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Randomised clinical trial: the beneficial effects of VSL#3 in obese children with non-alcoholic steatohepatitis

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SUMMARY

Background
Gut microbiota modifiers may have beneficial effects of non-alcoholic fatty liver disease (NAFLD) but randomised controlled trials (RCT) are lacking in children.

Aim
To perform a double-blind RCT of VSL#3 vs. placebo in obese children with biopsy-proven NAFLD.

Methods
Of 48 randomised children, 44 (22 VSL#3 and 22 placebo) completed the study. The main outcome was the change in fatty liver severity at 4 months as detected by ultrasonography. Secondary outcomes were the changes in triglycerides, insulin resistance as detected by the homoeostasis model assessment (HOMA), alanine transaminase (ALT), body mass index (BMI), glucagon-like peptide 1 (GLP-1) and activated GLP-1 (aGLP-1). Ordinal and linear models with cluster confidence intervals were used to evaluate the efficacy of VSL#3 vs. placebo at 4 months.

Results
At baseline, moderate and severe NAFLD were present in 64% and 36% of PLA children and in 55% and 45% of VSL#3 children. The probability that children supplemented with VSL#3 had none, light, moderate or severe FL at the end of the study was 21%, 70%, 9% and 0% respectively with corresponding values of 0%, 7%, 76% and 17% for the placebo group ($P < 0.001$). No between-group differences were detected in triglycerides, HOMA and ALT while BMI decreased and GLP-1 and aGLP1 increased in the VSL#3 group ($P < 0.001$ for all comparisons).

Conclusions
A 4-month supplement of VSL#3 significantly improves NAFLD in children. The VSL#3-dependent GLP-1 increase could be responsible for these beneficial effects. Trial identifier: NCT01650025 (www.clinicaltrial.gov)

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INTRODUCTION

The most recent World Health Organization (WHO) estimate indicates that obesity is increasing rapidly, particularly in children (http://www.who.int/mediacentre/factsheets/fs311/en/index.html, access October 2013). Childhood obesity not only causes long-term health problems that become obvious in adulthood like cardiovascular diseases and cancer but also short-term secondary complications, including dyslipidemia, insulin resistance and non-alcoholic fatty liver disease (NAFLD).[1–3] NAFLD in the paediatric repertoire comprises different diseases, ranging from simple intra-hepatic fat accumulation (fatty liver or NAFL) to a more severe pattern of liver damage (non-alcoholic steatohepatitis or NASH), which exhibits steatosis together with inflammation and ballooning, and eventually fibrosis.[4] The development of NAFL and its progression to NASH are the consequence of the interactions between a genetic background and progressive environment-driven epigenetic code changes and molecular alterations.[5] Indeed, environmental factors, like unhealthy diet (particularly Western dietary patterns) and low levels of physical activity, can drive epigenomic reprogramming of the host genome with post-translational modifications of gene expression ultimately determining phenotypic changes in the organism.[6, 7] For these reasons, lifestyle interventions currently represent the core of prevention and treatment of NAFLD in children.[8]

The understanding of the mechanisms related with NAFLD development and progression remains a major challenge. However, recent evidence demonstrated that the interaction between the liver and gut, the so called 'gut-liver axis', might play a major role among the factors leading to the phenotypic switching from the NAFL state to a more aggressive lesion including NASH and NASH-related fibrosis.[9, 10] In fact, in humans, NAFLD is associated with increased intestinal permeability (IP) and small intestinal bacterial overgrowth (SIBO), and these factors are associated with the severity of hepatic steatosis.[11] In NAFLD patients, the gut has been demonstrated to be 'leaky' because of a tight junctions disruption process that might explain the intestinal bacterial contribution to liver disease progression, since there is an increased exposure of the liver to gut-derived bacterial products.[12] Moreover, some recent studies have demonstrated an important role for aberrations in gut microbiota composition in promoting NAFLD in children.[13] These findings mirror those seen in animal models suggesting that gut microbiota composition may influence intra-hepatic fat accumulation as an environmental factor by several mechanisms, including increased monosaccharide absorption from the intestinal lumen, and production/release of hepatotoxic products and other molecules, which in turn may lead to a chronic low-grade inflammatory state.[14–16] Therefore, several authors have suggested the modulation of gut microbiota by probiotics, prebiotics and synbiotics as a possible approach for obesity and NAFLD. Their potential beneficial effects have been confirmed in several experimental studies.[17–19] In fact, it has been reported in animal models that probiotics administration was able to reset the 'leaky gut', and offered protection against NAFL development and its progression to NASH by modulating the expression of nuclear receptors and correcting insulin resistance in the liver and the adipose tissues.[18–20] VSL#3, a mixture of eight probiotic strains (*Streptococcus thermophilus*, bifidobacteria [*B. breve*, *B. infantis*, *B. longum*], *Lactobacillus acidophilus*, *L. plantarum*, *L. paracasei*, and *L. delbrueckii* subsp. *bulgaricus*), is the most studied probiotic in NAFLD.[19, 20] In mouse models of genetic dyslipidemia (Apo-E deficient mice) which fail to develop NASH-like lesions on a standard diet, it has been shown how destrane sulphate sodium (DSS)-induced intestinal inflammation and consequent increased IP, triggered the transition of steatosis to NASH, and how these disorders were efficiently prevented by a therapeutic intervention with VSL#3.[19] Interesting results were also obtained in animal models of high fat diet-induced NAFL/NASH that resulted in attenuated liver fibrosis after VSL#3 supplementation.[20] Despite a large number of animal data on the efficacy, as well as the well-known safety profile of the use of probiotics, randomised placebo-controlled trials in NAFLD are still lacking in humans.

On the basis of the possibility that VSL#3 could have disease-modifying effects in humans, we performed the first randomised controlled trial (RCT) of VSL#3 in children with NAFLD.

MATERIALS AND METHODS

Study design

We performed a parallel-arm double-blind RCT of VSL#3 vs. placebo, which were provided by VSL Pharmaceuticals Inc (Towson, MD, USA), in obese children with NAFLD enrolled at 'Bambino Gesù Children's Hospital'.

Obesity was diagnosed as body mass index (BMI) >85th percentile. The diagnosis of NAFLD was based on a combination of physical findings at examination,

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elevated aminotransferase (ALT) levels (up to 40 UI/L) of unknown origin and ultrasonographic) evidence of hepatic steatosis as well as histological evaluation of liver biopsies obtained at entry by an expert pathologist.

Exclusion criteria included the presence of liver disease due to any of the following: hypothyroidism, Wilson disease, viral hepatitis (HBV, HCV), acute systemic disease, cystic fibrosis, coeliac disease, suspicion of muscular dystrophy, alpha-1-antitrypsin deficiency, metabolic inherited diseases, autoimmune hepatitis, drug toxicity and drugs known to induce steatosis (e.g. valproate, amiodarone or prednisone).[21] Patients were also excluded if body weight and carbohydrate metabolism were altered by the use of parenteral nutrition, protein malnutrition, previous gastrointestinal surgery, structural abnormalities of the gastrointestinal tract or neurological impairment. Finally, the use of nonsteroidal anti-inflammatory drugs, antibiotics, probiotics or anti-secretory drugs capable of causing achlorhydria within 2 months preceding enrolment were also considered exclusion criteria.

Sample size was calculated on the basis of the change in NAFLD severity observed with docosahexaenoic acid (DHA) in a previous RCT.[22] Using proportional odds-logistic regression, we found that the log-odds of less severe vs. more severe NAFLD was 1.4 in the DHA group vs. the placebo group. This is the value associated with the treatmentXtime interaction in the proportional odds-logistic regression model used to analyse the DHA RCT.[22] We decided to enrol a number of subjects sufficient to detect such effect also for VSL#3 vs. placebo. We calculated that 24 subjects per group would ensure a power of 83% to detect such log-odds at an alpha level of 0.05.[23] Children were randomised to receive in blinded fashion, 1 sachet/day of VSL#3, or placebo, if the subject's age was less than 10 years old. Two sachets of VSL#3 or placebo were administered in children older than 10 years of age. A computer-generated randomisation sequence assigned participants in a 1:1 ratio to treatment with VSL #3 or placebo. A statistician blinded to participants' clinical data, and who did not perform the final analysis, generated the allocation sequence and randomly assigned participants to the VSL#3 or placebo group. Only the statistician had access to the treatment codes. Sachets were stored at the hospital pharmacy and dispensed at the baseline visit (randomisation) and bimonthly thereafter.

Treatment duration was for 4 months. Enrollees in both arms, treating physicians, and study coordinators were blinded to treatment as sachets provided were identical as was the visual aspect and taste of the study agent and the placebo. The envelopes were numbered, and all investigators were blinded for all the duration of the study.

A low calorie diet was prescribed to all patients during the entire study. Specifically, as recommended by the Italian Recommended Dietary Allowances, each patient received the following diet: carbohydrate, 50–60%; fat, 23–30%; fatty acid, two-thirds saturated, one-third unsaturated protein, 15–20%; for a total of 25–30 Kcal/kg body weight/day. In addition to the prescribed diet, a moderate programme of aerobic exercise (30–45 min at least 3 times a week) was also recommended and was tailored to individual preferences.[21.]

Compliance was monitored through monthly phone calls. Patients and patients' families were not asked to take VSL#3/placebo sachets with them during the calls and visits since the sachets had to be kept at a temperature of 4°C before oral use. Therefore, compliance to the treatments was also evaluated every visit (month 0, 1, 2, 3 and 4) by review of medication records in a patients' diary reporting sachets count and adverse events, and direct interview of patients by AA, GiBa and VG. Compliance was estimated as a percentage of sachets taken during the treatment and greater than 90% in both groups (mean values of 93% vs. 94%).

Complete medical histories were recorded for all participants. Any patients, parents or respective guardians reported about adverse events during the trial and the post-treatment follow-up.

Collection of anthropometrical data, biochemical and ultrasound (US) data were performed at baseline and after 4 months from the initiation of the trial.

The Hospital Ethics Research Committee approved the study, in accordance with the Declaration of Helsinki (as revised in Seoul, Korea, October 2008). Parents of the included patients gave their written informed consent for liver biopsy, treatment, diagnostic tests and publication of the results. The study was registered at Clinical Trials.gov (NCT01650025).

Anthropometry

Weight and height were measured following standard procedures.[24] BMI was calculated and standard deviations scores (SDS) were calculated using Italian reference data.[25]

Blood tests

Venous blood samples, obtained after an overnight 12-h fast, were used to measure biochemical parameters including: fasting glucose and insulin, total cholesterol,

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triglycerides, high-density lipoprotein (HDL) and low-density lipoprotein cholesterol (LDL), and levels of alanine transaminase (ALT) and aspartate transaminase (AST). The degree of insulin resistance/sensitivity was estimated with the homoeostatic model assessment (HOMA) equation, as follows: (fasting insulin * fasting glucose)/405.[26]

GLP-1 assay

Part of collected blood was centrifuged at 2000 *g* for 12 min and plasma was stored at −80°C pending further analysis. Plasma samples were used to perform specific sandwich enzyme-linked immunosorbent assay (ELISA) to determine the circulating levels of total and activated GLP-1 (Listarfish, Milan, Italy).

Liver ultrasound

Liver US was performed by the same experienced radiologist using an Acuson Sequoia C512 scanner equipped with a 15L8 transducer (Universal Diagnostic Solutions, Oceanside, CA, USA). Normal liver, absent steatosis (grade 0) was defined as having normal liver echo-texture; mild steatosis (grade 1) as slight and diffuse increase in fine parenchymal echoes with normal visualisation of diaphragm and portal vein borders; moderate steatosis (grade 2) was defined as moderate and diffuse increase in fine echoes with slightly impaired visualisation of diaphragm and portal vein borders; and severe steatosis (grade 3) was defined as fine echoes with poor or no visualisation of diaphragm, portal vein borders and posterior portion of the right lobe.

Liver histology

We performed percutaneous liver biopsy and with subsequent histology on liver sections from an 18G biopsy needle using standard procedures. Histological assessment was performed by an experienced pathologist (Rita De Vito) according to criteria proposed by NAFLD Clinical Research Network.[27] Briefly, steatosis was graded 0–3 as follows: 0 <5% steatosis; 1 = 5–33%; 2 = 33–66% and grade 3 >66%. Lobular inflammation was scored based on the number of inflammatory foci per 200X per field as follows: 0 = no inflammatory foci; 1 <2 foci; 2 = 2–4 foci and 3 >4 foci. Ballooning was scored as follows: 0 = none; 1 = few balloon cells present and 2 = prominent ballooning. Fibrosis was staged 0–4 as follows: 0 = no fibrosis; 1 = periportal or perisinusoidal; 1A = mild, Zone 3, perisinusoidal; 1B = moderate, Zone 3, perisinusoidal; 1C = portal/periportal; 2 = perisinusoidal and portal/periportal; 3 = bridging fibrosis and 4 = cirrhosis.

Statistical analysis

Most variables were not normally distributed and all are reported as medians and interquartile ranges (IQR). The effect of treatment on the main ordinal outcome (liver steatosis coded as 0 = none, 1 = light, 2 = moderate and 3 = severe), and the secondary continuous outcomes (triglycerides, ALT, BMI, HOMA, GLP-1 and aGLP-1) were evaluated using an ordinal generalised linear model (OGLM) and linear models (LM) employing treatment (0 = placebo; 1 = VSL#3), time (0 = baseline; 1 = 4 months), a treatmentXtime interaction (discreteX-discrete) and the baseline value of the outcome as predictors.[22, 28–31] When the treatmentXtime interaction equals 1 (treatment = 1 and time = 1), its size gives a measure of the change in the outcome of interest in the VSL#3 group relative to the placebo group. Repeated measures were taken into account by specifying cluster confidence intervals (CI) for each patient. The odds-ratio (OR) obtained from the OGLM is a measure of the odds of more severe vs. less severe steatosis. Probabilities estimated from the OGLM were plotted and continuous values estimated from the LMs tabulated to aid the clinical interpretation of the results. The analysis was intention-to-treat. Statistical significance was set to a *P*-value <0.05 and all statistical tests were two-tailed. Statistical analysis was performed using STATA 12.1 (Stata Corp, College Station, TX, USA) and StatXact 10 (Cytel Inc., Cambridge, MA, USA).

RESULTS

Patient characteristics at baseline

Between August 2012 and May 2013, we evaluated 116 Caucasian children with suspected NAFLD at our Out--patient Clinic. Fifty-seven of these children did not meet the inclusion criteria for the study. The remaining 59 children met the inclusion criteria and their parents agreed for respective children's study participation. Just before randomisation, the parents of 11 of these children decided not to permit their children to participate in the study, leaving 48 children for randomisation, 24 for the VSL#3 group and 24 for the placebo group. As four of the children were lost to follow-up after the study began – two in the VSL#3 group and two in the placebo group – the final analysis was performed with a total of forty-four study participants, i.e. 92% of the planned sample size of 48 patients.

Table 1 gives the baseline anthropometric and biochemical measurements of the children randomised to VSL#3 and placebo. Table 2 gives the baseline US and

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Table 1 | Baseline measurements of the children randomised to VLS#3 and placebo

	Placebo (n = 22; males = 14)			VSL#3 (n = 22; males = 10)		
	Median	IQR		Median	IQR	
Age (years)	11	10	12	10	9	12
Weight (kg)	53.9	47.8	65.0	60.5	55.7	70.5
Height (cm)	1.47	1.39	1.52	1.49	1.43	1.55
BMI (kg/m^2)	25.6	23.2	27.9	27.3	24.7	28.6
BMI (SDS)	1.69	1.23	2.12	2.01	1.63	2.37
Glucose (mg/dL)	85	78	89	84	79	91
Insulin (μU/mL)	16	12	21	18	14	27
HOMA	3.1	2.3	4.7	3.9	2.7	5.4
Cholesterol (mg/dL)	156	135	179	156	137	175
Triglycerides (mg/dL)	94	64	118	86	63	121
HDL cholesterol (mg/dL)	48	42	54	45	38	53
LDL cholesterol (mg/dL)	92	80	111	83	71	101
ALT (U/L)	32	23	42	27	20	50
AST (U/L)	63	53	74	56	51	70
GLP-1 (pmol/L)	2.1	1.8	2.6	2.9	1.9	2.3
aGLP-1 (pmol/L)	1.3	1.1	1.8	1.3	1.0	2.1

BMI, body mass index; SDS, standard deviation scores; HOMA, homoeostasis model assessment of insulin resistance; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ALT, alanine transaminase; AST, aspartate transaminase; GLP-1, glucagon-like peptide-1; aGLP-1, activated glucagon-like peptide-1.

histological features of the children randomised to VSL#3 and placebo.

Effects of VSL#3 on fatty liver: main outcome

The change in fatty liver after 4 months of supplementation of VSL#3 was the main outcome of the study. At baseline, moderate and severe NAFLD were present in 64% and 36% of placebo children and in 55% and 45% of VSL#3 children. The OR of more severe vs. less severe steatosis at 4 months was 0.001 (95% CI 0.0001–0.02) for the VSL#3 vs. the placebo group (OGLM, $P < 0.001$). Figure 1 plots the corresponding probabilities of steatosis in the VSL#3 and placebo groups.

The probability that children supplemented with VSL#3 had none, light, moderate or severe FL at the end of the study was 21%, 70%, 9% and 0% in the VSL#3 group and 0%, 7%, 76% and 17% for the placebo group. These probabilities sum to 100 as they are obtained from an ordinal model and take into account the baseline degree of fatty liver.

Effects on triglycerides, HOMA, ALT, BMI and GLP-1

In Table 3, we reported the baseline and 4-month values of the secondary outcomes as estimated by the LMs for repeated measures. The changes in triglycerides, HOMA, ALT and BMI were similar in the VSL#3 and placebo

Table 2 | Liver histopathology of the children randomised to VSL#3 and placebo

	Placebo		VSL#3	
	n	%	n	%
Fatty liver at US				
Moderate	14	63.6	12	54.5
Severe	8	36.4	10	45.5
Steatosis				
5–33%	3	13.6	2	9.1
33–66%	9	40.9	10	45.5
>66%	10	45.5	10	45.5
Inflammation				
<2 foci	7	31.8	7	31.8
2–4 foci	12	54.5	9	40.9
>4 foci	3	13.6	6	27.3
Ballooning				
Few balloon cells	13	59.1	15	68.2
Prominent balloon cells	9	40.9	7	31.8
Fibrosis				
Periportal or perisinusoidal	12	54.5	10	45.5
Perisinusoidal and portal/periportal	8	36.4	9	40.9
Bridging fibrosis	2	9.1	3	13.6
Non-alcoholic steatohepatitis score (NAS)				
3	1	4.5	2	9.1
4	3	13.6	4	18.2
5	5	22.7	3	13.6
6	9	40.9	6	27.3
7	4	18.2	5	22.7
8	0	0.0	2	9.1

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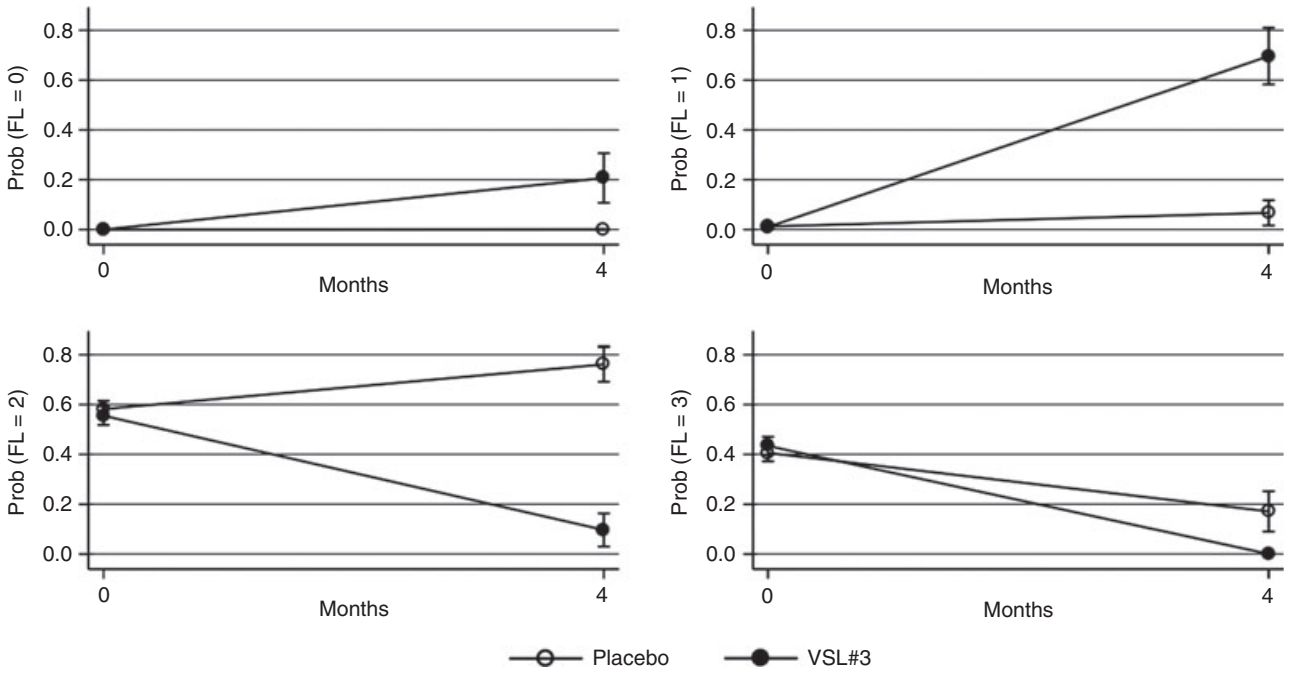

Figure 1 | Changes in the severity of fatty liver in the placebo and VSL#3 groups during the study. Values are mean probabilities and standard errors estimated from an ordinal logistic regression model for repeated measures (cluster confidence intervals). For this reason, probabilities at each time point sum to 100. FL = fatty liver; 0 = no fatty liver; 1 = light fatty liver; 2 = moderate fatty liver; 3 = severe fatty liver.

Table 3 | Changes in secondary outcomes during the study. Values are mean and standard errors estimated by a linear model for repeated measures (cluster confidence intervals)

	Placebo		VSL#3		
	Baseline	4 months	Baseline	4 months	*P*-value*
Triglycerides (mg/dL)	98 (3)	102 (10)	99 (4)	110 (9)	0.575
HOMA	4.7 (0.4)	3.5 (0.6)	4.3 (0.3)	3.3 (0.3)	0.693
ALT (U/L)	42 (1)	50 (5)	34 (1)	33 (1)	0.170
BMI (kg/m^2)	25.6 (0.01)	25.7 (0.24)	27.1 (0.01)	24.9 (0.2)	<0.001
BMI (SDS)	1.68 (0.01)	1.68 (0.04)	1.94 (0.01)	1.58 (0.04)	<0.001
GLP-1 (pmol/L)	2.25 (0.03)	2.17 (0.11)	2.20 (0.02)	3.24 (0.19)	<0.001
a-GLP1 (pmol/L)	1.44 (0.01)	1.42 (0.13)	1.58 (0.02)	2.20 (0.05)	<0.001

* Tests whether there is a change in time of the outcomes of interest for the VSL#3 vs. placebo group taking into account baseline values of the outcome (Wald test for treatmentXtime interaction, linear model for repeated measures).

groups. BMI values were decreased ($P < 0.001$) in VSL#3-supplemented children with respect to placebo group after the 4-month supplementation. Recently, Yadav *et al.* demonstrated that VSL#3 was able to suppress body weight gain and insulin resistance in mouse via modulation of the gut flora composition, which in turn enhanced the expression and the activity of the GLP-1 released by the intestinal L cells.[32] Therefore, we assessed the plasma concentration of the total form (GLP-1) and activated form (aGLP-1) of this factor in both study groups (Table 3).

Figure 2 reports the individual before-after plots for GLP-1 and aGLP-1. There was a clear trend towards increasing values of GLP-1 and aGLP-1 in the VSL#3 group.

DISCUSSION

Current medical and lifestyle interventions offer modest efficacy in the treatment of paediatric NAFLD and other therapeutic interventions are not approved for children.[3, 4] Clinical trials with safe and well-tolerated pharmacological agents that may improve insulin resistance

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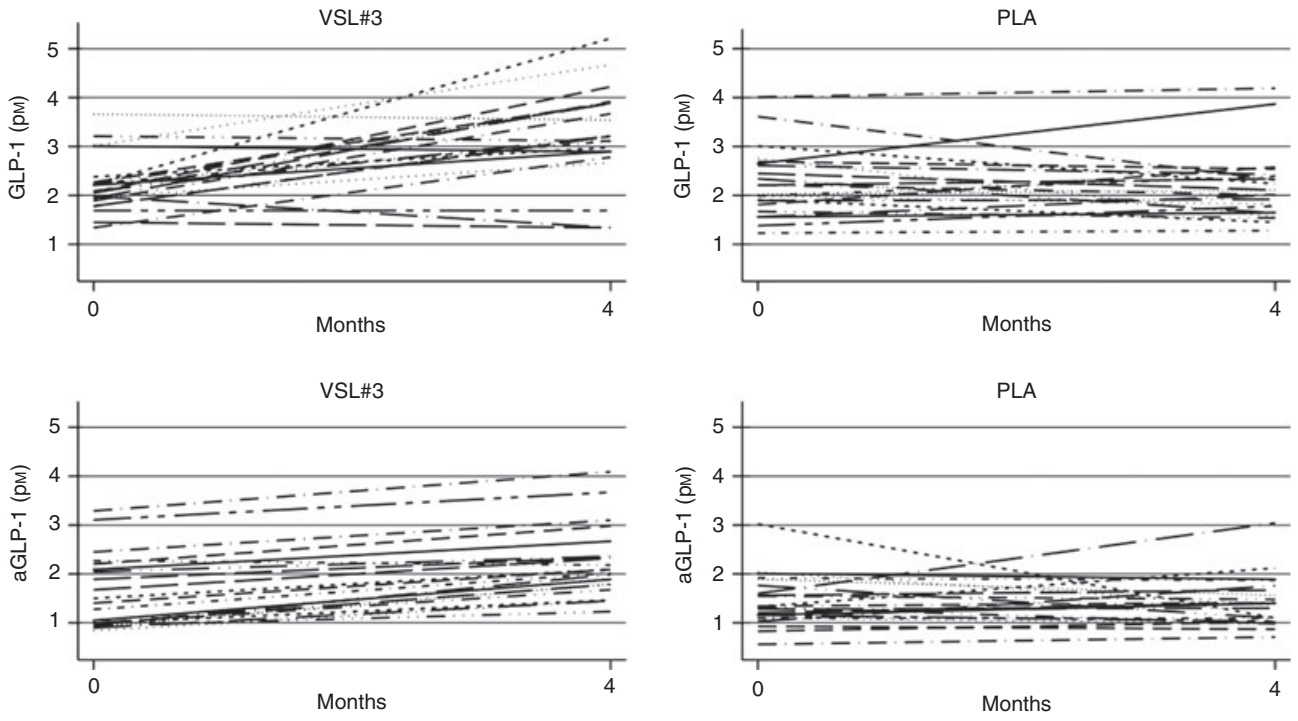

Figure 2 | Individual changes of GLP-1 and aGLP-1 in the placebo and VSL#3 groups during the study.

and oxidative stress, Metformin and vitamin E respectively, were completed by different tertiary centres with controversial efficacy.[21, 33, 34] Interestingly, Nobili et al. recently demonstrated that docosahexaenoic acid metabolically and histologically improves liver steatosis in children with NAFLD.[4, 22, 35, 36] However, to date there are no clear specific therapeutics targeting NAFLD-associated liver damage and associated long-term risks in children.

The role of the intestinal microbiota in NAFLD has garnered significant attention during the last 5 years, and several reports in rodent models suggest a crucial role of the intestinal microbiota composition in NAFLD development and progression.[37, 38] It is now well-recognised in humans that changes in microbiota composition, termed dysbiosis, is strongly associated with obesity and NAFLD.[13, 39] Furthermore, as the microbiota and host response to changes in flora metabolism can be strongly influenced by diet, the ingestion of foods higher in refined sugar and fats, thus linking the overwhelming presence of gut-derived microbial products, activation of innate immunity an inflammation with consequential NAFLD development.[40, 41]

Therefore, based on these recent findings it would seem rational to study several therapeutic strategies to modify the gut microbiota composition (i.e. antibiotics, probiotics and prebiotics) – reverse dysbiosis, with the ultimate goal of reversing NAFLD-related liver injury. Until our present study, most studies have been conducted in pre-clinical models, while there are limited studies in paediatric human NAFLD-related disease.[42–45]

A recent double-blind clinical trial demonstrated that obese children with NAFLD treated with Lactobacillus GG resulted in a significant decrease (up to normalisation in 80% of cases) in serum ALT values; and in titres of anti-peptidoglycan-polysaccharide antibodies, which are a ubiquitously detected as a result of exposure to bacterial cell wall antigen. Consequently, these antibodies are suitable as an indirect indicator of intestinal bacterial overgrowth.[44]

Interestingly, Loguercio *et al.* demonstrated that VSL#3 supplementation in patients affected by several types of chronic liver diseases, including NAFLD, may reduce liver damage and improve serum levels of various biomarkers In that study, however, the only NAFLD-related study endpoint was ALT.[45]

According to the data we acquired here following 4 months of supplementation with VSL#3, we observed improvement of FL, as evaluated by US, as well as a significant decrease in the BMI ofVSL#3-supplemented children compared to the placebo group.

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The dual beneficial effect on FL and body weight we observed could result from either direct or indirect consequences that result from VSL#3-dependent reversal of dysbiosis. The restoration of normal gut flora could result in reduced intestinal permeability, increased production of short chain fatty acids (SCFAs) and anorexogenic gut hormones (including GLP-1 and GLP-2), as well as enhancement of insulin sensitivity. Taken together, these beneficial effects would reduce the inflammatory state and insulin resistance, which are well known characteristics of human obesity.

In fact, interestingly, Yadav *et al.* recently demonstrated that VSL#3 in rats may modulate the gut flora composition (i.e. decreased firmicutes and increased bacteriodetes and bifidobacteria) and stimulate differential production of SCFAs, like butyrate, that have a beneficial effect on weight loss if administered to diet-induced obese mice[32] Therefore, it is quite conceivable that the effects of VSL#3 in our patients could be dependent on the restoration of normal gut microbiota as well as a consequent production of SCFAs. These findings merit further investigation by metagenomic and metabolomic approaches in VSL#3-supplemented obese children with biopsy-proven NAFLD. Yadav and colleagues also demonstrated that increased gut microbiota butyrate could enhance the expression and the activity of the GLP-1. GLP-1 has long been known to be an incretin secreted by the enterochromaffin cells of the small intestine and proximal colon.[26] Following ingestion of food, GLP-1 is released by these cells and stimulates the endocrine pancreas thus promoting insulin sensitivity, and therefore aids both glucose and fat metabolism. Indeed, circulating levels of GLP-1, both in total and active form are significantly increased in our VSL#3 patients after the 4-month supplementation.

Interestingly, over time evolutionary changes in the biology of GLP-1 have rendered its half-life in mammals rather short since the peptide is inactivated by dipeptidyl peptidase IV (DPPIV). Recently the role of GLP-1 and respective analogues – used currently for the treatment of type 2 diabetes mellitus (T2DM) – has been challenged. GLP-1 receptors are found ubiquitously in various mammalian organs, but controversy exists as to their presence on hepatocytes.[46] Importantly, however, there is an emerging consensus, following the production of long-acting GLP-1 analogues, that GLP-1 may have insulino-mimetic effects. Recent studies in animal models and NAFLD adults showed an effective role of GLP-1 receptor agonists (such as exenatide and liraglutide) as a new promising therapy in NAFLD for their ability in modulating fatty acid oxidation, decreasing lipogenesis and improving hepatic glucose metabolism.[47, 48] The cells responsible for synthesising the pre-propetide for GLP-1/GLP-2 are found in the distal small intestine and proximal colon and are termed L cells. L cells have G protein coupled receptors (GPCRs) which are known to avidly bind bile salts. While the recent FLINT trial appears promising, the effects of the long-term use of bile salt agonists in patients, especially children, is not currently known. A safer pharmacologic mechanism to enhance GLP protein secretion, as we have observed in this study raises the spectra of a safe alternative. Furthermore, a recent randomised, double-blind, placebo-controlled, multicentre clinical, demonstrated that a well-tolerated exenatide administered to obese adolescents resulted in a significant reduction in BMI compared with placebo that was confirmed during the open-label extension of the study.[49] Although, the long-term hepato-metabolic effects of GLP-1 agonists are still unknown, this latter study suggests that exenatide could be a plausible new potentially safe and efficacious drug that could in combination with VSL#3, be a treatment for obese children with NAFLD.

In summary, our human data corroborate findings in previous pre-clinical studies. Our clinical trial provides the first human clinical evidence demonstrating that a brief-course of supplementation with VSL#3 significantly improves fatty liver and BMI in children with NAFLD. The effect of long-term supplementation with VSL#3 with respect to reversal of hepatic injury in NASH, as well as metabolomic, analysis of gut microflora remain to be defined in a possible extension of this trial.

AUTHORSHIP

Guarantor of the article: Valerio Nobili.

Author contributions: Nobili V obtained funding. Nobili V and Alisi A contributed to study design. Alisi A, Baviera G and Giorgio V collected data. Bedogni G and Alisi A contributed to statistical analysis. Porro E, Paris C, Giammaria P and Reali E interpreted the data. Alisi A, Bedogni G and Baviera G drafted the manuscript. Anania F revised the manuscript for important intellectual content. All authors approved the final version of the manuscript.

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