dL)	1.08	−0.63 to 2.78	−2.58	−4.19 to −0.96	−3.66	−6.03 to −1.28	0.003
Insulin (μU/mL)	−2.58	−5.38 to 0.22	−4.21	−6.86 to −1.55	−1.63	−5.53 to 2.28	0.412
HOMA-IR	−0.50	−1.20 to 0.20	−1.04	−1.70 to −0.38	−0.54	−1.51 to 0.43	0.277
Total-C (mg/dL)	−6.53	−11.17 to −1.88	−0.29	−4.69 to 4.12	6.24	−0.23 to 12.71	0.059
HDL-C (mg/dL)	3.91	2.31 to 5.50	2.12	0.61 to 3.63	−1.79	−4.01 to 0.43	0.114
LDL-C (mg/dL)	−10.21	−15.74 to −4.68	4.58	−0.66 to 9.82	14.79	7.09 to 22.49	<0.001
Triglycerides (mg/dL)	11.34	−3.90 to 26.58	−26.35	−40.78 to −11.91	−37.69	−58.91 to −16.46	0.001

BMI, body mass index; C, cholesterol; CI, confidence interval.; DBP, diastolic blood pressure; HOMA-IR, homeostasis model to assess the insulin resistance index; SBP, systolic blood pressure; WC, waist circumference.
*Mean changes from baseline to end were adjusted for age, sex, and change in BMI.
†p values obtained by Wald statistics and multiple lineal regression models.

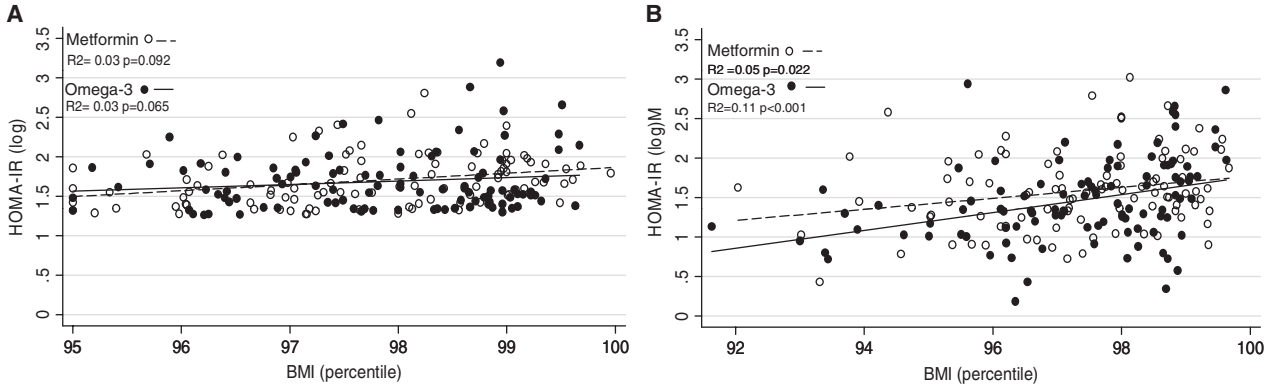

Fig. 2. Relationships between homeostasis model assessment-estimated insulin resistance (HOMA-IR) and body mass index (BMI) percentile in the metformin (Met) and omega-3 (ω-3) groups at baseline (**A**) and at the end of treatment (**B**).

had a direct association with HDL-C levels and an inverse association with triglycerides. In controlled clinical trials with adults, triglyceride reduction is dose dependent, and an effect is observed even for dosages of <1 g/d (33). In this study, we used a dosage of approximately 2 g/d that produced a triglyceride reduction similar to that reported in the literature (16, 34, 35). However, the ω-3 dosages used in this study did not modify the total cholesterol or LDL-C levels, even while inducing a modest but significant increase in HDL-C (16, 34).

The precise mechanism by which ω-3 improves fasting glucose and insulin values and HOMA-IR has not been completely elucidated. Likewise, the required dose to improve IR and triglycerides in obese children is also under study; it was recently reported that 900 mg/d for 4 wk reduced glucose, insulin, and HOMA-IR in obese children with IR (33).

It has been proposed that the above benefits are produced by the incorporation of ω-3 into skeletal muscle cell membranes, improvements in lipid capture in the liver, reductions in plasma glucose levels, decreased triglyceride synthesis, and increased lipid beta-oxidation. These benefits can also improve the depuration of very low-density lipoprotein particles from peripheral circulation (36–39).

Our study has several major limitations. First, the study's design was not double-blind; rather, the assignment of treatment was made according to the school that the child attended. Second, while the experiment was being performed, we did not intervene with dietary and exercise modifications. Although this is a limitation against reducing weight, this did

allow for us to observe the effects of each treatment more specifically. Third, the dosages and lengths of the treatments posed another limitation. While the Met dosages were considered low, the ω-3 dosages could be considered high; additionally, the duration of 12 wk used could be considered short. However, at least for ω-3, the duration of treatment is not associated with the effect observed, which suggests that once the maximal effect is achieved, it is maintained throughout the intervention period (34). Fourth, no antiinflammatory or proinflammatory cytokines or any other lipid metabolites were measured to ascertain the effects of Met and ω-3 on these parameters. Other studies have reported that ω-3 increases adiponectin concentrations (33, 40); consequently, it was not possible to establish the effect of these treatments on the inflammatory response. Finally, the onset of puberty was not measured; therefore, this variable, which has been described to be associated with IR even before the onset of puberty (41), was not controlled.

In conclusion, despite the many limitations of our study, our results indicate that, at low dosages and for a short duration, Met modestly improves several metabolic and anthropometric parameters in obese children but does not affect IR; the dose could be considered insufficient. ω-3 treatment improved IR, triglyceride levels, and weight loss, possibly because PUFAs were used at doses that potentially permitted their integration into the cellular membranes of tissues that modulate diverse molecular pathways and improve inflammatory and metabolic states (35).

The results of this study suggest that ω-3 may be useful as an adjuvant therapy for obese children with IR. These results do not exclude the promotion of the dietary intake of these fatty acids, or of regular exercise habits.

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References

1. World Health Organization. Obesity and overweight facts (available from http:www.who.inthprNPHdocsgs_obesity.pdf). Accessed on February 2008.
2. World Health Organization. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. WHO technical report series no 894, Geneva, 2000.
3. Olaiz-Fernández G, Rivera-Dommarco J, Shamah-Levy T et al. Encuesta Nacional de Salud y Nutrición 2006. Cuernavaca, México: Instituto Nacional de Salud Pública, 2006.
4. Cruz M, Torres M, Aguilar-Herrera B et al. Type 2 diabetes mellitus in children. An increasing health problem in Mexico. J Pediatr Endocrinol Metab 2004: 17: 183–190.
5. Chiarelli F, Marcovecchio ML. Insulin resistance and obesity in childhood. Eur J Endocrinol 2008: 159: S67–S74.
6. Juárez-López C, Klünder-Klünder M, Medina-Bravo P, Madrigal-Azcárate A, Mass-Díaz E, Flores-Huerta S. Insulin resistance and its association with the components of metabolic syndrome among obese children and adolescents. BMC Public Health 2010: 10: 318.
7. Posadas-Sánchez R, Posadas-Romero C, Zamora-González C, Mendoza-Pérez E, Cardoso-Saldaña G, Yamamoto-Kimura L. Lipid and lipoprotein profiles and prevalence of dyslipidemia in Mexican adolescents. Metabolism 2007: 56: 1666–1672.
8. Flores-Huerta S, Klünder-Klünder M, Reyes-de-la-Cruz L, Santos JI. Increase in body mass index and waist circumference is associated with high blood pressure in children and adolescents in Mexico City. Arch Med Res 2009: 40: 208–215.
9. Levy-Marchal C, Arslanian S, Cutfield W et al. Insulin resistance in children: consensus, perspective, and future directions. J Clin Endocrinol Metab 2010: 95: 5189–5198.
10. Pacifico L, Anania C, Martino F et al. Management of metabolic syndrome in children and adolescents. Nutr Metab Cardiovasc Dis 2011: 21: 455–466.
11. Wilson DM, Abrams SH, Aye T et al. Metformin extended release treatment of adolescent obesity. A 48-week randomized, double-blind, placebo-controlled trial with 48-week follow-up. Arch Pediatr Adolesc Med 2010: 164: 116–123.
12. Love-Osborne K, Sheeder J, Zeitler P. Addition of metformin to a lifestyle modification program in adolescents with insulin resistance. J Pediatr 2008: 152: 817–822.
13. Nobili V, Manco M, Ciampalini P et al. Metformin use in children with nonalcoholic fatty liver disease: an open-label, 24-month, observational pilot study. Clin Ther 2008: 30: 1168–1176.
14. Yanovski JA, Krakoff J, Salaita CG et al. Effects of metformin on body weight and body composition in obese insulin-resistant children. Diabetes 2011: 60: 477–485.
15. Hassanali Z, Ametaj BN, Field CJ, Proctor SD, Vine DF. Dietary supplementation of n-3 PUFA reduces weight gain and improves postprandial lipaemia and the associated inflammatory response in the obese JCR:LA-cp rat. Diabetes Obes Metab 2010: 12: 139–147.
16. Jacobson TA. Role of n–3 fatty acids in the treatment of hypertriglyceridemia and cardiovascular disease. Am J Clin Nutr 2008: 87 (suppl): 1981S–1990S.
17. Ramel A, Martinéz A, Kiely M, Morais G, Bandarra NM, Thorsdottir I. Beneficial effects of long-chain *n*-3 fatty acids included in an energy-restricted diet on insulin resistance in overweight and obese European young adults. Diabetologia 2008: 51: 1261–1268.
18. White PJ, Marette A. Is omega-3 key to unlocking inflammation in obesity? Diabetologia 2006: 49: 1999–2001.

19. Kuczmarski RJ, Ogden CL, Guo SS et al. 2000 CDC growth charts for the United States: methods and development. Vital Health Stat 11 2002: 246: 1–190.
20. García-Cuartero B, García-Lacalle C, Jiménez-Lobo C et al. Índice HOMA y QUICKI, insulina y péptido C en niños sanos. Puntos de corte de riesgo cardiovascular. An Pediatr (Barc) 2007: 66: 481–490.
21. World Health Organization. Waist circumference and waist–hip ratio: report of a WHO expert consultation, Geneva, 2008.
22. American Academy of Pediatrics, National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. Pediatrics 2004: 114: 555–576.
23. DeLong DM, DeLong ER, Wood PD, Lippel K, Rifkind BM. A comparison of methods for the estimation of plasma low- and very low-density lipoprotein cholesterol. The lipid research clinics prevalence study. JAMA 1986: 256: 2372–2377.
24. Park MH, Kinra S, Ward KJ, White B, Viner RM. Metformin for obesity in children and adolescents: a systematic review. Diabetes Care 2009: 32: 1743–1745.
25. Burgert TS, Duran EJ, Goldberg-Gell R et al. Short-term metabolic and cardiovascular effects of metformin in markedly obese adolescents with normal glucose tolerance. Pediatr Diabetes 2008: 9: 567–576.
26. Freemark M, Bursey D. The effects of metformin on body mass index and glucose tolerance in obese adolescents with fasting hyperinsulinemia and a family history of type 2 diabetes. Pediatrics 2001: 107: e55.
27. Wiegand S, l'Allemand D, Hübel H et al. Metformin and placebo therapy both improve weight management and fasting insulin in obese insulin-resistant adolescents: a prospective, placebo-controlled, randomized study. Eur J Endocrinol 2010: 163: 585–592.
28. Arslanian SA, Lewy V, Danadian K, Saad R. Metformin therapy in obese adolescents with polycystic ovary syndrome and impaired glucose tolerance: amelioration of exaggerated adrenal response to adrenocorticotropin with reduction of insulinemia/insulin resistance. J Clin Endocrinol Metab 2002: 87: 1555–1559.
29. Jones KL, Arslanian S, Peterokova MA, Park J-S, Tomlinson MJ. Effect of metformin in pediatric patients with type 2 diabetes. A randomized controlled trial. Diabetes Care 2002: 25: 89–94.
30. Srinivasan S, Ambler GR, Baur LA et al. Randomized, controlled trial of metformin for obesity and insulin resistance in children and adolescents: Improvement in body composition and fasting insulin. J Clin Endocrinol Metab 2006: 91: 2074–2080.
31. Brufani C, Fintini D, Nobili V, Patera PI, Cappa M, Brufani M. Use of metformin in pediatric age. Pediatr Diabetes 2011: 12: 580–588.
32. Quinn SM, Baur LA, Garnett SP, Cowell CT. Treatment of clinical insulin resistance in children: a systematic review. Obes Rev 2010: 11: 722–730.
33. López-Alarcón M, Martínez-Coronado A, Velarde-Castro O, Rendón-Macías E, Fernández J. Supplementation of n3 long-chain polyunsaturated fatty acid synergistically decreases insulin resistance with weight loss of obese prepubertal and pubertal children. Arch Med Res 2011: 42: 502–508.
34. Balk EM, Lichtenstein AH, Chung M, Kupelnick B, Chew P, Lau J. Effects of omega-3 fatty acids on serum markers of cardiovascular disease risk: a systematic review. Atherosclerosis 2006: 189: 19–30.
35. Mozaffarian D, Wu JHY. Omega-3 fatty acids and cardiovascular disease. Effects on risk factors, molecular pathways, and clinical events. J Am Coll Cardiol 2011: 58: 2047–2067.
36. Oliver E, McGillicuddy F, Phillips C, Toomey S, Roche HM. The role of inflammation and macrophage accumulation in the development of obesity-induced type 2 diabetes mellitus and the possible therapeutic effects of long-chain n-3 PUFA. Proc Nutr Soc 2010: 69: 232–243.
37. Taouis M, Dagou C, Ster C, Durand G, Pinault M, Delaure J. N-3 Polyunsaturated fatty acids prevent the defect of insulin receptor signaling in muscle. Am J Physiol Endocrinol Metab 2002: 282: E664–E671.
38. Figueras M, Olivan M, Busquets S, López-Soriano FJ, Argilés JM. Effects of eicosapentaenoic acid (EPA) treatment on insulin sensitivity in an animal model of diabetes: improvement of the inflammatory status. Obesity 2011: 19: 362–369.
39. Park Y, Harris WS. Omega-3 fatty acid supplementation accelerates chylomicron triglyceride clearance. J Lipid Res 2003: 44: 455–463.
40. Sneddon AA, Tsofliou F, Fyfe CL et al. Effect of a conjugated linoleic acid and ω-3 fatty acid mixture on body composition and adiponectin. Obesity 2008: 16: 1019–1024.
41. Jeffery AN, Metcalf BS, Hosking J, Streeter AJ, Voss LD, Wilkin TJ. Age before stage: insulin resistance rises before the onset of puberty. A 9-year longitudinal study (EarlyBird 26). Diabetes Care 2012: 35: 536–541.