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Gianfranco Donelli *Editor*

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Gianfranco Donelli
Editor

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Personalized Nutrition for Microbiota Correction and Metabolism Restore in Type 2 Diabetes Mellitus Patients

Tamara Meleshko, Roman Rukavchuk, Olga Levchuk, and Nadiya Boyko

Abstract

Type 2 diabetes is one of the most common noncommunicable diseases in the world. Recent studies suggest a link between type 2 diabetes and microbiota, as well as the ability to treat and prevent it using personalized approaches to nutrition. In this work, we conducted clinical studies on the effects of a personalized diet on 56 female patients. Biochemical, physical, and immunological parameters were measured by standard methods on days 1 and 18 of the experiment. Gut and oral microbiota studies were performed in dynamics on days 1, 7, 11, and 18 using real-time polymerase chain reaction. With the help of the developed information system, a personalized diet was developed for each participant of the experiment. In the group of patients following personalized diets a statistically significant decreasing levels of glucose, thymol test, creatinine, very low-density lipoprotein, urea, secretory IgA, and tumour necrosis factor-α, and improvement in all physical parameters were observed. There was a statistically significant increase in uric acid, sodium, and magnesium. Statistically significant changes in gut microbiota were observed in *Enterococcus faecalis*, *Escherichia coli* (lac+, lac−), *Lactobacillus* spp., and *Candida* spp. Such microorganisms of oral microbiota as *E. faecalis*, *Lactobacillus* spp., *Pseudomonas aeruginosa*, and *Candida* spp. demonstrated statistically significant changes. All these changes indicate an improvement in the patients' condition in the experimental group compared to the control group. Our algorithm used for the development of personalized diets for patients with diabetes type 2 demonstrated clinical efficacy of its implementation.

Keywords

Human microbiota · Metabolism regulation · Noncommunicable disease · Personalised diet · Prognostic correction

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1 Introduction

Type 2 diabetes mellitus (T2D) is a growing global health problem closely related to the epidemic of obesity. It is characterized by dysregulation of carbohydrate, lipid, and protein metabolism and results from impaired insulin secretion, insulin resistance, or a combination of both (DeFronzo et al. 2015). T2D is one of the noncommunicable diseases (NCDs) common among almost all people in the world (Raychaudhuri 2011) regardless of their age and region due to the changes in lifestyles, genetics, and environmental factors, all of which together influence the disorder (Raj et al. 2018).

Typical clinical markers of type 2 diabetes include glucose and glycosylated haemoglobin, increased cholesterol, triglycerides, low-density lipoprotein, very low-density lipoprotein, and decreased high-density lipoprotein (Krauss 2004). Metabolic parameters such as urea, uric acid, creatinine, bilirubin, calcium, magnesium, sodium, alanine aminotransferase, and others involved in lipid profile regulation are an additional source of complete information about the biochemical status of the human body. Recent researches have demonstrated that the development of low-grade inflammation is a consequence of gut microbiota alteration, which is closely related to metabolic disorders such as obesity and T2D (Cani et al. 2012; Minihane et al. 2015). In particular, in the majority of patients suffering from diabetes the levels of *Bifidobacterium* and *Lactobacillus* decrease, which leads to an increase in the levels of *Bacteroides, Prevotella, Peptococcus, Clostridium, Proteus, Staphylococcus,* and *Candida*. Importantly, T2D subjects have smaller amounts of butyrate producing bacteria, such as *Roseburia intestinalis* and *Faecalibacterium prausnitzii*, and a mucus-degrading bacterium *Akkermansia muciniphila* (Tilg and Moschen 2014).

Studies conducted within the "Human Microbiome" project (Group et al. 2009) demonstrated that intestinal microbiome can be dominated by different ratios of beneficial microorganisms and still perform identical functions. Thus, it is not only the species composition of the microbiome, but also its "function" that is important. Herewith, it is obvious that the microbiome of each individual is unique.

Recently, numerous research studies have been conducted to find a relationship between nutrition and its impact on human health. Nevertheless, today a balanced diet principle remains practically unapplied. The reason is, on the one hand, that people misunderstand (underestimate) the role of food as a source of essential balanced nutrients. On the other hand, there are huge amounts of data on "proper nutrition" (rational nutrition) available and they are often contradictory, scientifically unsubstantiated, and clinically unconfirmed. A new modern challenge is the use of P4 (predictive, preventive, personalised, and participatory) approaches, in particular personalized nutrition, in medical practice.

The diet-microbiome interplay is currently the basis for personalized nutrition introduction and microbiota composition is the key factor affecting responsiveness to nutritional interventions that will soon take into account initial stratification of individuals on the basis of microbiota (Ercolini and Fogliano 2018).

The health benefits of adherence to the Mediterranean diet, as well as the relationship between microbiota and its associated metabolome in people consuming varied diets ranging from vegan to omnivorous, are now evidence-based (Shanahan et al. 2017).

In our opinion, the most promising way of individual microbiome correction, as well as prognostic modulation of local immune response, is the use of complete personalized diets rather than individual components. The most popular diets whose positive health effects on the human body are considered to be established include the Mediterranean diet, vegetarian/vegan diet, high-fibre diet, and high-protein diet.

The antioxidant and anti-inflammatory effects of the Mediterranean diet on the whole as well as the effects of this diet's individual components, in particular olive oil, fruits and vegetables, whole grains, and fish, have a beneficial impact on abdominal obesity, lipids levels, glucose metabolism, and blood pressure levels (Kastorini et al.

2011). Gut microbiota in individuals following the Mediterranean diet is characterized by high levels of *Lactobacillus* spp., *Bifidobacterium* spp., and *Prevotella* spp. and low levels of *Clostridium* spp., which relates to weight loss, improvement of the lipid profile, and decreased inflammation (Singh et al. 2017).

For vegetarians and vegans, the most relevant risk factors for chronic disease, such as body mass index (BMI), lipid variables, and fasting glucose, are significantly lower. People following a plant-based dietary pattern demonstrate significantly lower levels of BMI, total cholesterol, LDL-cholesterol, triglycerides, and blood glucose when vegetarians were compared to nonvegetarians, and lower levels of BMI, total cholesterol, and LDL-cholesterol when vegans were compared to nonvegans (Dinu et al. 2017). People following vegan and vegetarian diets rich in fermentable plant-based foods were reported to have a microbiota characterized by a lower abundance of *Bacteroides* spp. and *Bifidobacterium* spp. (Wu et al. 2016).

High fibre intake is associated with lower serum cholesterol concentrations, lower risk of coronary heart disease, reduced blood pressure, enhanced weight control, better glycaemic control, reduced risk of certain forms of cancer, and improved gastrointestinal function (Anderson et al. 2009). One study revealed that three diets containing different fibre-rich whole grains (barley, brown rice, or a combination of both) increased microbial diversity, the Firmicutes/Bacteroidetes ratio, and the abundance of the genus *Blautia* in faecal samples (Oriach et al. 2016).

High-protein diet decreases weight, fasting glucose, and insulin concentrations as well as total and abdominal fat. In addition, this diet significantly decreases LDL cholesterol concentrations (Parker et al. 2002). Dietary protein intake in humans has been associated with the *Bacteroides* enterotype (Oriach et al. 2016).

In previous studies, we obtained data demonstrating that extracts of certain edible plants rich in biologically active substances (BAS) specifically stimulate the immune response and have anti-inflammatory properties. We also proved that these extracts are able to specifically modulate intestinal microbiota (Bati and Boyko 2013).

In our previous studies involving different mouse models, we showed the molecular mechanism by which different gut commensal representatives modulate local immune response at mucosal sites in a strain- or species-specific manner. We were able to analyse *in vitro* the effects of individual commensal bacteria on human monocyte-derived dendritic cells (moDCs)-mediated inflammation and effector T-lymphocyte priming conditions mimicking unique intestinal microenvironment. Human moDCs expressing peroxisome proliferator-activated receptor gamma (PPARγ) also regulate cell surface expression of type I and II CD1 glycoprotein receptors as well as mucosa-associated CD103 protein differently in the absence or presence of all-trans-retinoic acid (ATRA), when ATRA provides a tolerogenic effect. In other words, this makes the pro- and anti-inflammatory reprogramming of this population of immune cells possible (Bene et al. 2017).

However, applying all these observations in practice taking into consideration patients' microbiome uniqueness is a challenge.

Additionally, it is known that the geographical location of plant food ingredients' growth affects the quantitative and qualitative composition of their BAS. Also, geographical location determines people's lifestyles, their habits and traditions, and diets.

Previously, within the BaSeFood project, we conducted a study of priority dishes in the Black Sea region, including Ukrainian ones. We determined the nutritional value and composition of food products, which formed the basis for the creation of the First National Composite Database of Food (Costa et al. 2013). One of the tasks of this work was to investigate the fundamental possibility of creating or developing personalized (individual) approaches (diet plans) using traditional dishes (based on traditional dishes) of our region as a source of BAS selected for their known biological effects on the microbiome and local immune response and that could be used to treat T2D in a controlled diet study (Danesi et al. 2013; Pallah et al. 2019).

Following to numerous *in vitro* studies (Pallah et al. 2019; Bati and Boyko 2016, 2017) and based on *in vivo* experiments data about main influences of various plant originated compounds and defined beneficial lactic acid bacteria (LAB) strains on gut microbiota, mucosal immune response and lipid metabolism of tested mice and rats (Bati and Boyko 2016; Meleshko et al. 2020) the selection procedure of most promising ethnical foods had been performed.

Thus, the aim of this study was to investigate the possibility of correction of lipid metabolism of patients with T2D using a personalised diet based on the most important microbial, biochemical, and immunological biomarkers of chronic inflammation.

To achieve this goal, we focused on lipid metabolism, immune, and microbiome biomarkers as a whole, as well as patients' individual characteristics (differences), to be able to regulate those indices that are considered major evidence-based determinants of T2D.

2 Materials and Methods

Patients of the Mukachevo Central District Hospital, Therapy Department, took part in the controlled clinical trial; all participants gave written informed consent.

Women aged 39–68 years with T2D were selected according to the criteria typical of this nosology (DeFronzo et al. 2015). Exclusionary criteria involved smoking, alcohol or drug abuse, pregnancy, and unstable medical status. No participants had clinically significant cardiovascular, renal or liver disease, a history of cancer or any other comorbidities. Patients who participated in the study did not take any other drugs.

Eligibility requirements were fulfilled and enrolment procedures were performed in accordance with the EU Clinical Trials Regulation (Regulation (EU) No 536/2014). The study protocol was approved by the Uzhhorod National University, Research Ethics Committee.

To confirm the effectiveness of personalized diet plans, a randomized controlled trial was conducted in two parallel groups. Group I (experimental one) included patients who followed an 18-day personalized diet, which included individually selected products rich in BAS and yogurts with unique microbial starters. Group II (control one) involved patients who, for 18 days, ate berries and yogurt prepared without microbial starters in the morning. Patients were not instructed to do additional physical exercise. The experimental group consisted of 35 patients and the control one of 21 patients. The study lasted for roughly a month. Before and after the diet course we measured five groups of parameters (total 62 parameters): (1) patients' biochemical status; (2) gut microbiota; (3) oral microbiota; (4) immune status; and (5) physical parameters of patients (measurement of body weight, circumference of waist, thighs, and upper thighs). Gut and oral microbiota studies were performed in dynamics on days 1, 7, 11 and 18 of the experiment.

In order to conduct measurements, that is to determine the condition (severity and course of the disease), for each individual we identified typical to this disease diagnostic markers for the detection of T2D, such as blood glucose, lipid profile (cholesterol, LDL, HDL, VLDL, triglycerides, and atherogenicity levels), glycosylated haemoglobin, total protein, and bilirubin levels, as well as typical diagnostic enzymes (amylase, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, gamma-glutamyl transferase, and total creatine kinase), thymol test, and indicators measuring the state of the excretory system (albumin, urea and uric acid, and total creatinine) and micro- (iron) and macronutrient (potassium, magnesium, calcium, sodium) blood composition, as an evidence of existing metabolic disorders. Regarding immune parameters, we limited ourselves to the well-known indicators of inflammatory processes, that is markers of inflammation and their agonists (IL-1β, Il-10, TNF-α). However, we also considered previously identified (selected) local inflammation markers, such as levels of total and secretory immunoglobulin A in serum (IgA, SIgA). During the study of intestinal and oral microbiome we focused on

such target groups of microorganisms as (1) typical intestinal commensals and the so-called beneficial microorganisms (Enterobacteriaceae family, genera *Staphylococcus, Streptococcus*, *Lactobacillus, Candida, Clostridium* spp.); (2) opportunistic microorganisms - *Pseudomonas aeruginosa, E. faecalis, Staphylococcus* spp., Enterobacteriaceae; (3) markers of metabolic disorders that we identified earlier (*E. coli* lac+, *E. coli* lac-, *Bifidobacterium* spp., *Enterococcus* spp.) (Petrov and Boyko 2014).

Blood formula (red and white blood cells, monocytes, lymphocytes, platelet assay, and eosinophils) was identified using Mythic 22 Orphee S.A. (Switzerland) Haematology system. Erythrocyte sedimentation rate (ESR) was measured using the Westergren method. Haemoglobin was identified calorimetrically. All biochemical parameters were assayed using Cobas c 311 (Roche/Hitachi) Switzerland.

Intestinal and oral microbes were studied according to our own method using the following nutrient media: Mitis Salivarius Agar, Bile Esculin Agar, Mannitol Salt Agar, Endo Agar, Bismuth Sulphite Agar, HiCrome Clostridial Agar, Sabouraud Dextrose Agar, Lactobacillus MRS Agar, Bifidobacterium Agar, Bacteroides bile esculin agar, Propionibacter Isolation Agar, L.D. Esculin HiVegTM Agar (manufactured by HiMedia Laboratories, India), UriSelect™ 4 Medium (Bio-Rad Laboratories, Inc., USA), and Blaurock semi-liquid modified hepatic medium (manufactured by Liofilchem, Italy). Identification of isolated microorganisms was performed using biochemical test systems ANAERO-23, ENTERO-24, NEFERM-test, Candida-23, STAPHY-16, and STREPTO test 24 (Erba Lachema s.r.o., Czech Republic). Microbiome studies were also performed using real-time polymerase chain reaction (qPCR). Immune parameters were measured using indicator immunosorbent systems Vector-Best (Russian Federation); results were read at a wavelength of 450 nm using a plate immunosorbent assay BioTek Elx800.

With the help of the developed information system and created an algorithm based on linear programming approaches, which allows selecting food for any individual (patient) in accordance with the state of her gut microbiota and immune and biochemical parameters, a personalized diet was developed for each participant of the experiment. Developed diets included products that contain functioning groups of biologically active substances such as polyphenols, anthocyanins, and flavonoids as well as unique microbial starters for fermentation. Sequenced strains of *Lactobacillus casei* IMB B-7412, *Lactobacillus plantarum* IMB B-7414, and *Lactobacillus plantarum* IMB B-7413 were used to prepare yogurts. The selection of food products was based on WHO recommendations (https://www.who.int/nutrition/publications/nutrient/en/), taking into account individual wishes and contraindications, as well as when determining the portion size - individual characteristics of patients such as the level of physical activity, body mass index, etc.

Statistical analyses were performed using the statistical program GraphPad Prism version 3.00 (GraphPad Software, USA). All data are presented as the mean ± SD or mean ± SE. For normally distributed data, checked used Shapiro-Wilk test, comparisons were tested using ANOVA. The two-tailed Mann-Whitney U-test was used for comparisons between the groups. P values <0.05 were considered statistically significant.

3 Results

On the first day of the experiment, in all patients diagnosed with type 2 diabetes there was an increase in the level of biochemical parameters observed: glucose (the real average value is 8 times higher than the allowed excess of the average value of the norm), LDH (the real average value is 5 times higher than the allowed excess of the average value of the norm), HbA1C (the real average value is 1,5 times higher than the allowed excess of the average value of the norm), and immunological indicator IL-10 (the real average value is 2 times higher than the allowed excess of the average value of the norm), as well as physical parameters such as BMI (the

real average value is 5,5 times higher than the allowed excess of the average value of the norm). Also, a decrease in HDL levels was observed: the real average value is 1.5 times lower than the allowed decrease in the average value of the norm (see Table 1, Fig. 1).

The composition of the intestinal microbiota on day 1 of the experiment demonstrated a predominance of enterococci and lactobacilli with a significant variety of commensal and opportunistic microorganisms, namely enterobacteria, pseudomonads, streptococci, staphylococci, bacilli, and candida. We observed an increase level of *E. faecalis* (the real average value is 2 times higher than the allowed excess of the average value of the norm), a decrease in levels of *E. coli* (lac+) (the real average value is more than 8 times lower than the allowed decrease in the average value of the norm), *E. coli* (lac−) (the real average value is 4 times lower than the allowed decrease in the average value of the norm) and *Lactobacillus* spp. (the real average value is more than 1,5 times lower than the allowed decrease in the average value of the norm) (see Figs. 2 and 3). The oral microbiota was characterized by a predominance of lactobacilli, enterococci, and streptococci, as well as a number of other bacteria, such as *E. coli* (lac+), *Citrobacter* spp., *E. cloacae*, *P. aeruginosa*, *S. epidermidis*, *Bacillus* spp., and *Candida* spp. We observed an increased level of *E. faecalis* (the real average value is 4 times higher than the allowed excess of the average value of the norm), *Lactobacillus* spp. (the real average value is 7,5 times higher than the allowed excess of the average value of the norm), *P. aeruginosa* and *Candida* spp. (the real average value is 2 times higher than the allowed excess of the average value of the norm) (see Figs. 2 and 4). On the first day of the experiment, no statistically significant difference was observed between the control and experimental groups.

After 18 days of the experiment, no statistically significant changes in parameters were observed in the control group, but there were changes in blood and physical parameters and microbiota composition in the experimental group. According to the data obtained, there was a decrease in the levels of such biochemical parameters as glucose, bilirubin, thymol test, cholesterol, HDL, LDL, VLDL, iron, gamma-glutamyl transferase, total protein, urea, creatinine, LDH, HbA1C, and triglycerides as well as changes in all immune and physical parameters. Also, an increase in amylase, alkaline phosphatase, calcium, creatine kinase, aspartate transferase, alanine aminotransferase, uric acid, sodium, magnesium, albumin, and atherogenicity levels was observed. Herewith, on day 18 of the experiment all indicators were almost unchanged in the control group (see Table 1).

After adherence to a personalized diet, in the experimental group patients there was a statistically significant reduction in the following parameters: glucose, thymol test, VLDL, urea, creatinine, sIgA, and TNF-a, as well as all physical parameters. There was a statistically significant increase in such biochemical parameters as uric acid, sodium, and magnesium. Regarding intestinal microbiota indicators, there was a decrease in the levels of all microbiota members except lactobacilli. Statistically significant changes were observed in *Enterococcus faecalis, Escherichia coli* (lac+), *Escherichia coli* (lac−), *Lactobacillus* spp., and *Candida* spp. The oral microbiota was characterized by a decrease in the number of all representatives except lactobacilli. Such microorganisms as *E. faecalis, Lactobacillus* spp., *P. aeruginosa,* and *Candida* spp. demonstrated statistically significant changes.

Statistically significant changes in the concentration of microorganisms (in dynamics) were observed in both the intestinal microbiota (*E. faecalis, E. coli* (lac+), *E. coli* (lac−), *Lactobacillus* spp., and *Candida* spp.) and oral microbiota (*E. faecalis, Lactobacillus* spp., *P. aeruginosa,* and *Candida* spp.) (see Figs. 5 and 6). Dynamic intestinal microbiota changes in the experimental group (see Fig. 4) demonstrate that the average concentration of *E. faecalis* remained unchanged until day 11 while a statistically significant difference compared to the first day appeared on day 11 and the tendency to a decrease remained on day 18 of the experiment. *E. coli* (lac+) is characterized by a decrease in concentration throughout the

Table 1 Measured parameters in experiment

Parameter, units	Experimental group, mean ± SD		Control group, mean ± SD		Reference range
	Day 1	Day 18	Day 1	Day 18	
Blood parameters					
Amylase, u/l 37°C	42.076 ± 2.133	46.076 ± 3.789	42.302 ± 1.204	42.056 ± 1.002	< 90
Alkaline phosphatase, u/l 37°C	59.990 ± 2.423	63.004 ± 5.841	59.684 ± 1.528	59.447 ± 1.472	35–104
Bilirubin, Mol/l	17.141 ± 2.849	16.774 ± 2.295	16.622 ± 1.499	16.493 ± 1.854	< 21
Glucose, mmol/l	12.316 ± 2.186[a]	9.961 ± 2.063[a]	12.187 ± 1.32	11.812 ± 1.257	4.1–5.9
Calcium, Mol/l	2.451 ± 0.038	2.530 ± 0.081	2.458 ± 0.02	2.462 ± 0.027	2.25–2.75
Thymol test, u/l	3.219 ± 0.592[a]	2.229 ± 0.601[a]	3.214 ± 0.389	3.173 ± 0.328	0–4
Cholesterol, mmol/l	5.427 ± 0.913	5.055 ± 0.581	5.454 ± 0.52	5.499 ± 0.487	2.9–5.2
HDL cholesterol, mmol/l	1.378 ± 0.233	1.314 ± 0.242	1.404 ± 0.134	1.411 ± 0.119	> 1.68
LDL cholesterol, mmol/l	3.383 ± 0.727	2.933 ± 0.398	3.382 ± 0.422	3.413 ± 0.39	< 3.34
VLDL, mmol/l	0.967 ± 0.233[a]	0.781 ± 0.284[a]	0.958 ± 0.161	0.942 ± 0.149	0.26–1.04
Creatine kinase, mmol/l	66.994 ± 15.466	73.219 ± 18.382	66.794 ± 9.489	67.681 ± 8.507	26–192
Iron, μmol/l	15.789 ± 2.915	15.464 ± 1.852	15.312 ± 1.578	15.059 ± 1.893	8.95–30.43
Gamma-glutamyl transferase, u/l 37°C	31.210 ± 5.256	24.747 ± 6.33	30.825 ± 3.197	32.512 ± 2.661	6–42
Aspartate transferase, u/l 37°C	17.415 ± 2.547	20.646 ± 4.87	17.377 ± 1.573	17.196 ± 1.52	< 32
Alanine aminotransferase, u/l 37°C	21.159 ± 3.846	27.396 ± 7.653	20.921 ± 2.23	20.877 ± 2.249	< 32
Total protein, g/l	67.829 ± 2.834	67.381 ± 1.186	68.126 ± 1.294	68.559 ± 1.443	66–87
Urea, mmol/l	5.558 ± 0.715[a]	4.530 ± 0.716[a]	5.583 ± 0.451	5.754 ± 0.404	2.76–8.07
Uric acid, μmol/l	265.097 ± 45.594[a]	290.966 ± 51.233[a]	263.386 ± 26.167	274.948 ± 19.444	150–350
Potassium, Mol/l	4.409 ± 0.202	4.410 ± 0.138	4.438 ± 0.097	4.477 ± 0.12	3.5–5.5
Creatinine, μmol/l	76.980 ± 4.462[a]	68.290 ± 3.662[a]	77.808 ± 2.18	78.289 ± 2.601	45–84
LDH, u/l 37°C	366.829 ± 53.892	344.897 ± 46.811	367.829 ± 33.487	383.114 ± 29.803	135–214
Sodium, mmol/l	136.181 ± 2.488[a]	139.41 ± 1.204[a]	136.496 ± 1.469	136.631 ± 1.822	132–145
Glycosylated haemoglobin, %	8.892 ± 0.731	8.235 ± 0.956	8.866 ± 0.395	8.782 ± 0.468	< 7.0
Magnesium, mmol/l	0.701 ± 0.08[a]	0.767 ± 0.058[a]	0.717 ± 0.036	0.726 ± 0.049	0.66–1.07
Albumin, g/l	44.467 ± 1.671	45.021 ± 0.898	44.612 ± 0.865	44.828 ± 0.932	35–52
Atherogenic coefficient, mmol/l	2.842 ± 0.52	2.975 ± 0.626	2.798 ± 0.314	2.796 ± 0.31	< 3.0
Triglycerides, mmol/l	2.118 ± 0.509	1.747 ± 0.664	2.099 ± 0.351	2.064 ± 0.324	< 2.26
Immune parameters					
IgA, g/l	3.187 ± 0.871	2.624 ± 0.44	3.113 ± 0.492	2.877 ± 0.481	0.7–4.0
sIgA, mg/l	4.636 ± 1.572[a]	3.399 ± 1.215[a]	4.675 ± 0.997	4.29 ± 0.885	1.60–5.48

(continued)