Contemporary Clinical Trials Communications 11 (2018) 55–62

Contents lists available at ScienceDirect

Contemporary Clinical Trials Communications

journal homepage: www.elsevier.com/locate/conctc

The impact of probiotics' administration on glycemic control, body composition, gut microbiome, mitochondria, and other hormonal signals in adolescents with prediabetes – A randomized, controlled trial study protocol

Charikleia Stefanaki[a,*], Flora Bacopoulou[a,b], Athanasios Michos[b]

[a] Choremeion Research Laboratory, First Department of Pediatrics, Faculty of Medicine, National and Kapodistrian University of Athens, "Aghia Sophia" Children's Hospital, Athens, Greece

[b] Center for Adolescent Medicine and UNESCO Chair on Adolescent Health Care, First Department of Pediatrics, Faculty of Medicine, National and Kapodistrian University of Athens, "Aghia Sophia" Children's Hospital, Athens, Greece

ARTICLE INFO

Keywords:
Gut microbiome
Prediabetes
Probiotics
Gut barrier
RCT
Mitochondrion

ABSTRACT

Background: Recent studies have demonstrated that a significant proportion of adolescents exhibit abdominal obesity in early–middle adolescence, and impaired glucose metabolism. Dysregulation of glucose metabolism is aggravated by the existing osteosarcopenia not only in obese but also in overweight youth. Biochemical inflammation, derived from glucose metabolism dysregulation, in combination with increased stress levels lead to the accumulation of reactive oxygen species, also known as ROS, which seem to afflict the integrity of the gastrointestinal wall, gut mucosa, and commensal, intestinal gut microflora. The current scientific protocol aims to assess the administration of probiotics in prediabetic adolescents in relation with their glycemic control, body composition, and intestinal microbiome.

Methods/Design: This is a study protocol of a two-armed RCT, that recruits adolescents with prediabetes, who will receive either a 4-month, life-style intervention, or a life-style intervention along with a probiotic supplement. The primary outcome is the differences in gut microbiome synthesis, body composition analysis parameters, and concentrations of hormones, before and after the intervention.

Discussion: This study aims to halt the progression of obesity and diabetes and aspires to contribute new evidence for upgraded treatment of obesity and diabetes.

Trial registration: Australian New Zealand Clinical Trial Registry (ACTRN12615000470594).

1. Introduction

Hormonal changes occurring during adolescence have various effects on glucose metabolism and body composition changes [1]. Sex steroids, also, lead to the development of secondary sexual characteristics and growth acceleration of puberty.

During this phase, a well-described physiological decrease in insulin sensitivity occurs, which resolves after completion of puberty, independently of changes in body mass index (BMI) [2]. The presence of hyperglycemia i.e. elevated pre-, or postprandial serum glucose concentrations, is due to impaired glucose metabolism, and it usually heralds the onset of Diabetes Mellitus (DM) [3,4]. Hyperglycemia that does not fulfil the diagnostic criteria for DM is called prediabetes (preDM). It results from a combination of defects in insulin secretion, insulin action, or both [5].

Recent studies have demonstrated that a significant proportion of adolescents exhibit abdominal obesity in early–middle adolescence, and impaired glucose metabolism [6,7]. The problem is on the rise, due to the contemporary sedentary lifestyle [8]. Dysregulation of glucose metabolism is aggravated by the existing osteosarcopenia not only in obese but also in overweight youth [9,10].

Current studies suggest the direct link of the hyperglycemic elements of the prediabetic state with micro and macro-vascular complications [11–15]. PreDM suggests unhealthy lifestyle, frequently autoimmunity [16,17], and low-grade, subclinical inflammation [18–22]. Low-grade inflammation is equivalent to biochemical, and psychological stress [23–28]. Biochemical inflammation in combination with increased stress levels lead to the accumulation of reactive oxygen species, also known as ROS, which seem to afflict the integrity of the gastrointestinal wall, gut mucosa, and commensal, intestinal gut

* Corresponding author. First Department of Pediatrics, Faculty of Medicine, National and Kapodistrian University of Athens, Thivon & Levadeias Streets, Goudi, Athens, 11527, Greece.

E-mail addresses: cstefanaki@gmail.com, cstefanak@med.uoa.gr (C. Stefanaki).

https://doi.org/10.1016/j.conctc.2018.06.002
Received 12 January 2018; Received in revised form 20 May 2018; Accepted 1 June 2018
Available online 02 June 2018

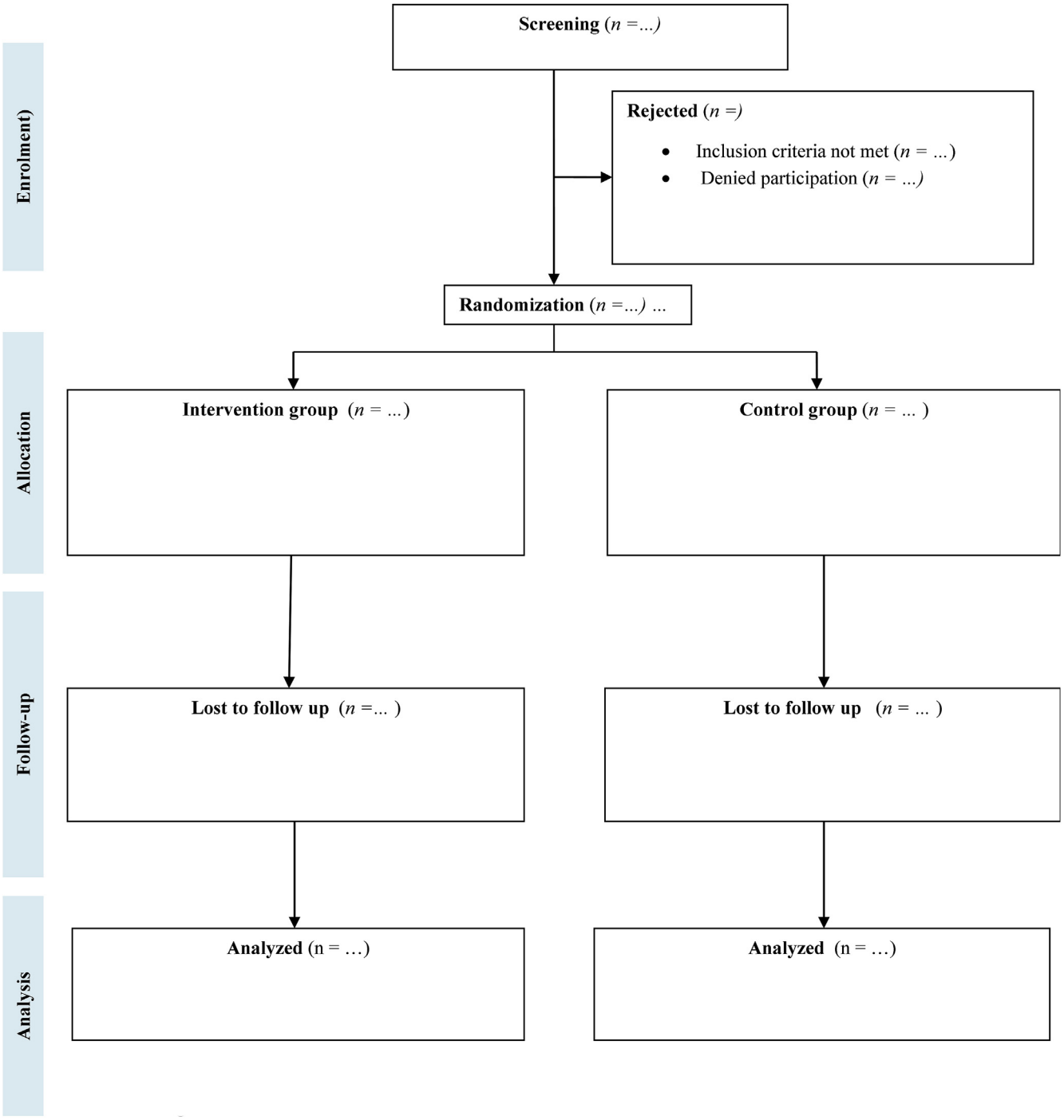

Fig. 1. Recruitment process flow chart.

microflora [29–32].

Commensal bacteria seem to confer metabolic, protective, and trophic functions to the host through fermentation of non-digestible carbohydrates, vitamin production, bile acids biotransformation, barrier protection against opportunistic pathogens, control against gut epithelial cell proliferation and maturation and stimulation of both local and systemic immunity. Once the balance between the intestinal microbiota antigen production and immune cell activation is lost, combined with increased gut permeability, innate and adaptive immune responses are deregulated towards a chronic pro-inflammatory response either locally (gut) or systemically (subclinical, inflammatory responses in obesity, and T2DM) [33]. The imbalance in the gut intestinal flora is called dysbiosis.

Dysbiosis is characterized by either reduced diversity, decreased abundance of butyrate-producing bacteria and an increase in opportunistic pathogens, increased relative abundance of urease- and uricase-harboring, indole- and p-cresol-forming bacteria, reduced short-chain fatty acid producing bacteria, enrichment of mucin-degrading, or low efficient butyrate producing bacteria. Dysbiosis is also present in prediabetic patients. Recent studies and current medical literature reviews have demonstrated that gut dysbiosis might be a triggering point for the preDM and DM onset and it consists a continuous stimulus, serving as a constant inflammatory source in the low-grade inflammation process, which characterizes the metabolic diseases [34–41].

2. Objectives

The current scientific protocol aims to assess the administration of probiotics in prediabetic adolescents in relation with their glycemic control, body composition, and intestinal microbiome. Secondary objectives of this trial are to evaluate the efficacy of the oral administration of probiotics to normalize glycemic control and body composition, along with other hormonal parameters, in adolescents with prediabetes.

3. Methods

This randomized, controlled trial takes place at the Center for Adolescent Medicine and UNESCO Chair on Adolescent Health Care, First Department of Pediatrics, National and Kapodistrian University of Athens, Athens, Greece. The study protocol was approved by the Institutional Review Board of Research and Ethics of the "Aghia Sophia" Children's Hospital (Number of scientific protocols repository: 28931/11.02.2015). It is in accordance with the Helsinki Declaration for Human Studies. The parents and legal guardians of all participants provide informed written consent prior to study participation. The trial is registered at the Australian New Zealand Clinical Trial Registry (ACTRN12615000470594).

3.1. Study design

This study is a two-arm randomized, controlled trial that randomizes in a 1:1 allocation scheme, adolescents aged 12–20 years with prediabetes (according to ADA criteria) to: 1) administration of probiotics, and weekly counseling to promote a healthier lifestyle (Group A); and 2) weekly counseling to promote a healthier lifestyle, alone (Group B). Both interventions are 4 months long and involve two individuals and 8 individual sessions. Participants randomized to Group B will not receive probiotics. Study outcomes will be assessed at baseline, and every month for 4 months.

3.2. Setting and recruitment

Participants are recruited from a senior adolescent medicine specialist at the Center for Adolescent Medicine and UNESCO Chair on Adolescent Health Care, First Department of Pediatrics, National and Kapodistrian University of Athens, Athens, Greece (Fig. 1).

3.3. Participants

Adolescents are screened for eligibility over a routine visit at the Center for Adolescent Medicine and UNESCO Chair on Adolescent Health Care, First Department of Pediatrics, National and Kapodistrian University of Athens, Athens, Greece.

Inclusion criteria: Adolescent males, and females, aged 12–20 years, with willingness to participate in the study and diagnosed with prediabetes, as defined by American Diabetes Association (ADA):

1Hb1AC ranging from 5.7% to 6.4%, and/or
2Fasting serum glucose concentrations ranging from 100 mg/dl to 125 mg/dl, and/or
32 h serum glucose on Oral Glucose Tolerance Test: ranging from 140 mg/dl to 199 mg/dl.

Participants must also understand, read, and write in Greek and plan to reside in the Attiki area for the 4-month duration of the intervention of the study.

Exclusion criteria:

1. Short-bowel syndrome with concurrent D-lactic acidosis.
2. Current hospitalization for any reason.
3. Co-morbid infection of any kind.
4. Co-morbid hereditary and/or acquired immunodeficiency.
5. Genetic defects of insulin action, diseases of the exocrine pancreas, gestational diabetes, endocrinopathies (Cushing's syndrome, acromegaly, glucagonoma, pheochromocytoma, somatostatinoma, aldosteronoma, hyperthyroidism), diabetes induced by drugs or chemicals (vacor, pentamidine, nicotinic acid, glucocorticoids, thyroid hormone, diazoxide, b-adrenergic agonists, thiazides, clozapine, protease inhibitors), or Stiffman's syndrome.
6. Treatment with antibiotics within the past month, prior enrolment and/or intake of yogurts and products containing probiotics or any immuno-modulating agent, whatsoever.
7. Diarrhea, due to the administration of probiotics, that lasts more than 3 days and/or causes severe dehydration.
8. Seropositivity for HBsAg, HBcAb and/or established diagnosis of any hepatic disorder.
9. Inability to commit to clinical trial follow-up.
10. Atopic dermatitis.
11. Epilepsy.
12. Established or possible pregnancy.
13. Presence of metal prostheses, due to orthopedic surgery or otherwise or implanted devices such as pacemakers.
14. Chronic life-threatening disease, such as neoplasia.

3.4. Interventions

3.4.1. Probiotics

Probiotic supplements are administered daily in 2 sachets, postprandially (one sachet after lunch, and the other one after dinner) for 4 months. Each sachet of probiotics contains 450×10^9 CFUs of *Streptococcus thermophilus* (DSM24731), *Bifidobacteria breve* (DSM24732) *Bifidobacteria longum* (DSM24736), *Bifidobacteria infantis* (DSM24737), *Lactobacillus acidophilus* (DSM 24735) *Lactobacillus plantarum* (DSM24730), *Lactobacillus paracasei* (DSM24733), *Lactobacillus delbreuckii* subspecies *bulgaricus* (DSM24734; now available in EU as Vivomixx® and in USA as Visbiome®).

3.4.2. Weekly counseling of a healthier way of life

Weekly counseling to promote a healthier way of life, to restore gut ecology, and glycemic homeostasis, is provided to each adolescent, along with the administration of probiotics.

An accredited dietician counsels the participants to adopt a healthier way of life, once a week. The role of the traditional, Mediterranean diet is emphasized first. Advise is given for high intake of extra virgin (cold pressed) olive oil, vegetables including leafy green vegetables, fruits, cereals, nuts and pulses/legumes, moderate intake of fish and meat, dairy products and red wine, and low intake of eggs and sweets. More specifically, the advice on Mediterranean diet is based on the 1999 Greek Dietary guidelines: olive oil will be the main added lipid/day; vegetables: 6 servings/day; fruits: 3 servings/day; breads and cereals: 8 servings/day, maximum intake; legumes, and nuts: 3–4 servings weekly; fish/seafood: 5–6 servings weekly; eggs: 3 servings weekly; poultry: 4 servings weekly; dairy foods: 2 servings/day; red meat: 8 servings/monthly; no sweets; no wine and no refreshments.

Practice of daily exercise is another pillar of this intervention. Study participants are counselled to perform for a minimum of 30 min, moderate to vigorous physical activity on each school day. Moderate to vigorous exercise is defined as the exercise causing "some increase in breathing and heart rate usually associated (in a healthy person) with brisk walking, dancing, swimming, or cycling on flat terrain." In exercise physiology terms, the energy expended is at least 3 metabolic equivalents (METS) [42–45].

3.4.3. Oral glucose tolerance test

At enrollment, each adolescent will undergoes screening with a standard OGTT, ingesting glucose (APL) dissolved in water in a dosage

of 1.75 g glucose/kg body weight (maximum 75 g) [46], after a 12-h overnight fast. Blood samples are collected at baseline (0 min) and at 30, 60, 90, and 120 min post glucose intake. Blood samples for determination of glucose are collected in vacutainer tubes. Blood samples for determination of insulin are collected in EDTA-containing vacutainer tubes. Tubes are immediately placed on ice and centrifuged at 4 C for 10 min. Plasma is stored in a biobank at −80 C° until analysis.

3.5. Data collection and outcome measures

There is one study site involved in the trial: The Center for Adolescent Medicine and UNESCO Chair on Adolescent Health Care of the First Department of Pediatrics, of Medical School of the National and Kapodistrian University of Athens in Greece. Recruitment occurs at the study site which is an “adolescent friendly” health service where adolescents present either by themselves or following referral by other health professionals. Adolescents and their parents/guardians are informed about the study upon arrival. If they are interested in participating in the study, they are screened for eligibility, after provision of written consent by the adolescents over 18 years of age or by their parents/guardians for the younger teens. The initial assessment includes blood sampling in the morning following 12- hours fast for assessment of glucose and HbA1c. If the adolescents meet the diagnostic criteria for preDM, they provide fecal samples, and afterwards, they undergo an additional screening for preDM with an OGTT. A blood sample of 5 ml is also stored for each participant. Adolescents are clinically evaluated, and counselled every month, for 4 consecutive months.

3.6. Randomization-blinding

An online randomization internet site (www.random.org) is used to assign the participants to intervention and control groups. The randomness comes from atmospheric noise. Random allocation sequence is implemented by a designated clinical assistant who is not otherwise associated with the trial. No blinding, or concealment is used within the groups.

3.7. Sample size

As this is a pilot trial of administration of probiotics in adolescents with preDM in Greece, the aim is to recruit between 12 and 25 participants per group, as medical literature demonstrates, for this number of participants the observed power for a pilot study is 80% [47–50]. Plus, the main aim is to obtain preliminary data that can be used for planning definitive studies, and to is to identify important safety concerns [51].

3.8. Statistical analyses

Statistical analysis will be performed with StatSoft Statistica v.10 and the level of statistical significance will be set to two-sided $P < 0.05$. Characteristics of the study sample will be presented by group, in terms of mean, standard deviation, for quantitative variables, and absolute numbers and percentages, for qualitative variables, in case of normal distribution. In case of abnormal distribution, the respective non-parametric descriptive and inferential statistical tests will be employed. For group comparisons, independent and paired Student's *t*-test, for quantitative variables, in order to evaluate statistically significant differences between the two groups and within the two groups, respectively, before and after the intervention, and x^2 tests, or Fisher's exact tests for qualitative variables will be employed. Pearson's correlation co-efficients will be performed to assess correlation between the variables. Data will be analyzed using the intention-to-treat analysis. No stratification for age groups will be employed due to the small number of anticipated participants.

3.9. Gut microbiome measures

3.9.1. GI effects 2200 kit – GENOVA diagnostics

Semi-quantitative determination of the levels of microorganisms in participants' stool samples is performed with the use of PCR and the incorporation of SYBR green for result determination. According to manufacturer's guidelines stool samples are transferred to the laboratory in C&S media. Stool concentration is determined and recorded as grams stool/ml of C&S media. Genomic DNA is then extracted from a 0.2 ml aliquot of the stool sample.

Twenty-four separate assays for the stated microorganisms are performed under conditions required for specific amplification of each microorganism. The conditions are determined and confirmed using genomic DNA specific for each of the microorganisms (genus and/or species level). The conditions yielding a dose dependent increase in the SYBR green incorporation that coordinate with increasing genomic DNA input are deemed appropriate for analysis. Additionally, appropriate dilutions of patient sample DNA are determined for each of the assays. Two separate dilutions for each patient sample are assayed for each of the 24 microorganism assays.

Once the required number of amplification cycles is complete an aliquot of the PCR product is evaluated spectrophotometrically for the incorporation of SYBR green. Incomplete intercalation is removed via incubation with urea. Results are compared to a genomic standard within each batch that has been amplified at the same time under the same conditions as the patient samples. Two controls are included with each batch to monitor amplification. The two controls have concentrations representative of the patients' results for each of the two dilutions within the assay. The two levels of controls along with the no target control are evaluated and if deemed acceptable reporting of the results will follow. Each of the controls must be within the acceptable range established for the assay and the no target control must not indicate the presence of a contaminant.

For result reporting and comparison to the reference interval, the pg/microliter result of the DNA concentration is converted into CFU/g feces. This is accomplished using the genome size equivalent for each of the microorganisms as well as the initial stool concentration and assay specific sample dilution.

Reference intervals have been determined by screening of 350 individuals that yielded 96 individuals identified as healthy. The assay was performed on the samples submitted by these individuals. The resulting data underwent statistical analysis for outlier removal, quintile distribution and 95% reference interval determination. The reference interval provided on the report and the quintile distribution scale utilized for patient result depiction are derived from this analysis [52].

3.10. Body composition measures

3.10.1. BIA-ACC©

Body composition is assessed in each study participant using the BIA-ACC device (BIOTEKNA Biomedical Technologies, Italy) [53,54].This device applies alternating currents using two different frequencies, 50 and 1.5 kHz (bi-frequency measurement method), to measure body composition, based on a multi-compartment model (2C, 3C, 4C, 5C) [55]. The subjects lay supine on an examination bed, while there is no skin contact with metallic elements. Two skin patches—with a horizontal distance of 10 cm between them—are applied on the dorsal surface of the right hand and an additional pair of skin patches on the dorsal surface of the right foot (Hand-to-Foot). The skin patches are connected to the electrodes of the BIA-ACC® device. The formulas used for computations have been described in detail elsewhere [54]. Fat free mass (skeletal muscles, bones), fat mass, total body water, extracellular body water, intracellular body water, and phase angle are extracted from the raw data output file, for analysis.

3.11. Glycemic control measures

Glycemic control is determined by fasting, morning blood glucose every month and by evaluation of HbA1c at baseline and after the 4-month period of the study.

3.12. Additional hormonal parameters

Additional blood is collected from each study participant into tubes containing SST-Gel, and centrifuged for serum separation. Serum and blood samples are stored in multiple aliquots (serum samples at −80 °C and whole blood at −20 °C) until measurement of additional metabolic parameters. Before analysis, they will be thawed, while protected from light, and auto-oxidation. Additional metabolic parameters that will be assessed in this trial are:

3.12.1. Adiponectin

Adiponectin is mainly secreted by white fat tissue, but it can also be produced by brown adipose tissue to a small extent [56]. It is the most abundant product of adipocytes, and is strongly correlated with cardiometabolic risk. Several previous studies have shown that adiponectin levels are positively associated with insulin sensitivity, and inversely associated with the development of diabetes and progression from prediabetes to type 2 DM. The potential role of adiponectin in modulating early glucose abnormalities during transition from normoglycemia to prediabetes is a subject of significant interest [57,58]. Adiponectin will be assessed in each adolescent at baseline, and after the 4-month period of the study.

3.12.2. Leptin

Leptin is a hormone that has received considerable attention in relation to the fat-bone connection, despite its better-known role in appetite regulation. In humans, production of the adipocyte-derived peptide leptin has been linked to adiposity, insulin, and insulin sensitivity. There are studies that support the notion of its active role in the cardiometabolic health of adolescents [59,60]. Leptin will be assessed in each adolescent at baseline, and after the 4-month period of the study.

3.12.3. Glucagon-like peptide – 1 (GLP-1)

Gut peptides including (glucagon-like peptidesGLPs) have been reported to control epithelial barrier proliferation and integrity. GLP-1 has also been implicated in obesity, and hyperglycemic states [61]. GLP-1 will be assessed in each adolescent at baseline, and after the 4-month period of the study.

3.12.4. MOTS – c

It is a mitochondrial-derived peptide that seems to have profound, and distinct biological activities, providing a paradigm-shifting concept of active mitochondrial-encoded signals that act at the cellular and organism level. MOTS - c has been shown to target the skeletal muscle, enhancing glucose metabolism. Consequently, MOTS-c has implications in the regulation of obesity, diabetes, exercise, and longevity, representing an entirely novel mitochondrial signaling mechanism to regulate metabolism within, and between cells [62–64]. MOTS - c will be assessed in each adolescent at baseline, and after the 4-month period of the study.

4. Discussion

An increasing body of literature reports associations between the gut microbiome, body composition, and chronic, non-communicable diseases, building the groundwork for a hypothesis that suggests modulation of the intestinal microbiota as the factor linking the environment with host genetics. Intestinal epithelial cells emerge as key mediators in the inflammation, and immunity of mucosal tissues [65].

Control of intestinal epithelial stemness is crucial for tissue homeostasis. Disturbances in epithelial function are implicated in inflammatory diseases of the gastrointestinal tract [66]. Human mitochondria are heirs of microbes and altered mitochondrial function has been implicated in processes, ranging from ageing to diabetes [67,68]. Products of bacterial metabolism in the gut are interconnected with the metabolism of mitochondria. The possibility of microbiome-induced changes in human nutrient metabolism that may further impact mitochondrial metabolism is not yet specifically investigated, but indirect evidence supports this argument. Dysfunctional mitochondria seem to be associated with obesity, and they are also observed in numerous metabolic disorders, such as PCOS [69–73]. Given the wide range of possibilities, a useful way forward could be to look at possible intersections between health, mitochondria and gut microbiota [74]. These reasons led to the measurement of MOTS-c concentrations, and body composition in our prediabetic adolescents.

Adiponectin and leptin are well known hormones, deriving mainly from adipocytes. Adiponectin has many actions in several tissues, among which it has been found to improve glucose utilization and fatty acid oxidation in myocytes, increasing energy expenditure in skeletal muscle [75]. Leptin acts primarily in the brain, especially the hypothalamus, where its action is integrated with that of other adipokines, gastrokines, and other signals to coordinate energy homeostasis. Serum leptin increases in proportion to body fat percentage [76]. The administration of probiotics may activate different metabolic pathways, which may affect the metabolism of the adipose tissue. In rat models, the administration of probiotics has demonstrated anti-inflammatory effects [77], or in some cases, even loss of weight, and fat mass [78].

Body composition analysis has been proven to be an accurate means of providing evidence for health status [9,53]. Data from recent studies suggest significant relationships between bone, fat tissue and glucose metabolism in pediatric patients with type I diabetes. These results demonstrate that poor metabolic control is associated with reduced bone formation. On the other hand, fat, fat-free and bone tissues can influence glucose metabolism, potentially in an insulin-dependent manner [79,80]. Similar results have been observed in adults [81]. There is virtually no data about prediabetes and body composition in adolescence. One study that examined a simple, standardized, fitness program in Southwest American Indian adolescents has demonstrated effectiveness in reducing fasting lipids and adiposity, as well as in improving glycemic indices over the course of six months [80]. Last, in another study, administration of metformin, an exercise program, and a structured, reduced energy diet, which was either high-carbohydrate or moderate-carbohydrate with increased-protein, demonstrated clinically significant improvements in obese adolescents at risk of type 2 diabetes [82].

World Health Organization defines probiotics as live microorganisms, which when administered in adequate amounts confer a health benefit to the host. A substantial number of mechanisms seem to be associated with probiotic beneficial effects, such as production of inhibitory substances like H_2O_2, bacteriocins, organic acids, blocking of adhesion sites for pathogenic bacteria, competition with the pathogenic microorganisms for nutrients, degradation of toxins as well as blocking of toxin receptors, and, finally, modulation of immune responses [83]. Growing evidence suggests administration of probiotics as a useful prevention in pre-DM, T1DM and T2DM onset. Probiotic administration is minimally invasive, and it may prove to be successful in ameliorating glycemic control, or even reverse the pathogenic process in these patients, by ameliorating their body composition, or even their cellular structure. The disease burden is high. Its prevalence is estimated about 366 million of patients, globally, for the age group 20–79 years, alone. The 11% of health expenditures is attributed to diabetes and it is the cause of 465 million of USD loss, annually [84].

In Saudi Arabia, the prevalence of prediabetes is 5.4%, based on oral glucose tolerance test (OGTT), and 21.9% based on HbA1c [85]. According to epidemiological data from the UK, the prevalence rate of

prediabetes in the general population increased from 2003 to 2011 [86]. An increase in the prevalence of prediabetes has also been observed in China, where prediabetes has risen in the general population [87]. In a US national cohort study of a representative sample of adolescents, it was estimated that the national population-based prevalence rates of IFG, IGT, and prediabetes among adolescents, aged 12–19 years were 13.1%, 3.4%, and 16.1%, respectively [88]. Worldwide, epidemiological trends demonstrate increases in the prevalence of prediabetes in the general population; adolescents seem to follow these trends, irrespectively of race.

There is still no clear consensus on the definition of prediabetes, or metabolic syndrome in adolescents. In this study, we use the criteria of prediabetes in adults. However, there are only a few observational studies about prediabetes in adolescence [89]. Moreover, most adolescents are screened for preDM, using inadequate, or inaccurate means. But, given that the progression of the glucose homeostasis alterations in adolescents are usually rapid and insidious, BMI seems to be inadequate for prediabetes screening in adolescence. In overweight and obese subjects, measuring only fasting plasma glucose concentrations and HbA1c ratio are also inadequate means for prediabetes screening. OGTT remains the gold standard for the direct evaluation of insulin secretion, and the indirect evaluation of the degree of insulin resistance [1].

A challenges of the present study is the recruitment procedure, since the discrimination between an evolving preDM condition and physiologic insulin resistance is arduous, rendering pre-DM as a rare condition in adolescence [1]. We are also using the Center for Adolescent Medicine and UNESCO Chair on Adolescent Health Care of the First Department of Pediatrics of the National and Kapodistrian University of Athens, as recruitment and delivery of treatment site, and despite experience, our methods will still need to be tested, and refined in that locality. Having broad inclusion criteria should help in recruitment, and these may be adjusted over time if recruitment rates are low. Another barrier of recruitment is the period of 4 month that might prove long, and may result in loss to follow-up.

To the best of our knowledge, this is the first study, to evaluate the impact of probiotics on the glycemic control of prediabetic adolescents, and possibly to halt the progression of obesity and diabetes that aspires to contribute new evidence for upgraded treatment of obesity and diabetes.

Conflicts of interest

None to disclose.

Author's contributions

This study is part of the PhD thesis of CS. CS designed the study, and wrote the initial draft of the manuscript, FB is responsible for recruitment of study participants. FB and AM supervised the study and revised the manuscript.

Acknowledgements

GENOVA Diagnostics provides the fecal microbiome kits for the study, and AM Health, Greece provides the probiotics sachets. Companies have not participated in the study design and have not intervened in the analysis of the study results.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.conctc.2018.06.002.

References

[1] C. Stefanaki, F. Bacopoulou, M. Peppa, Prediabetes and adolescence—trends, causes, effects, and screening, US Endocrinol. 12 (2) (2016) 94–98.
[2] C.M. Burt Solorzano, C.R. McCartney, Obesity and the pubertal transition in girls and boys, Reproduction 140 (3) (2010) 399–410.
[3] M. Roden, Diabetes mellitus: definition, classification and diagnosis, Wien Klin. Wochenschr. 128 (Suppl 2) (2016) S37–S40.
[4] A.A. Kazi, L. Blonde, Classification of diabetes mellitus, Clin. Lab. Med. 21 (1) (2001) 1–13.
[5] N. Unwin, J. Shaw, P. Zimmet, K.G. Alberti, Impaired glucose tolerance and impaired fasting glycaemia: the current status on definition and intervention, Diabet. Med. 19 (9) (2002) 708–723.
[6] P.C. Brar, P. Patel, S. Katz, The relationship between insulin resistance and endothelial dysfunction in obese adolescents, J. Pediatr. Endocrinol. Metab. 30 (6) (2017) 635–642.
[7] F. Bacopoulou, G. Landis, A. Rentoumis, A. Tsitsika, V. Efthymiou, Mediterranean diet decreases adolescent waist circumference, Eur. J. Clin. Invest. 47 (6) (2017) 447–455.
[8] A.K. Tsitsika, E.K. Andrie, T. Psaltopoulou, C.K. Tzavara, T.N. Sergentanis, I. Ntanasis-Stathopoulos, F. Bacopoulou, C. Richardson, G.P. Chrousos, M. Tsolia, Association between problematic internet use, socio-demographic variables and obesity among European adolescents, Eur. J. Publ. Health 26 (4) (2016) 617–622.
[9] C. Stefanaki, M. Peppa, D. Boschiero, G.P. Chrousos, Healthy overweight/obese youth: early osteosarcopenic obesity features, Eur. J. Clin. Invest. 46 (9) (2016) 767–778.
[10] M.R. Laurent, V. Dubois, F. Claessens, S.M. Verschueren, D. Vanderschueren, E. Gielen, F. Jardi, Muscle-bone interactions: from experimental models to the clinic? A critical update, Mol. Cell. Endocrinol. 432 (2016) 14–36.
[11] S.R. Zatalia, H. Sanusi, The role of antioxidants in the pathophysiology, complications, and management of diabetes mellitus, Acta Med. Indones. 45 (2) (2013) 141–147.
[12] H. Mangiapane, Cardiovascular disease and diabetes, Adv. Exp. Med. Biol. 771 (2012) 219–228.
[13] C. Campos, Chronic hyperglycemia and glucose toxicity: pathology and clinical sequelae, Postgrad. Med. 124 (6) (2012) 90–97.
[14] A.D. Baron, Impaired glucose tolerance as a disease, Am. J. Cardiol. 88 (6A) (2001) 16H-9H.
[15] R.E. Pratley, The early treatment of type 2 diabetes, Am. J. Med. 126 (9 Suppl 1) (2013) S2–S9.
[16] H. Tfayli, S. Arslanian, Pathophysiology of type 2 diabetes mellitus in youth: the evolving chameleon, Arq. Bras. Endocrinol. Metabol. 53 (2) (2009) 165–174.
[17] H. Tfayli, F. Bacha, N. Gungor, S. Arslanian, Islet cell antibody-positive versus -negative phenotypic type 2 diabetes in youth: does the oral glucose tolerance test distinguish between the two? Diabetes Care 33 (3) (2010) 632–638.
[18] D.C. Ziemer, P. Kolm, J.K. Foster, W.S. Weintraub, V. Vaccarino, M.K. Rhee, R.M. Varughese, C.W. Tsui, D.D. Koch, J.G. Twombly, K.M. Narayan, L.S. Phillips, Random plasma glucose in serendipitous screening for glucose intolerance: screening for impaired glucose tolerance study 2, J. Gen. Intern. Med. 23 (5) (2008) 528–535.
[19] H. Tsui, S. Winer, Y. Chan, D. Truong, L. Tang, J. Yantha, G. Paltser, H.M. Dosch, Islet glia, neurons, and beta cells, Ann. N. Y. Acad. Sci. 1150 (2008) 32–42.
[20] E.C. Eringa, E.H. Serne, R.I. Meijer, C.G. Schalkwijk, A.J. Houben, C.D. Stehouwer, Y.M. Smulders, V.W. van Hinsbergh, Endothelial dysfunction in (pre)diabetes: characteristics, causative mechanisms and pathogenic role in type 2 diabetes, Rev. Endocr. Metab. Disord. 14 (1) (2013) 39–48.
[21] S.M. Grundy, Pre-diabetes, metabolic syndrome, and cardiovascular risk, J. Am. Coll. Cardiol. 59 (7) (2012) 635–643.
[22] W. Xie, L. Du, Diabetes is an inflammatory disease: evidence from traditional Chinese medicines, Diabetes Obes. Metabol. 13 (4) (2011) 289–301.
[23] I.J. Elenkov, G.P. Chrousos, Stress system–organization, physiology and immunoregulation, Neuroimmunomodulation 13 (5–6) (2006) 257–267.
[24] I.J. Elenkov, D.G. Iezzoni, A. Daly, A.G. Harris, G.P. Chrousos, Cytokine dysregulation, inflammation and well-being, Neuroimmunomodulation 12 (5) (2005) 255–269.
[25] I.J. Elenkov, G.P. Chrousos, Stress hormones, proinflammatory and antiinflammatory cytokines, and autoimmunity, Ann. N. Y. Acad. Sci. 966 (2002) 290–303.
[26] Z. Stankovic, M. Jasovic-Gasic, M. Zamaklar, Psycho-social and clinical variables associated with depression in patients with type 2 diabetes, Psychiatr. Danub. 23 (1) (2011) 34–44.
[27] M.L. Marcovecchio, F. Chiarelli, The effects of acute and chronic stress on diabetes control, Sci. Signal. 5 (247) (2012) pt10.
[28] C.E. Lloyd, P.H. Dyer, R.J. Lancashire, T. Harris, J.E. Daniels, A.H. Barnett, Association between stress and glycemic control in adults with type 1 (insulin-dependent) diabetes, Diabetes Care 22 (8) (1999) 1278–1283.
[29] K.A. Lee, W.J. Lee, Drosophila as a model for intestinal dysbiosis and chronic inflammatory diseases, Dev. Comp. Immunol. 42 (1) (2014) 102–110.
[30] L.C. Yu, J.T. Wang, S.C. Wei, Y.H. Ni, Host-microbial interactions and regulation of intestinal epithelial barrier function: from physiology to pathology, World J. Gastrointest. Pathophysiol. 3 (1) (2012) 27–43.
[31] G. Faria, J. Preto, A.B. Almeida, J.T. Guimaraes, C. Calhau, A. Taveira-Gomes, Fasting glycemia: a good predictor of weight loss after RYGB, Surg. Obes. Relat. Dis. 10 (3) (2014) 419–424.
[32] S. Grenham, G. Clarke, J.F. Cryan, T.G. Dinan, Brain-gut-microbe communication in

health and disease, Front. Physiol. 2 (2011) 94.
[33] C. Stefanaki, M. Peppa, G. Mastorakos, G.P. Chrousos, Examining the gut bacteriome, virome, and mycobiome in glucose metabolism disorders: are we on the right track? Metabolism 73 (2017) 52–66.
[34] J.M. Sosenko, J.S. Skyler, C.A. Beam, J.P. Krischer, C.J. Greenbaum, J. Mahon, L.E. Rafkin, D. Matheson, K.C. Herold, J.P. Palmer, T. Type 1 Diabetes, G. Diabetes Prevention Trial-Type 1 Study, Acceleration of the loss of the first-phase insulin response during the progression to type 1 diabetes in diabetes prevention trial-type 1 participants, Diabetes 62 (12) (2013) 4179–4183.
[35] R. Burcelin, C. Chabo, V. Blasco-Baque, M. Serino, J. Amar, Intestinal microbiota and novel therapeutic perspectives for the treatment of metabolic diseases, Med. Sci. (Paris) 29 (8–9) (2013) 800–806.
[36] J. Amar, M. Serino, C. Lange, C. Chabo, J. Iacovoni, S. Mondot, P. Lepage, C. Klopp, J. Mariette, O. Bouchez, L. Perez, M. Courtney, M. Marre, P. Klopp, O. Lantieri, J. Dore, M. Charles, B. Balkau, R. Burcelin, D.E.S.I.R.S. Group, Involvement of tissue bacteria in the onset of diabetes in humans: evidence for a concept, Diabetologia 54 (12) (2011) 3055–3061.
[37] X. Zhang, D. Shen, Z. Fang, Z. Jie, X. Qiu, C. Zhang, Y. Chen, L. Ji, Human gut microbiota changes reveal the progression of glucose intolerance, PLoS One 8 (8) (2013) e71108.
[38] O.Y. Kim, J.H. Lee, G. Sweeney, Metabolomic profiling as a useful tool for diagnosis and treatment of chronic disease: focus on obesity, diabetes and cardiovascular diseases, Expert Rev. Cardiovasc Ther. 11 (1) (2013) 61–68.
[39] S. Brugman, F.A. Klatter, J.T. Visser, A.C. Wildeboer-Veloo, H.J. Harmsen, J. Rozing, N.A. Bos, Antibiotic treatment partially protects against type 1 diabetes in the Bio-Breeding diabetes-prone rat. Is the gut flora involved in the development of type 1 diabetes? Diabetologia 49 (9) (2006) 2105–2108.
[40] F. Greenway, S. Wang, M. Heiman, A novel cobiotic containing a prebiotic and an antioxidant augments the glucose control and gastrointestinal tolerability of metformin: a case report, Benef. Microbes 5 (1) (2014) 29–32.
[41] C. Rebello, F.L. Greenway, N.V. Dhurandhar, Functional foods to promote weight loss and satiety, Curr. Opin. Clin. Nutr. Metab. Care 17 (6) (2014) 596–604.
[42] R.R. Pate, M.G. Davis, T.N. Robinson, E.J. Stone, T.L. McKenzie, J.C. YoungP.A. American heart association Council on Nutrition, Metabolism, Y. Council on Cardiovascular Disease in the, N. Council on Cardiovascular, Promoting physical activity in children and youth: a leadership role for schools: a scientific statement from the American heart association Council on nutrition, physical activity, and metabolism (physical activity committee) in collaboration with the councils on cardiovascular disease in the young and cardiovascular nursing, Circulation 114 (11) (2006) 1214–1224.
[43] G.P. August, S. Caprio, I. Fennoy, M. Freemark, F.R. Kaufman, R.H. Lustig, J.H. Silverstein, P.W. Speiser, D.M. Styne, V.M. Montori, S. Endocrine, Prevention and treatment of pediatric obesity: an endocrine society clinical practice guideline based on expert opinion, J. Clin. Endocrinol. Metab. 93 (12) (2008) 4576–4599.
[44] G. Horgan, Healthier lifestyles series: 1. Exercise for children, J. Fam. Health Care 15 (1) (2005) 15–17.
[45] S. Nguyen, A.L. Hacker, M. Henderson, T. Barnett, M.E. Mathieu, L. Pagani, J.L. Bigras, Physical activity programs with post-intervention follow-up in children: a comprehensive review according to categories of intervention, Int. J. Environ. Res. Publ. Health 13 (7) (2016).
[46] A. Forslund, J. Staaf, J. Kullberg, I. Ciba, M. Dahlbom, P. Bergsten, Uppsala longitudinal study of childhood obesity: protocol description, Pediatrics 133 (2) (2014) e386–e393.
[47] S.A. Julious, Sample size of 12 per group rule of thumb for a pilot study, Pharmaceut. Stat. 4 (2005) 287–291.
[48] J. Sim, M. Lewis, The size of a pilot study for a clinical trial should be calculated in relation to considerations of precision and efficiency, J. Clin. Epidemiol. 65 (3) (2012) 301–308.
[49] C.G. Moore, R.E. Carter, P.J. Nietert, P.W. Stewart, Recommendations for planning pilot studies in clinical and translational research, Clin Transl Sci 4 (5) (2011) 332–337.
[50] A.L. Whitehead, S.A. Julious, C.L. Cooper, M.J. Campbell, Estimating the sample size for a pilot randomised trial to minimise the overall trial sample size for the external pilot and main trial for a continuous outcome variable, Stat. Meth. Med. Res. 25 (3) (2016) 1057–1073.
[51] A. Jacobs, Power calculations for pilot studies, Br. Med. J. 325 (2002) 491.
[52] M.A. Stefanaki C, E. Roma-Giannikou, E. Mantzou, G. Paltoglou, D. Landis, F. Bacopoulou, Adolescents with prediabetes lack key species by quantitative, core gut microbiome analysis - a case-control study, in: T.E. Society (Ed.), Endocrine Society's 98th Annual Meeting and Expo, Massachusetts, Boston, 2016.
[53] M. Peppa, C. Stefanaki, A. Papaefstathiou, D. Boschiero, G. Dimitriadis, G.P. Chrousos, Bioimpedance analysis vs. DEXA as a screening tool for osteosarcopenia in lean, overweight and obese Caucasian postmenopausal females, Hormones (Basel) 16 (2) (2017) 181–193.
[54] C. Tsigos, C. Stefanaki, G.I. Lambrou, D. Boschiero, G.P. Chrousos, Stress and inflammatory biomarkers and symptoms are associated with bioimpedance measures, Eur. J. Clin. Invest. 45 (2) (2015) 126–134.
[55] Z.M. Wang, R.N. Pierson Jr., S.B. Heymsfield, The five-level model: a new approach to organizing body-composition research, Am. J. Clin. Nutr. 56 (1) (1992) 19–28.
[56] Z.V. Wang, P.E. Scherer, Adiponectin, the past two decades, J. Mol. Cell Biol. 8 (2) (2016) 93–100.
[57] J.L. Parker-Duffen, K. Walsh, Cardiometabolic effects of adiponectin, Best Pract. Res. Clin. Endocrinol. Metabol. 28 (1) (2014) 81–91.
[58] J.C. Winer, T.L. Zern, S.E. Taksali, J. Dziura, A.M. Cali, M. Wollschlager, A.A. Seyal, R. Weiss, T.S. Burgert, S. Caprio, Adiponectin in childhood and adolescent obesity and its association with inflammatory markers and components of the metabolic syndrome, J. Clin. Endocrinol. Metab. 91 (11) (2006) 4415–4423.
[59] K.V. Giudici, J.M. Kindler, B.R. Martin, E.M. Laing, G.P. McCabe, L.D. McCabe, D.B. Hausman, L.A. Martini, R.D. Lewis, C.M. Weaver, M. Peacock, K.M. Hill Gallant, Associations among osteocalcin, leptin and metabolic health in children ages 9-13 years in the United States, Nutr. Metab. 14 (2017) 25.
[60] F. Bacha, D. Edmundowicz, K. Sutton-Tyrell, S. Lee, H. Tfayli, S.A. Arslanian, Coronary artery calcification in obese youth: what are the phenotypic and metabolic determinants? Diabetes Care 37 (9) (2014) 2632–2639.
[61] D.C. Lau, H. Teoh, Current and emerging pharmacotherapies for weight management in prediabetes and diabetes, Can. J. Diabetes 39 (Suppl 5) (2015) S134–S141.
[62] C. Lee, K.H. Kim, P. Cohen, MOTS-c: a novel mitochondrial-derived peptide regulating muscle and fat metabolism, Free Radic. Biol. Med. 100 (2016) 182–187.
[63] C. Lee, J. Zeng, B.G. Drew, T. Sallam, A. Martin-Montalvo, J. Wan, S.J. Kim, H. Mehta, A.L. Hevener, R. de Cabo, P. Cohen, The mitochondrial-derived peptide MOTS-c promotes metabolic homeostasis and reduces obesity and insulin resistance, Cell Metabol. 21 (3) (2015) 443–454.
[64] K. Zarse, M. Ristow, A mitochondrially encoded hormone ameliorates obesity and insulin resistance, Cell Metabol. 21 (3) (2015) 355–356.
[65] A. Michos, A. Terzidis, M. Kanariou, V. Kalampoki, C. Koilia, M. Giannaki, M. Liatsis, A. Pangalis, E. Petridou, Association of allergic sensitization with infectious diseases burden in Roma and non-Roma children, Pediatr. Allergy Immunol.: Off. Publ. Eur. Soc. Pediatr. Allergy Immunol. 22 (2) (2011) 243–248.
[66] E. Berger, E. Rath, D. Yuan, N. Waldschmitt, S. Khaloian, M. Allgauer, O. Staszewski, E.M. Lobner, T. Schottl, P. Giesbertz, O.I. Coleman, M. Prinz, A. Weber, M. Gerhard, M. Klingenspor, K.P. Janssen, M. Heikenwalder, D. Haller, Mitochondrial function controls intestinal epithelial stemness and proliferation, Nat. Commun. 7 (2016) 13171.
[67] P. Bajpai, A. Darra, A. Agrawal, Microbe-mitochondrion crosstalk and health: an emerging paradigm, Mitochondrion 39 (2018 Mar) 20–25.
[68] S. Shinjo, S. Jiang, M. Nameta, T. Suzuki, M. Kanai, Y. Nomura, N. Goda, Disruption of the mitochondria-associated ER membrane (MAM) plays a central role in palmitic acid-induced insulin resistance, Exp. Cell Res. 359 (1) (2017) 86–93.
[69] K. Boengler, M. Kosiol, M. Mayr, R. Schulz, S. Rohrbach, Mitochondria and ageing: role in heart, skeletal muscle and adipose tissue, J. Cachexia, Sarcopenia Muscle 8 (3) (2017) 349–369.
[70] J.C. Bournat, C.W. Brown, Mitochondrial dysfunction in obesity, Current opinion in endocrinology, Diabetes Obes. 17 (5) (2010) 446–452.
[71] J.W. Gordon, V.W. Dolinsky, W. Mughal, G.R. Gordon, J. McGavock, Targeting skeletal muscle mitochondria to prevent type 2 diabetes in youth, Biochem. Cell Biol. = Biochimie et biologie cellulaire 93 (5) (2015) 452–465.
[72] A. Gonzalez-Franquesa, M.E. Patti, Insulin resistance and mitochondrial dysfunction, Adv. Exp. Med. Biol. 982 (2017) 465–520.
[73] J. Rieusset, Contribution of mitochondria and endoplasmic reticulum dysfunction in insulin resistance: distinct or interrelated roles? Diabetes Metabol. 41 (5) (2015) 358–368.
[74] W. Xu, S. Ghosh, S.A. Comhair, K. Asosingh, A.J. Janocha, D.A. Mavrakis, C.D. Bennett, L.L. Gruca, B.B. Graham, K.A. Queisser, C.C. Kao, S.H. Wedes, J.M. Petrich, R.M. Tuder, S.C. Kalhan, S.C. Erzurum, Increased mitochondrial arginine metabolism supports bioenergetics in asthma, J. Clin. Invest. 126 (7) (2016) 2465–2481.
[75] A.E. Achari, S.K. Jain, Adiponectin, a therapeutic target for obesity, diabetes, and endothelial dysfunction, Int. J. Mol. Sci. 18 (6) (2017).
[76] M.B. Allison, M.G. Myers Jr., 20 years of leptin: connecting leptin signaling to biological function, J. Endocrinol. 223 (1) (2014) T25–T35.
[77] I. Novotny Nunez, C. Maldonado Galdeano, A. de Moreno de LeBlanc, G. Perdigon, Lactobacillus casei CRL 431 administration decreases inflammatory cytokines in a diet-induced obese mouse model, Nutrition 31 (7–8) (2015) 1000–1007.
[78] L. Crovesy, M. Ostrowski, D. Ferreira, E.L. Rosado, M. Soares-Mota, Effect of Lactobacillus on body weight and body fat in overweight subjects: a systematic review of randomized controlled clinical trials, Int. J. Obes. 41 (11) (2017) 1607–1614.
[79] A. Wedrychowicz, M. Stec, K. Sztefko, J.B. Starzyk, Associations between bone, fat tissue and metabolic control in children and adolescents with type 1 diabetes mellitus, Exp. Clin. Endocrinol. Diabetes: Off. J. German Soc. Endocrinol. German Diabetes Assoc. 122 (8) (2014) 491–495.
[80] L. Colip, M.R. Burge, P. Sandy, D. Ghahate, J. Bobelu, T. Faber, V. Shah, Exercise intervention improves the metabolic profile and body composition of southwestern American indian adolescents, J. Diabetes Obes. 3 (3) (2016).
[81] M. Rabijewski, L. Papierska, P. Piatkiewicz, An association between bone mineral density and anabolic hormones in middle-aged and elderly men with prediabetes, Aging Male: Off. J. Int. Soc. Study Aging Male 20 (3) (2017) 205–213.
[82] S.P. Garnett, M. Gow, M. Ho, L.A. Baur, M. Noakes, H.J. Woodhead, C.R. Broderick, K. Chisholm, J. Briody, S. De, K. Steinbeck, S. Srinivasan, G.R. Ambler, C.T. Cowell, Improved insulin sensitivity and body composition, irrespective of macronutrient intake, after a 12 month intervention in adolescents with pre-diabetes; RESIST a randomised control trial, BMC Pediatr. 14 (2014) 289.
[83] K.R. Pandey, S.R. Naik, B.V. Vakil, Probiotics, prebiotics and synbiotics- a review, J. Food Sci. Technol. 52 (12) (2015) 7577–7587.
[84] D.R. Whiting, L. Guariguata, C. Weil, J. Shaw, IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030, Diabetes Res. Clin. Pract. 94 (3) (2011) 311–321.
[85] E. Al Amiri, M. Abdullatif, A. Abdulle, N. Al Bitar, E.Z. Afandi, M. Parish, G. Darwiche, The prevalence, risk factors, and screening measure for prediabetes and diabetes among Emirati overweight/obese children and adolescents, BMC Public Health 15 (2015) 1298.
[86] A.G. Mainous 3rd, R.J. Tanner, R. Baker, C.E. Zayas, C.A. Harle, Prevalence of

prediabetes in England from 2003 to 2011: population-based, cross-sectional study, BMJ open 4 (6) (2014) e005002.
[87] S. Li, S. Guo, F. He, M. Zhang, J. He, Y. Yan, Y. Ding, J. Zhang, J. Liu, H. Guo, S. Xu, R. Ma, Prevalence of diabetes mellitus and impaired fasting glucose, associated with risk factors in rural Kazakh adults in Xinjiang, China, Int. J. Environ. Res. Publ. Health 12 (1) (2015) 554–565.
[88] C. Li, E.S. Ford, G. Zhao, A.H. Mokdad, Prevalence of pre-diabetes and its association with clustering of cardiometabolic risk factors and hyperinsulinemia among U.S. adolescents: national Health and Nutrition Examination Survey 2005-2006, Diabetes Care 32 (2) (2009) 342–347.
[89] S. Cook, M. Weitzman, P. Auinger, M. Nguyen, W.H. Dietz, Prevalence of a metabolic syndrome phenotype in adolescents: findings from the third National Health and Nutrition Examination Survey, 1988-1994, Arch. Pediatr. Adolesc. Med. 157 (8) (2003) 821–827.