

Dimethylguanidino valeric acid is independently associated with intrahepatic triglyceride content in patients with nonalcoholic fatty liver disease (NAFLD) and decreased after long-term exercise

Jia Li

The First Affiliated Hospital of Xiamen University <https://orcid.org/0000-0003-4233-9021>

Yinxiang Huang

The First Affiliated Hospital of Xiamen University

Caixin Huang

The First Affiliated Hospital of Xiamen University

Yan Zhao

The First Affiliated Hospital of Xiamen University

Xiulin Shi

The First Affiliated Hospital of Xiamen University

Zhen Chen

The First Affiliated Hospital of Xiamen University

Liyang Wang

The Affiliated Hospital of Xiamen University

Lili Han

Fujian Medical University

Zhong Chen

Xiamen University

Xuejun Li (✉ lixuejun@xmu.edu.cn)

<https://orcid.org/0000-0002-0578-2633>

Research article

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Abstract

Backgrounds: Dimethylguanidino valeric acid (DMGV) is closely associated with nonalcoholic fatty liver disease (NAFLD), the most recommended therapy of NAFLD is Exercise. Our aim was to investigate the correlation between DMGV concentrations and clinical characters in patients with NAFLD, and assessed the effect on DMGV concentrations changes after 6 month exercise training.

Methods: NAFLD individuals (n = 220) were selected and randomly divided into control group (n = 74), moderate exercise group (n = 73) and vigorous exercise group (n = 73) with 6 month followed-up. Clinical characteristics were obtained from our previous clinical trial, serum DMGV levels were determined by a validated ultrahigh performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) method.

Results: On baseline, DMGV levels were positive associated with age, visceral fat and intrahepatic triglyceride (IHTG) content, and inversely associated with fasting blood glucose and diastolic blood pressure. In addition, the association between DMGV levels and IHTG content remained significant after adjusting other main clinical characters (β coefficient = 0.174, $P = 0.018$). After 6 month exercise training, IHTG was decreased by both exercise intensities without a significant difference ($P = 0.45$), however, moderate exercise was more efficient on DMGV decreasing than vigorous exercise (-9.96 to 2.27 ng/ml, $P < 0.001$ for moderate exercise; -4.53 to 4.56 ng/ml, $P = 0.762$ for vigorous exercise) with a significant difference ($P = 0.047$).

Conclusions: DMGV was a potential indicator during development and progression of NAFLD, and moderate exercise was more efficient on metabolic changes than vigorous exercise with equal IHTG improvement, suggesting a priority of exercise intensity during NAFLD treatment.

ClinicalTrials.gov Identifier: NCT01418027, August 16, 2011

Background

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease and has become an increasingly pandemic disease as the improvement of people's life, which is also the hepatic manifestation of the metabolic syndrome and a risk factor for many metabolic diseases^{1,2}. Exercise was a major recommended treatment for NAFLD by decreasing visceral adipose tissue, liver fat, body fat and weight as well as improvement of cardiovascular risk factors^{3,4}. Our previous clinical trial⁵ found that intrahepatic triglyceride (IHTG) content was decreased significantly by long term moderate and vigorous exercise in NAFLD subjects without a significant difference, while the metabolic profile changes during exercise was unknown, which exercise intensity was more efficient on metabolic alteration was unclear.

With the development of analytical technologies, metabolomic study has been widely used to discover a panel of biomarkers in development and progression of NAFLD^{6,7}. Recently, Targeted and non-targeted

as a marker of liver fat and predicted future diabetes up to 12 years in 3 distinct human cohorts⁸, in addition, plasma DMGV levels had a strong relationship with visceral adiposity and decreased insulin sensitivity, and was recognized as an early marker of cardiometabolic dysfunction in health white people⁹. However, DMGV' character in NAFLD status and its response to exercise were limited.

This current study aimed to figure out the relationship between DMGV levels and clinical parameters among NAFLD patients, especially IHTG content, and compare the DMGV levels changes after 6 month moderate and vigorous exercise training.

Methods

Study protocol

The study protocol, informed consent form and all steps from blood extraction to analysis were approved by a steering committee, institutional review boards of Xiamen University and the First Affiliated Hospital of Xiamen University in China, and written informed consent was obtained from all participants. All methods were carried out in accordance with the relevant guidelines and regulations.

Study participant and protocol were described in our previous study (Trial registration number: NCT01418027)⁵, briefly, a total of 220 individuals with NAFLD were selected and randomly assigned to control group (n = 74), moderate exercise group (n = 73, brisk walking 150 minutes per week at 45%-55% of maximum heart rate) and vigorous-moderate exercise group (n = 73, jogging 150 minutes per week at 65%-80% of maximum heart rate for 6 months and brisk walking 150 minutes per week at 45%-55% of maximum heart rate for another 6 months) with a 12-month followed-up. At 8:00 am after an overnight fast, blood samples were collected, and then were centrifuged at 3000 × g for 10 min, serum were stored at -80 °C until analysis. In this present study, only 6 month exercise intervention was concerned.

Determination Of Serum Dmgv Concentration

50 µL serum was added 200 µL acetonitrile and 5 µL inner standard (IS) solution (L-phenyl-d5-alanine, 10 µg/mL), after centrifuging at 19,000 × g for 10 min at 4 °C, 50 µL of the supernatant was then mixed with 200 µL initial mobile phase, the aliquot of which (5 µL) was injected into the ultrahigh performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) system for analysis.

DMGV (purity > 97%) was synthesized by Nanjing Fanyida Biotechnology co. LTD (Nanjing China). LC-MS/MS was run on an Agilent 6460 triple stage quadrupole mass spectrometer equipped with an ESI ion source and an Agilent 1290 HPLC system with auto-sampler (Agilent Technologies, Santa Clara, CA, USA). DMGV was separated on an HSS T3 column (2.1 × 100 mm, 1.8 µm, Waters, Milford, USA) at 30 °C. The mobile phase consisting of water with 2.5 mM ammonium formate and 0.1% formic acid (Solvent A) and acetonitrile (Solvent B) was used with a gradient elution: 0-4.5 min, 3–5% B at a flow rate of 0.2 mL/min, Loading [MathJax]/jax/output/CommonHTML/jax.js ilibrated by 3% B for another 1.5 min, respectively. MS/MS

conditions were as follows: Gas temperature 325 °C, Gas flow 10 L/min, Nebulizer 30 psi, Sheath gas temperature 400 °C, Sheath gas flow 11 L/min, Capillary 3500 V, Nozzle voltage 2000 V. Quantification was obtained by using multiple reaction monitoring (MRM) mode in positive ion mode (DMGV: m/z 202.1–71.1, Fragmentor 123 V, Collision Energy 25 V, Cell Cell Accelerator Voltage 0 V; IS: m/z 171.1–125.1, Fragmentor 83 V, Collision Energy 10 V, Cell Cell Accelerator Voltage 0 V). Mass Hunter workstation software (Version B. 05. 00, Agilent Technologies, Santa Clara, CA, USA) was employed for data acquisition and processing.

Statistical Analysis

Pearson's correlation coefficient (for normally distributed variables) and Spearman's rank correlation (for non-normally distributed variables) were performed to analyze the correlation between clinical characteristics and DMGV levels in NAFLD subjects at baseline. The subjects were classified into four quartiles according to DMGV levels, other parameters in different groups were compared using an ANOVA test. DMGV levels changes after exercise testing were assessed by a paired t test in each group. All statistical analyses were performed using SPSS 22.0 software. P value less than 0.05 was considered statistically significant.

Results

Subjects characteristics

A total of 220 NAFLD participants were recruited and followed up for 12 months⁵, only 6 month intervention were concerned in the present study (flow diagram was showed in Fig. S1). There were no significant difference in basic anthropometric data among three groups (Table S1); after 6 month exercise training, main clinical characteristics changed (Table S2), particularly, IHTG content were significant decreased (by 5.0% in the vigorous exercise, $P < 0.001$; 4.2% in the moderate exercise, $P < 0.001$), but high-intensity exercise offered no additional benefit to moderate intensity exercise in decreasing IHTG ($P = 0.45$)^{5,10}.

Uhplc-ms/ms Method Validation

For determination of DMGV concentration in human serum, serum sample preparation, LC condition and MS parameters of m/z 202.1→71.1 was carefully optimized. The lower limit of detection (LLOD) of DMGV in human serum was 1 ng/ml, and the lower limit of quantification (LLOQ) was 5 ng/ml. Calibration curve, accuracy, precision of intra-day and inter-day, recovery and matrix effect were carefully validated and all within the acceptable limit (data were not shown).

Correlation Between Dmgv Levels And Clinical Characteristics At Baseline

There were no significant difference on DMGV concentration among three groups at baseline ($P= 0.842$). Serum DMGV levels were positive associated with age, visceral fat and IHTG content, and inversely associated with fasting blood glucose, diastolic blood pressure (Table 1). After adjusting age, sex, BMI (body mass index) and visceral fat, correlation with IHTG remained highly significant (β coefficient = 0.169, $P= 0.021$), moreover, DMGV was still significant positive associated with IHTG after additional adjusting weight, subcutaneous fat, fasting blood glucose and DBP (β coefficient = 0.174, $P= 0.018$).

Table 1
Bivariate analysis between DMGV concentration and clinical characteristics in NAFLD subjects at baseline (n = 220).

Variables	r	P
Age	0.177	0.013
Fasting blood glucose	-0.193	0.007
Total cholesterol (TC)	0.052	0.479
Total Triglyceride (TG)	-0.016	0.833
High-density lipoprotein-cholesterol (HDL-C)	0.087	0.231
Low-density lipoprotein-cholesterol (LDL-C)	-0.064	0.384
Alanine transaminase (ALT)	0.11	0.139
Aspartate aminotransferase (AST)	0.122	0.095
γ -Glutamyltransferase (GGT)	-0.097	0.186
Systolic blood pressure (SBP)	-0.073	0.31
Diastolic blood pressure (DBP)	-0.156	0.03
Weigh	-0.135	0.061
Body Mass Index (BMI)	-0.121	0.095
Waist circumference	-0.082	0.257
Visceral fat	0.16	0.027
Subcutaneous fat	0.128	0.077
Total Fat	0.11	0.13
Intrahepatic triglyceride (IHTG) content	0.19	0.009

Subjects were divided into four quartiles according to DMGV levels, TG, LDL-C, ALT, AST, GGT, SBP, weigh, BMI, subcutaneous fat and total fat were did not different in these four groups (Table 2), visceral fat and IHTG content were elevated with higher DMGV levels ($P < 0.001$). Moreover, DMGV levels were elevated with higher IHTG content when subjects were divided into four quartiles according to IHTG content (Fig. 1).

Table 2

Clinical characteristics as DMGV levels were divided into four quartiles (data were presented as mean \pm SD).

Variables	Quartile 1 (n = 55)	Quartile 2 (n = 55)	Quartile 3 (n = 55)	Quartile 4 (n = 55)	P
DMGV, ng/ml	18.3 \pm 4.3	29.6 \pm 4.8 ^{***}	60.0 \pm 10.0 ^{***}	84.9 \pm 6.6 ^{***}	< 0.001
Age, years	51.7 \pm 7.1	54.7 \pm 6.9 [*]	53.1 \pm 7.4	56.7 \pm 5.2 ^{**}	0.001
Fasting blood glucose, mg/dl	106.7 \pm 9.6	104.4 \pm 9.5	101.8 \pm 9.8 [*]	101.1 \pm 9.7 ^{**}	0.022
TC, mg/dl	222.6 \pm 36.0	232.8 \pm 33.8	241.3 \pm 40.1 ^{**}	222.8 \pm 37.0	0.023
TG, mg/dl	168.1 \pm 79.4	175.5 \pm 68.7	168.2 \pm 70.1	159.2 \pm 47.3	0.689
HDL-C, mg/dl	45.0 \pm 7.9	47.7 \pm 6.6	52.4 \pm 9.9 ^{***}	47.4 \pm 7.7	< 0.001
LDL-C, mg/dl	144.9 \pm 29.3	143.7 \pm 27.6	152.0 \pm 43.4	133.5 \pm 35.7	0.051
ALT, U/L	23.8 \pm 6.9	24.9 \pm 8.4	26.1 \pm 10.9	25.3 \pm 8.3	0.602
AST, U/L	22.6 \pm 3.4	23.7 \pm 5.4	23.4 \pm 5.98	24.3 \pm 5.3	0.420
FFT, U/L	39.8 \pm 17.9	36.6 \pm 20.1	33.9 \pm 17.1	32.3 \pm 12.7 [*]	0.117
SBP, mmHg	134.5 \pm 15.4	134.4 \pm 14.5	128.6 \pm 13.1 [*]	133.1 \pm 15.9	0.120
DBP, mmHg	82.0 \pm 10.2	82.4 \pm 8.3	77.6 \pm 8.7 [*]	79.5 \pm 9.1	0.020
Weigh, kg	72.8 \pm 8.6	72.0 \pm 9.8	69.5 \pm 8.6 [*]	70.1 \pm 8.1	0.177
BMI, kg/m ²	28.2 \pm 2.9	27.8 \pm 2.6	27.5 \pm 2.1	27.6 \pm 2.5	0.431
Waist circumference, cm	96.3 \pm 7.1	95.6 \pm 6.4	92.9 \pm 5.2 ^{**}	96.0 \pm 6.6	0.026
Visceral fat, cm ²	116.1 \pm 32.9	126.9 \pm 33.0	141.6 \pm 37.5 ^{***}	146.1 \pm 29.4 ^{***}	< 0.0001
Subcutaneous fat, cm ²	230.4 \pm 78.7	213.6 \pm 55.2	232.5 \pm 58.3	244.8 \pm 69.3	0.116
Total Fat, kg	23.5 \pm 5.4	22.1 \pm 3.8	23.1 \pm 3.7	24.6 \pm 4.9	0.051
IHTG content, %	12.8 \pm 5.6	13.8 \pm 6.1	17.9 \pm 6.5 ^{***}	20.4 \pm 8.5 ^{***}	< 0.0001
P value was calculated among four groups by ANOVA analysis					
* P < 0.05 ** P < 0.01 *** P < 0.001 vs Quartile 1					

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DMGV Levels Changes After Exercise Intervention

After 6 month exercise training, DMGV levels were decreased by moderate (-9.96 to 2.27 ng/ml, $P < 0.001$) and vigorous exercise training (-4.53 to 4.56 ng/ml, $P = 0.762$) (Fig. 2). Compared to control, moderate exercise was more efficient on decreasing DMGV concentration than vigorous exercise with a significant difference ($P = 0.047$) (Fig. 3).

Correlation between baseline DMGV levels and longitudinal changes of clinical characteristics

Baseline DMGV levels were inversely correlated with the changes of waist circumference ($r = -0.312$, $P = 0.010$) and subcutaneous fat ($r = -0.278$, $P = 0.022$), and positive correlated with changes of LDL-C ($r = 0.286$, $P = 0.018$) after 6 month moderate exercise training, and it was not associated with changes of IHTG content ($r = 0.189$, $P = 0.122$). We then investigate the correlations between changes of DMGV levels and changes of clinical parameters, unfortunately, there were no strong correlation (data were not shown).

Discussion

This study has two principal findings. First, DMGV levels were positive associated with IHTG content, and elevated with higher IHTG content in NAFLD subjects. After 6 month exercise intervention, DMGV levels were decreased in both exercise groups, and moderate exercise was more efficient on decreasing DMGV levels than vigorous exercise. Taken together, these findings highlight the important role of DMGV in NAFLD progression, and moderate was a preferred exercise intensity in NAFLD treatment.

DMGV was a product of asymmetric dimethylarginine (ADMA) metabolized by alanine-glyoxylate aminotransferase 2 (AGXT2) and participated in nitric oxide signaling as a part of the arginine metabolism in human¹². DMGV was higher in individuals with nonalcoholic steatohepatitis and confirmed a strong positive correlation with liver fat⁸. Moreover, DMGV was a beneficial metabolic intervention, since its level was decreased in participants after weight loss surgery. Recent study proposed that DMGV levels were positively associated with body fat, abdominal visceral fat, TG and an inverse associated with insulin sensitivity, LDL-C and HDL-C in healthy people⁹; however, DMGV remains an incompletely understood metabolite with few data in NAFLD. For DMGV concentration quantitation in human serum in our study, LC condition, MS/MS parameters and serum sample preparation was carefully optimized, method validation was strictly performed. Acetonitrile was chosen for proteins participate in serum sample preparation since it was widely used and relatively simple before LC-MS/MS analysis^{11, 15}. Isotope-labeled DMGV was the best inner standard for DMGV quantitation¹⁶, unfortunately, there was no commercial reference and it had to be synthesized, therefore, L-phenyl-d5-alanine was used as inner standard because of its widely used in other similar metabolomics studies¹¹,

¹⁷. In this work, the relationship between DMGV levels and clinical parameters in NAFLD subjects was investigated, and it was demonstrated a positive association with age, IHTG content and visceral fat, and an inverse association with fasting blood glucose and DBP for the first time. In particular, DMGV had a close positive relationship with IHTG content after adjusting other main clinical characteristics in NAFLD participants. In addition, DMGV concentration and IHTG content were elevated with each other according to the four quartiles analysis, which indicated that DMGV might be a potential indicator during NAFLD development and progression (IHTG content > 5% was diagnosed as NAFLD in clinic). These results further supported DMGV as independent biomarker in NAFLD.

Exercise benefited NAFLD in different exercise intensities without a significant difference on decreasing IHTC content in our previous clinical trial, but the metabolic profile changes were poorly understood. A previous metabolomics study found a panel of altered serum metabolites after 6 month vigorous exercise training ¹¹, however, whether these metabolites altered by moderate exercise was unknown, which exercise intensity was more efficient on metabolites changes was unclear. Thus, in the present study, DMGV was used to evaluate the effect on metabolic changes under different exercise intensities. We found that DMGV concentration was decreased after 6 month exercise training, which was corresponding to another similar study ⁹. Interestingly, compared to control, moderate exercise was much more efficient on decreasing DMGV than vigorous exercise with an equal effect on IHTG reduction, though vigorous exercise was more efficient on improvement of weight, waist circumference, body fat and visceral fat. It was confirmed that various exercise intensities impacted plasma metabolic profile differently ¹⁸⁻²⁰, and low-intensity exercise favors a fat oxidation rate than in the high intensity exercise group with a greater decrease in body mass and fat mass ²¹, thus, we considered that moderate exercise benefits DMGV metabolism better than vigorous exercise might due to the better improvement on enzyme related to DMGV metabolism and oxidation ability, which needed further investigation.

This work has several limitations. All participants in this study were diagnosed as NAFLD by protonmagnetic resonance spectroscopy (IHTG content \geq 5%) without healthy subjects. Another limitation was a lack of specific pathologies of NAFLD (steatosis, nonalcoholic steatohepatitis, liver fibrosis and cirrhosis) without liver biopsy. Moreover, DMGV levels altered differently response to different exercise intensities, the inner mechanism were needed to figure out in further study.

Conclusions

To the best of our knowledge, this is the first study to demonstrate that DMGV levels was closely associated with main clinical characteristics and decreased after 6 month exercise training in NAFLD subjects. Compared to vigorous exercise, moderate exercise was more efficient on decreasing DMGV concentration with an equal IHTG improvement. Overall, this finding provided a meaningful metabolic indicator in the development and progression in NAFLD, and optimized the clinical guideline in NAFLD treatment by long term exercise.

Abbreviations

NAFLD: nonalcoholic fatty liver diseases

DMGV: Dimethylguanidino valeric acid

UHPLC-MS/MS: ultrahigh performance liquid chromatography-tandem mass spectrometry

IHTG: intrahepatic triglyceride

IS: inner standard

BMI: body mass index

TG: Total Triglyceride

TC: Total cholesterol

HDL-C: High-density lipoprotein-cholesterol

LDL-C: Low-density lipoprotein-cholesterol

ALT: Alanine transaminase

AST: Aspartate aminotransferase

GGT: γ -Glutamyltransferase

SBP: Systolic blood pressure

DBP: Diastolic blood pressure

ADMA: asymmetric dimethylarginine

AGXT2: alanine-glyoxylate aminotransferase 2

Declarations

Ethics approval and consent to participate: The study protocol, informed consent form and all steps from blood extraction to analysis were approved by a steering committee, institutional review boards of Xiamen University and the First Affiliated Hospital of Xiamen University in China. Written informed consent was obtained from all participants and which was clearly stated in section “Study protocol”. All methods were carried out in accordance with the relevant guidelines and regulations.

Consent for publication: not applicable.

Availability of data and materials: Data sharing is not applicable to this article as no datasets were generated.

Competing interests: All authors declare no conflicts relative to this manuscript.

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Authors' contributions: JL, XL and Zhong C contributed the study conception and design. JL and was responsible for data analysis and wrote the manuscript. XL, YH, YZ, Zheng C, XS, LW and LH were participated in clinical trial study, clinical characteristics detection, data analysis and blood sample collection. All authors have read and approved the manuscript

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Additional Figure Legend

Fig. S1 Flow diagram of clinical trial in this work.

Figures

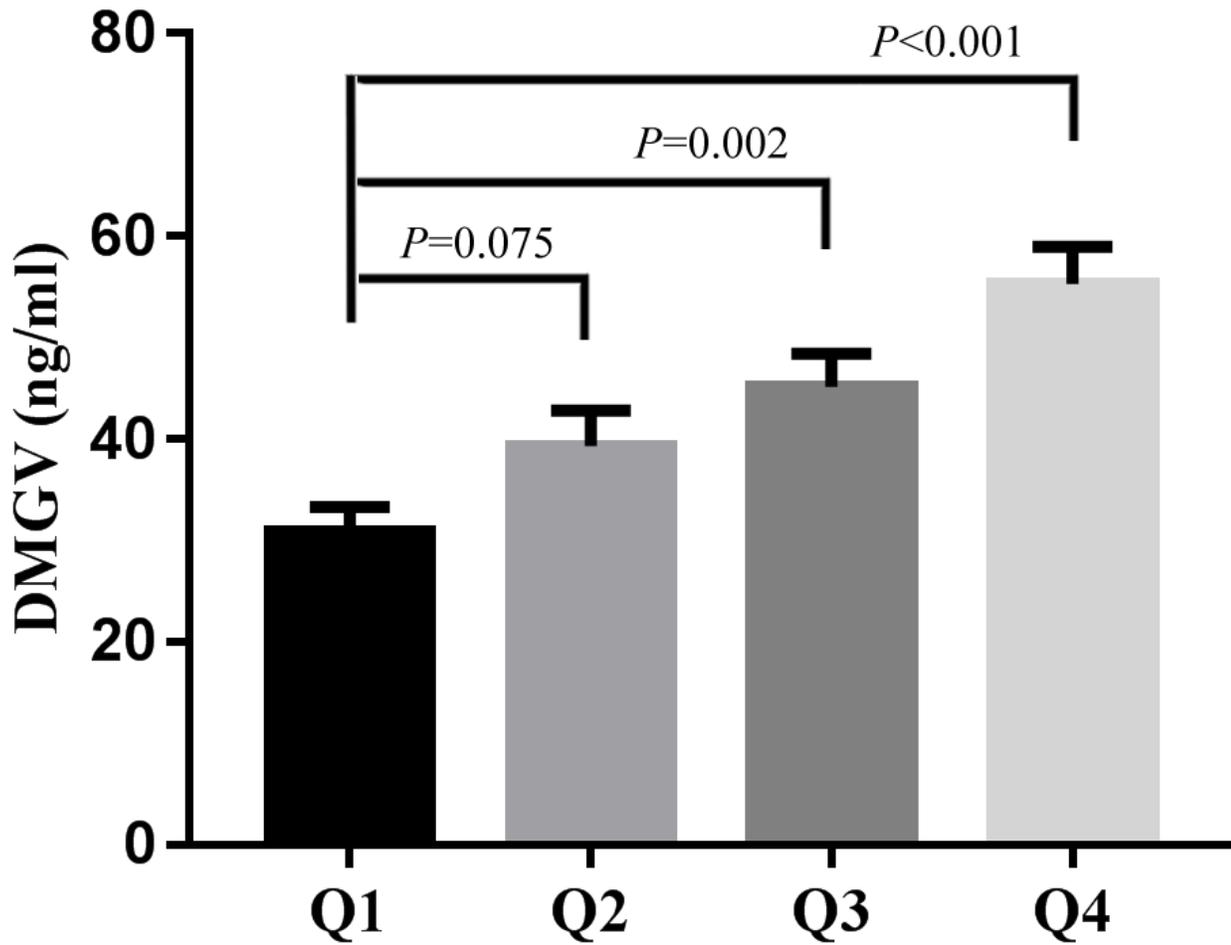


Figure 1

DMGV concentration in each quartile (Q) when subjects were divided into four quartiles according to IHTG content.

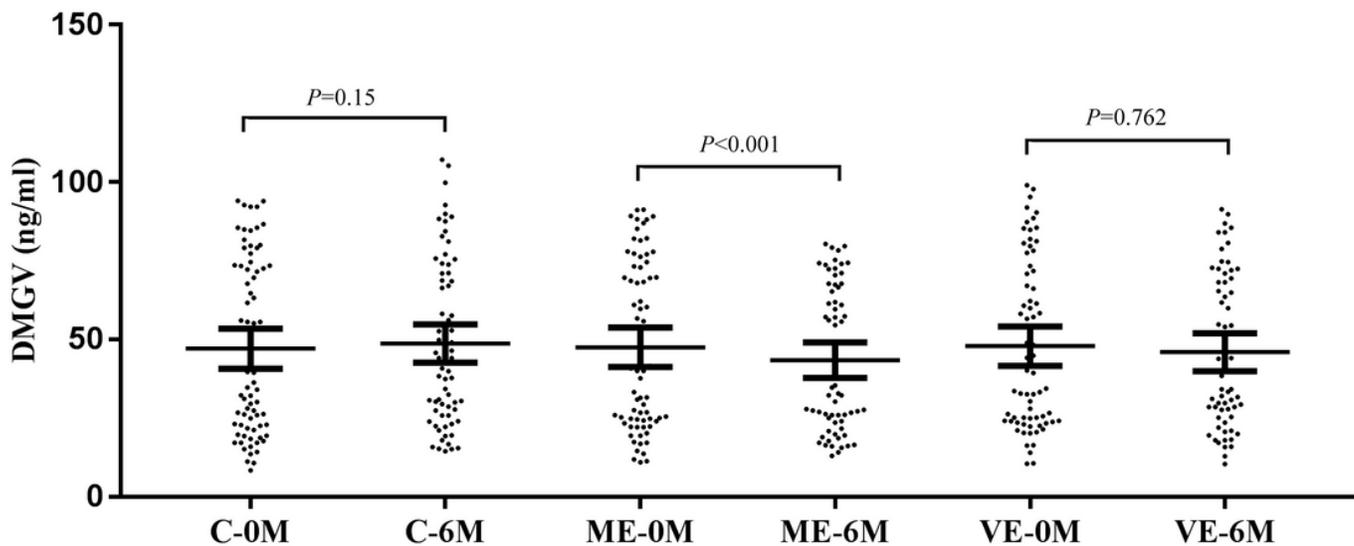


Figure 2

DMGV concentration in control (C), moderate exercise (ME) and vigorous exercise (VE) groups on baseline (0 M) and 6 month (6 M). (Data were compared by a paired t test in each group, and presented as mean with 95% confidence interval)

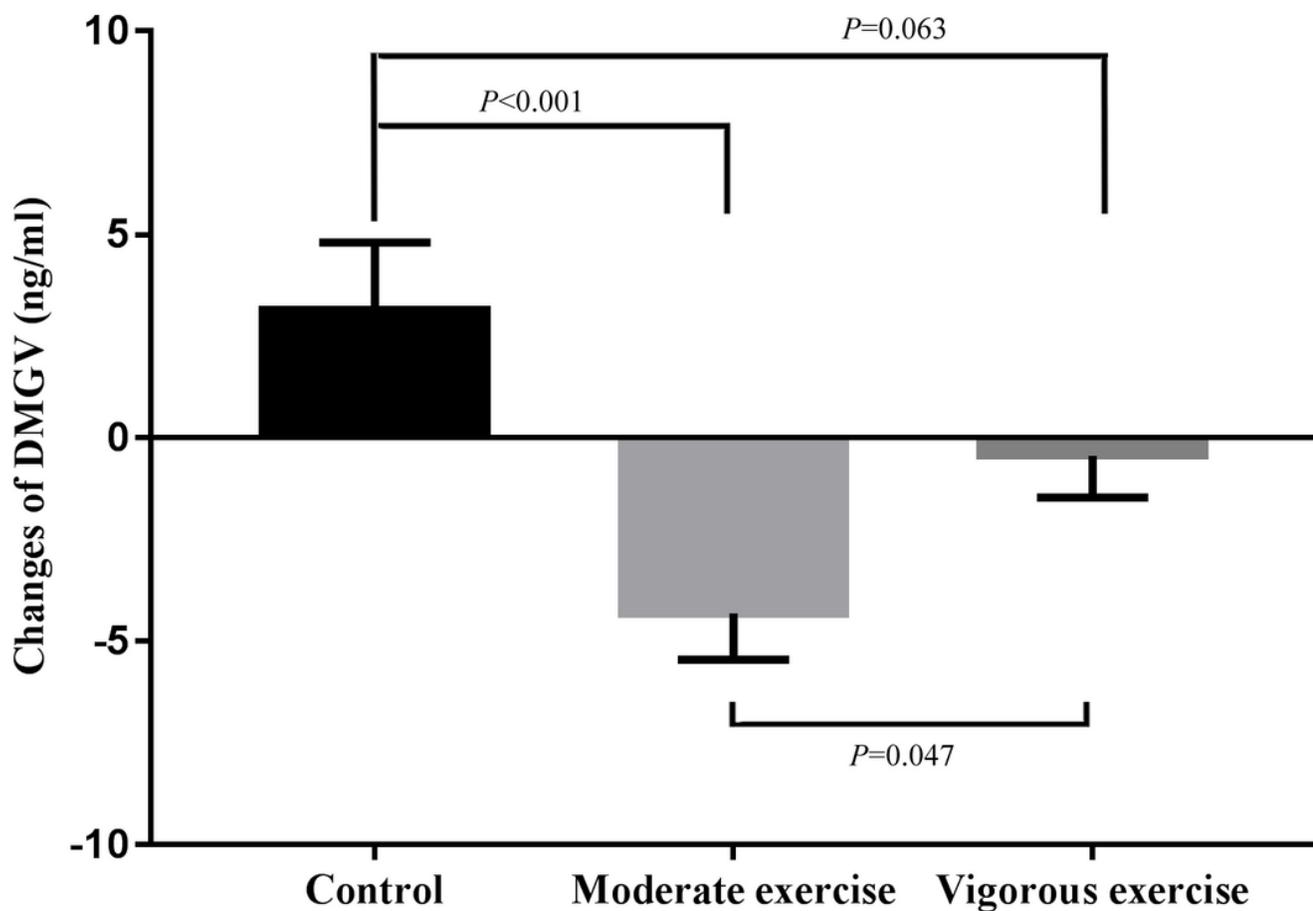


Figure 3

DMGV concentration changes after 6 month exercise intervention in control, moderate exercise and vigorous exercise groups (mean \pm SEM).

Supplementary Files

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