The Effect of A 4-Week Vegan Diet on Adipokine Levels in Healthy Male and Female Participants: A Randomized-Controlled Trial

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Abstract

Background Vegan diet (VD) is reported to show beneficial health effects including cardiovascular and anti-inflammatory protection, but the underlying mechanisms remain unclear. We hypothesized that adipokines, a special type of cytokine produced by the white adipose tissue with known effects on metabolism and the immune system, may contribute to the observed anti-inflammatory effects of VD.

Methods A parallel group interventional trial was designed to evaluate the effect of VD compared to meat-rich diet (MD) on serum levels of two central adipokines, leptin and adiponectin. Fifty-three healthy, omnivore participants (62% female, average age 31 years and BMI 23.1 kg/m^2) were randomly assigned to VD or MD for 4 weeks.

Results End value comparison between VD group and MD group showed a significantly lower level of adiponectin in the MD group (11.6 vs. 15.5 µg/mL, p = 0.025) indicating a moderate effect size (Cohen's d = 0.524). Participants' sex affected adipokine levels requiring a separate analysis of male and female participants. Leptin was increased by MD only in male participants (p = 0.019) whereas adiponectin remained stable. Female participants in VD group showed higher adiponectin levels at the end of trial (compared to VD-baseline, p = 0.023, as well as compared to MD group, p = 0.015). The end concentration of adiponectin depended on diet in female participants (p = 0.010).

Conclusion The results of our trial suggest that plasma concentration of leptin and adiponectin do not explain the immunomodulatory potential of VD in healthy participants, but it appears that diet modifies adipokine levels in a sex-specific manner.

Trial registration: German Clinical Trial register (DRKS00011963), registered 30 March 2017, https://www.drks.de/drks_web/navigate.do?navigationId=trial.HTML&TRIAL_ID=DRKS00011963.

Background

Up to a third of the body mass of a healthy, normal-weight human being consists of white adipose tissue, which is able to influence major body functions including metabolism, immune system and regulation of circulation. The white adipose tissue produces cytokines, so-called adipokines, which contribute in regulating appetite and energy homeostasis [1]. Several adipokines are known, but leptin and adiponectin are the most popular ones being the focus of thousands of scientific publications. The circulating concentration of adipokines depends on the amount of white adipose tissue [2]. The concentration of leptin is positively correlated with the body mass index (BMI) [3], and the concentration of adiponectin is negatively correlated with the BMI [4]. In normal-weight individuals low levels of leptin are responsible for feeling hungry, whereas high levels decrease appetite [5]. Obese individuals develop leptin resistance leading to an ineffective leptin regulation [3]. Leptin has, in addition, immunoregulatory functions being involved in proliferation and differentiation of different types of immune cells [2,6] and leptin appears to be increased in patients with auto-inflammatory diseases such as rheumatoid arthritis [2,7]. Adiponectin is also reported to be associated with disease activity of patients with rheumatoid arthritis [7,8].

Furthermore, low adiponectin concentrations are associated with metabolic syndrome, obesity-linked insulin resistance and atherosclerosis [4,9]. Several publications examined the influence of dietary intake on the concentration of leptin and adiponectin in plasma, yet these works did not investigate the effect of diet composition except for caloric intake [10–12]. One recent study indicated that long-term vegan and vegetarian diets might affect leptin concentrations of normal-weight individuals, specifically leading to lower leptin concentrations than average [13]. The comparison of two 12-week lasting protein-rich vegan diet with a control diet in rats revealed higher levels of adiponectin in the vegan group [14]. Plant-based diets have been reported to mediate anti-inflammatory effects [15–17]. Despite the established interplay of metabolism and immune function, the underlying mechanism of these diet-induced anti-inflammatory effects have been insufficiently studied. In 2017, we started a clinical trial aiming to broadly map the influence of a vegan diet on the immune system to clarify the underlying mechanism leading to an anti-inflammatory effect of a vegan diet [18,19]. Based on previous studies by us and others and the essential role of metabolism in immune function, we addressed this question in a randomized clinical trial and hypothesized that the introduction of a vegan diet might lead to lower levels of leptin and higher levels of adiponectin, a combination that would be favorable for adequate immune function.

Methods

A monocentric, controlled, randomized trial with parallel group design with healthy participants was performed at the Center for Complementary Medicine, University Medical of Freiburg, Germany, between April and June 2017. Methods have been described previously in detail [18,19]. The analysis of adipokines is a subgroup analysis of a clinical trial evaluating the effect of VD on immune system, Vitamin B12 metabolism and gut microbiome.

Criteria of inclusion were: Healthy, omnivorous, normal weight subjects, between 18 and 60 years of age, no regular intake of a medication as well as no clinically relevant allergies. Participants declared having no history of an eating disorder, participation in another clinical trial and blood donation in the last 4 weeks before the start of this trial. Abuse of drugs, nicotine or alcohol was not allowed. Participants had to be able to speak and understand German and to complete a nutritional protocol.

Newspaper announcement and bulletins were used for recruitment. After phone call, eligible subjects were invited for a personal visit to check eligibility criteria in detail. Precondition for inclusion was a written informed consent. After inclusion, each participant received an extensive training to keep his/her own balanced mixed diet according to the recommendations of the German Nutrition Association (DGE) [20]. After this one-week-lasting run-in phase, fasting baseline parameters were taken early in the morning. Afterwards, participants were randomly assigned to either a meat-rich (> 150 g of meat per day; any meat of their choice) or a strict vegan diet for four weeks. Every subject received another extensive training on the assigned diet and detailed written information and a recipe book. Fasting parameters were taken again after four weeks early in the morning. Participants finished the trial after blood sampling. Participants were free to choose their food within their assigned diet, but filling out a weekly nutritional protocol was mandatory for all participants. Results of nutritional protocols were used to evaluate diet

adherence. Additionally, all participants had to control their weight daily as weight changes of more than 2 kg were not allowed. Weekly follow ups were scheduled between the study staff and the participants by phone or e-mail.

Before onset, the trial was approved by the ethical committee of the University Medical Center of Freiburg, Germany (EK Freiburg 38/17), and was performed according to the principles of the declaration of Helsinki. The trial was registered at the German Clinical Trial register (DRKS00011963). The randomization list was created electronically block-wise (block size 13) by a third independent person (Python Software), sealed envelopes were used for implementation, and randomization was assigned directly after taking baseline blood samples by two members of the study group.

Serum from each participant was collected blinded for diet-assignment. Aliquots were prepared in 1.5-mL cryovials without information about the type of diet on the labels and stored at -20 °C until dry-iced cooled transport to the Department of Rheumatology and Clinical Immunology, Bad Nauheim, Germany. Adiponectin and leptin proteins were measured by ELISA as described by the manufacturer (Quantikine ELISA Human DRP300, DLP00 Bio-Techne R&D Systems, Wiesbaden, Germany). Assay Range: Adiponectin 3.9−250 µg/ml, sensitivity 0.891 ng/ml. Leptin 15.6−1,000 pg/ml, sensitivity 7.8 pg/ml. Measurement of amino acids was performed by the Laboratory of Clinical Biochemistry and Metabolism, Department for Pediatrics, University Hospital of Freiburg as previously described [18,19,21]. Measurement of leukocytes, monocytes and C-reactive protein was performed by the Central Laboratory of the University Hospital of Freiburg.

Statistical analysis

For the primary purpose of the study it was calculated that 48 participants (24 of each group) would be necessary to detect a statistical difference of $p < 0.05$ considering a statistical power of 80% and a hypothesized large effect size [18,19]. Analysis of adipokines was an exploratory aim. Therefore, to evaluate the effect of Time, Diet and Sex and their interactions on the levels of adipokines, with an estimated large effect size, at a significant alpha level of 0.05, and number of participants equal to 53 (males: 8 VD and 12 MD; females: 18 VD and 15 VD) a post hoc statistical power of 81% was calculated.

Data was entered and analyzed blinded for diet assignation via IBM SPSS (version 25.0). Baseline characteristics were evaluated by t-test, Mann-Whitney-U-Test and Fisher's exact test. Because some biochemical markers were not normally distributed, Mann-Whitney-U-test was used for comparison of group differences. Adjustment for baseline was considered by using ANCOVA. Multiple linear regression was performed to evaluate dependency effects of age, BMI (end concentration), weight changing (weight change of more than one SD (± 1.3 kg) compared to baseline weight), sex and diet on end concentration of adipokines. A three-way mixed ANOVA was used for evaluating between-subject effects of diet (VD, MD) and sex (male, female), within-subject effect of time (baseline, end), and their interactions. Correlation analysis was calculated using Spearman-Rho. Bonferroni correction was performed for all tests. Power analysis was calculated using G*Power (version 3.1.9.7).

Results

The flow of the study and baseline characteristics of the chosen participants are herein described in a condensed form as they were previously published [18,19]. Out of 150 interested persons 103 were invited for an interview after checking eligibility criteria by phone-call. Sixty-one interested persons fulfilled all criteria for inclusion and started the run-in phase. Eight of these had to be excluded before randomization because of consent withdrawal or acute illness. Overall, 26 participants were allocated to VD and 27 participants were allocated to MD for four weeks (Table 1). All participants completed the study as per protocol. At both time points (baseline and end), BMI did not differ significantly between the groups. The intake of energy was similar in both groups (Table 1) and was within the recommendations set forth by the DGE for healthy adults [18–20]. The results of nutritional protocols were previously published in detail [18].

Table 1: Demographic data of all participants in VD group and in MD group
(SD = Standard deviation, p-value from t-test/Mann-Whitney-U-Test/Fisher's exact test)

	Vegan ± SD (n = 26)	Meat-rich ± SD (n = 27)	p-value
Age (years)	33.2 ± 11.2	29.9 ± 9.5	0.407
Baseline: Body mass index (kg/m^2)	22.9 ± 2.2	23.3 ± 2.6	0.444
End of study: Body mass index (kg/m^2)	22.7 ± 2.0	23.4 ± 2.6	0.240
Baseline: Weight (kg)	68.3 ± 10.5	70.0 ± 13.3	0.762
End of study: Weight (kg)	67.8 ± 10.2	70.3 ± 13.2	0.593
Intake of energy (kcal)	2240.8 ± 894.8	2242.3 ± 762.7	0.213
Sex (n male/n female)	8/18	12/15	0.229

Systemic leptin and adiponectin before and after vegan and meat-rich diet

Comparison of baseline and end concentrations of leptin as well as of adiponectin between VD group and MD group did not reach statistical significance (Table 2). However, end value comparison between VD group and MD group showed a significantly lower level of adiponectin in the MD group (11.6 vs. 15.5 µg/mL, p = 0.025, Table 2). The effect size was moderate (Cohen's d = 0.524).

The results of mixed ANOVA presenting main and interaction effects are shown in Table 2. Results of leptin and adiponectin measurement were significantly affected by participants' sex (Table 2).

Table 2. Concentration of leptin and adiponectin before and at the end of the trial in and between both groups as well as main and interaction effects

(MD = Meat-rich diet, VD = Vegan diet, SD = Standard deviation, *Wilcoxon signed-rank-test, [+]Mann-Whitney-U-Test, °Mixed ANOVA, [†]adjusted for baseline).

		Leptin ± SD (ng/mL)		Adiponectin ± SD (µg/mL)	
		Baseline	*End*	*Baseline*	*End*
Vegan (VD)		17.3 ± 11.2	15.8 ± 11.5	13.4 ± 6.9	15.5 ± 7.6
Meat-rich (MD)		16.6 ± 14.7	16.9 ± 14.8	12.3 ± 7.2	11.6 ± 7.3
VD* p (Baseline vs. end value)		0.158		0.059	
MD* p (Baseline vs. end value)		0.387		0.400	
VD vs. MD[+] p (Baselines)		0.545		0.341	
VD vs. MD[+] p (End values)			$0.549^{†}$		$0.025^{†}$
Main & interaction effects°					
p-value	time	0.879		0.595	
	diet	0.427		0.581	
	sex	**<0.001**		**0.001**	
	time x diet	0.636		0.170	
	time x sex	0.368		0.555	
	time x diet x sex	0.993		**0.014**	

Results of multiple linear regression

The effect of multiple predictors on end concentration of adipokines is shown in Table 3. The end concentration of leptin depended on participants' end BMI.

Participants' sex affected the concentration of leptin and adiponectin as male participants had significantly lower levels of both adipokines compared to female participants (Table 3 and 4).

Table 3. Multiple linear regression of end concentration of leptin (R^2 = 0.629, Cohen's f^2 = 1.69, post hoc power analysis = 100%) and of adiponectin (R^2 = 0.298, Cohen's f^2 = 0.43, post hoc power analysis = 95%).
(*"Changing of weight" is defined as weight change of more than one SD (± 1.3 kg) compared to baseline weight;
β = standardized beta coefficient).

	Leptin			Adiponectin		
	β	95% CI	p	β	95% CI	p
Age	-0.118	-0.4-0.1	0.202	0.106	-0.1-0.3	0.401
Sex	0.702	13.9-24.0	**<0.001**	0.347	1.4-9.4	**0.010**
BMI	0.570	2.1-4.3	**<.0001**	-0.238	-1.6-0.1	0.073
Diet	0.034	-4.0-5.7	0.714	-0.170	-6.4-1.3	0.185
Changing of weight*	0.096	-2.3-7.6	0.290	0.113	-2.2-5.7	0.367

Table 4. Concentration of leptin and adiponectin before and at the end of the trial in female (n = 33) and in male (n = 20) participants (merged VD and MD groups)
(SD = Standard deviation, *Mann-Whitney-U-Test).

	Female (n = 33)	Male (n = 20)	p*
Leptin (ng/mL) ± SD			
Before	23.8 ± 11.7	5.7 ± 4.2	**0.002**
After	22.1 ± 13.6	6.9 ± 4.0	**0.001**
Adiponectin (µg/mL) ± SD			
Before	15.1 ± 7.1	9.1 ± 5.2	**<0.001**
After	16.1 ± 7.3	9.3 ± 6.2	**<0.001**

Influence of diet on adipokine levels of female participants

Female participants in the VD group (n = 18) lost on average 0.6 kg (range -2.2 - +3.4 kg; baseline 64.4 kg, end 63.9 kg, p = 0.033) body weight during the trial. In contrast, females in the MD group (n = 15) had a non-significant weight gain of 0.4 (range -0.8 - +2.0 kg; baseline 61.7 kg, end 62.1 kg, p = 0.172). BMI of the VD group was 22.6 ± 1.9 kg/m^2 before the trial and 22.4 ± 1.8 kg/m^2 at the end of the trial (p = 0.033). BMI of MD group was 22.6 ± 2.8 kg/m^2 before the trial and 22.8 ± 2.7 kg/m^2 at the end of the trial (p = 0.156).

Comparison of baseline and end concentration of leptin between VD and MD female subgroups did not reach statistical significance (Table 5 and Figure 1, Panel A). In female participants, the end concentration of leptin correlated significantly with the end BMI (r = 0.742, p < 0.001, Figure 2, Panel A). The end concentration of leptin depended on female participants' end BMI (p < 0.001, Table 5).

Table 5. Concentrations of leptin and adiponectin as well as main and interaction effects in female participants
(MD = Meat-rich diet, VD = Vegan diet, SD = Standard deviation, *Wilcoxon signed-rank-test, [+]Mann-Whitney-U-Test, °Mixed ANOVA, [†]adjusted for baseline).

	Leptin ± SD (ng/mL)		Adiponectin ± SD (µg/mL)	
	Baseline	*End*	*Baseline*	*End*
Vegan (VD, n = 18)	22.3 ± 9.0	20.2 ± 11.1	15.5 ± 6.2	18.8 ± 6.5
Meat-rich (MD, n = 15)	25.4 ± 14.4	24.6 ± 16.1	14.6 ± 8.2	12.7 ± 7.0
VD* p (Baseline vs. end value)	0.157		**0.023**	
MD* p (Baseline vs. end value)	0.955		0.173	
VD vs. MD[+] p (Baselines)	0.682		0.421	
VD vs. MD[+] p (End values)		0.510[†]		**0.001**[†]
Main & interaction effects°				
p-value	time	0.488	0.379	
	diet	0.325	0.131	
	time x diet	0.750	**0.004**	

Comparison of baseline and end concentration of adiponectin showed a significantly higher concentration in the female VD subgroup at the end of the trial (p = 0.023, Table 5 and Figure 1, Panel B). Furthermore, the end concentration of adiponectin was significantly higher in VD than in MD (18.8 vs. 12.7 µg/mL, p = 0.001). The end concentration of adiponectin depended on diet in female participants (p = 0.010, Table 6). The results of mixed ANOVA revealed an interaction effect between time and diet (time x diet p = 0.004) regarding the adiponectin concentration (Table 5). In female participants, the end concentration of adiponectin did not correlate with the end BMI (r = -0.240, p = 0.178, Figure 2, Panel B).

Table 6. Multiple linear regression of end concentration of leptin (R^2 = 0.620, Cohen's f^2 = 1.63, post hoc power analysis = 100%) and of adiponectin (R^2 = 0.290, Cohen's f^2 = 0.41, post hoc power analysis = 78%) in female participants.
(*"Changing of weight" is defined as weight change of more than one SD (± 1.3 kg) compared to baseline weight).

	Leptin			Adiponectin		
	β	95% CI	p	β	95% CI	p
Age	-0.251	-0.7-0.1	0.054	0.017	-0.2-0.3	0.923
BMI	0.802	3.2-6.4	**<0.001**	-0.289	-2.1-0.2	0.110
Diet	0.484	-5.1-8.2	0.632	-0.454	-11.4--1.7	**0.010**
Changing of weight*	-0.055	-7.7-7.3	0.957	0.281	-1.1-9.9	0.115

Influence of diet on adipokine levels of male participants

Male participants in the VD group (n = 8) had a non-significant weight loss of 0.5 kg (range -2.2 - +2.2 kg; baseline 77.1 kg, end 76.6 kg, p = 0.182) during the trial. Males in MD group (n = 12) had a non-significant weight gain of 0.4 kg (range -2.9 - + 2.3 kg; baseline 80.3 kg, end 80.7 kg, p = 0.214). BMI of VD group was 23.6 ± 2.7 kg/m^2 before the trial and 23.4 ± 2.5 kg/m^2 at the end of the trial (p = 0.208). BMI of MD group was 24.1 ± 2.2 kg/m^2 before the trial and 24.2 ± 2.3 kg/m^2 at the end of the trial (p = 0.214).

Leptin concentration increased significantly during the trial in MD group (p = 0.019, Table 7 and Figure 3, Panel A). The results of mixed ANOVA did not show any main effects of time or diet as well as interaction effects between diet and time on leptin concentration in male participants (Table 7).

Table 7. Concentrations of leptin and adiponectin as well as main and interaction effects with and without consideration of BMI and age in male participants
(MD = Meat-rich diet, VD = Vegan diet, SD = Standard deviation, *Wilcoxon signed-rank-test, [+]Mann-Whitney-U-Test, °Mixed ANOVA, [†]adjusted for baseline).

	Leptin ± SD (ng/mL)		Adiponectin ± SD (µg/mL)	
	Baseline	*End*	*Baseline*	*End*
Vegan (VD, n = 8)	5.9 ± 6.0	6.3 ± 5.0	8.6 ± 6.1	7.9 ± 2.7
Meat-rich (MD, n = 12)	5.5 ± 2.5	7.3 ± 3.4	9.5 ± 4.7	10.2 ± 7.7
VD* p (Baseline vs. end value)	0.735		0.889	
MD* p (Baseline vs. end value)	**0.019**		0.754	
VD vs. MD$^+$ p (Baselines)	0.734		0.571	
VD vs. MD$^+$ p (End values)		0.245†		0.427†
Main & interaction effects°				
p-value	time	0.063	0.968	
	diet	0.857	0.532	
	time x diet	0.236	0.446	

In male participants, the end concentration of leptin correlated significantly with the end BMI (r = 0.596, p = 0.006, Figure 4, Panel A), but multiple linear regression did not reveal any dependency on end concentration of leptin (Table 8).

Adiponectin concentration remained stable during the trial in male participants (Table 7 and Figure 3, Panel B). The results of mixed ANOVA did not show any main effects of time or diet as well as interaction effects between diet and time on adiponectin concentration in male participants (Table 7). In male participants, the end concentration of adiponectin did not correlate with the end BMI (r = -0.293, p = 0.211, Figure 4, Panel B). Multiple linear regression did not reveal any dependency on end concentration of adiponectin (Table 8), but the results are limited due to the poor test quality.

Table 8. Multiple linear regression of end concentration of leptin (R^2 = 0.486, Cohen's f^2 = 0.946, post hoc power analysis = 86%) and of adiponectin (R^2 = 0.273, Cohen's f^2 = 0.38, post hoc power analysis = 44%) in male participants.
(*"Changing of weight" is defined as weight change of more than one SD (± 1.3 kg) compared to baseline weight).

	Leptin			Adiponectin		
	β	95% CI	p	β	95% CI	p
Age	0.128	-0.1-0.2	0.512	0.370	-0.1-0.5	0.124
BMI	0.421	-0.1-1.5	0.061	-0.388	-2.4-0.4	0.137
Diet	0.045	-2.9-3.6	0.815	0.261	-2.7-9.1	0.261
Changing of weight*	-0.405	-6.8-0.3	0.073	-0.125	-8.1-5.0	0.623

Influence of age on concentration of leptin and adiponectin

The average age of participants was 31 years, and the median age of participants was 27 years. In order to be able to divide the comparison groups evenly, we initially used median value for further calculation. As median value Twenty-seven participants were 27 years old or even younger (12 VD and 15 MD), whereas 26 participants were older than 27 years (14 VD and 12 MD). Leptin concentration and adiponectin concentrations did not differ significantly in both groups before and at the end of the trial. The results of mixed ANOVA did not show any main or interaction effects of time or diet on leptin concentration in participants younger and older than 27 years. Mixed ANOVA revealed an interaction effect of time x diet on adiponectin concentration of younger participants (p = 0.003), but concentration of adiponectin after the trial did not differ significantly between VD (14.7 ± 8.8 μg/mL) and MD (11.4 ± 7.5 μg/mL) in these participants (p = 1.000).

Forty participants were 35 years old or younger (19 VD and 21 MD), and 13 participants were older than 35 years (7 VD and 6 MD). Only older participants showed an interaction effect of diet x time on leptin concentration (p = 0.023), but concentration of leptin after the trial did not differ significantly between VD (12.7 ± 5.5 ng/mL) and MD (21.1 ± 21.7, p = 1.000) in participants of 35 years of age. Participants younger than 35 years showed an interaction effect of diet x time on adiponectin concentration (p = 0.007), and the concentration of adiponectin after the trial differed significantly between VD (15.2 ± 7.6 μg/mL) and MD (10.9 ± 6.8 μg/mL, p = 0.044) in younger participants.

As mentioned before (Tables 3, 6 and 8) multiple linear regression did not reveal any dependency of age and end concentration of adipokines. There was no correlation between age and adipokine concentration in male and female participants (Figure 5 and 6).

Correlation of leptin and adiponectin with nutritional intake

All results of nutritional protocols were previously published [18,19]. Leptin concentration at the end of the trial did not show any correlation with nutritional intake of saturated, mono- or polyunsaturated fatty acids as wells as with total fat, cholesterol or total energy. We found a significant correlation of end adiponectin concentration with saturated fat intake (r = -0.364, p = 0.017), but not of mono- or polyunsaturated fatty acids as well as total fat and total energy. The intake of cholesterol was negatively

correlated with the end concentration of adiponectin (r = -0.474, p = 0.001) showing differences in VD group (r = -0.535, p = 0.010) and MD group (r = -0.036, p = 0.876). We found no sex-specific differences.

Correlation of adipokines with immune parameters and branched-chain amino acids

As we were able to previously show significantly lower levels of immune cells (leukocytes and monocytes) in VD compared to MD [19], we correlated the concentration of adipokines with these immune cells. Due to the immunomodulatory potential of adipokines we also performed a correlation of adipokines with C-reactive protein (CRP). Neither correlation of leptin with leukocytes (r = 0.252, p = 0.069), monocytes (r = 0.137, p = 0.327) and CRP (r = 0.171, p = 0.393) nor correlation of adiponectin with leukocytes (r = -0.103, p = 0.461), monocytes (r = -0.134, p = 0.339) and CRP (r = 0.167, p = 0.406) revealed significant results.

Furthermore, we found an association of lower serum concentrations of branched-chain amino acids (BCAA; valine, leucine and isoleucine) with lower numbers of immune cells in VD group at the end of the trial. Therefore, wee next evaluated the relationship between adipokines and BCAA. The baseline concentration of leptin correlated inversely with the baseline serum concentrations of valine (r = -0.302, p = 0.028), leucine (r = -0.306, p = 0.026) and isoleucine (r = -0.365, p = 0.007). At the end of the trial there was only an inverse correlation of leptin with valine (r = -0.301, p = 0.029, Figure 7, Panel A), which was more attributable to a correlation in MD group (r = -0.408, p = 0.034) than in VD group (r = -0.244, p = 0.230).

The baseline concentration of adiponectin correlated inversely with the baseline serum concentrations of valine (r = -0.412, p = 0.002), leucine (r = -0.311, p = 0.023) and isoleucine (r = -0.402, p = 0.003). The correlation between adiponectin and branched-chain amino acids remained visible at the end point measurement (valine: r = -0.569, p < 0.001, leucine: r = -0.478, p < 0.001, isoleucine: r = -0.575, p < 0.001, Figure 8), and was attributable to the VD group (valine: r = -0.637, p < 0.001, leucine: r = -0.670, p < 0.001, isoleucine: r = -0.438, p = 0.012), not to MD group (valine: r = -0.358, p = 0.066, leucine: r = -0.330, p = 0.092, isoleucine: r = -0.353, p = 0.071).

Subgroup analysis revealed that the correlation between adiponectin and BCAA at the end of the trial was more attributable to the VD group (valine: r = -0.637, p < 0.001, leucine: r = -0.483, p = 0.012, isoleucine: r = -0.670, p < 0.001) and to female participants (valine: r = -0.582, p < 0.001, leucine: r = -0.415, p = 0.016, isoleucine: r = -0.576, p < 0.001).

Discussion

Adipokines are factors derived from white adipose tissue that regulate metabolism and immune function [22]. Leptin acts as a modulator of appetite whereas adiponectin sensitizes cells to insulin. The direct control of central metabolism by adipokines results in pro- and anti-inflammatory effects [22]. This study was driven by the hypothesis that the adipokines leptin and adiponectin contribute to the immunomodulatory effects observed in an earlier study upon intervention of healthy subjects with a

balanced vegan diet. Our results suggest that leptin concentration is not modifiable by short-term VD. Instead, a comparison of baseline and end concentration of leptin in MD participants revealed a significant increase of this adipokine in male participants. Adiponectin was higher in the VD group after the trial and this was not dependent on initial baseline concentrations. Further analysis of adiponectin changes within the VD group showed sex-dependency as only adiponectin of female participants differed significantly at the start and end of the trial. Female participants in VD group had a slight weight-loss, which might confound the increase in adiponectin after the trial since the difference might not be caused by diet, but by weight loss. Weight-loss is discussed to be related to changing of adipokine levels. Published reports indicate effects of weight-loss on serum leptin, but the same has not been unequivocally seen with respect to serum adiponectin concentration [12]. Therefore, we performed a subgroup analysis excluding patients with a weight change of more than 1.3 kg. Interestingly, the difference of adiponectin between VD and MD remained statistically significant suggesting a not-weight related effect of diet on adiponectin levels in female participants. In male participants, who had no weight-loss during the trial, adiponectin remained stable, but comparability of male and female participants is limited.

Sex-dependent differences in adipokine levels are known for a long time [23–25], and the results of our trial also show sex-dependent differences leading to the performed subgroup analyses. The results of our and other studies emphasize not only the previously known fundamental difference of adipokine levels in males and females, but also a supposed sex-difference in alterability by diet or specific nutritional components [26]. This is also supported by the results of Vučić Lovrenčić et al. as they observed differences of adipokine concentration in female vegetarians compared to female omnivores but not in male vegetarians compared to male omnivores [27]. Apart from sex-related differences, the general impact of specific diets or of specific nutritional components on adipokines levels of normal-weight human beings remains still unclear, since the majority of publications dealt with adipokine changes in weight-losing subjects or in animals [28]. A systematic review by Eichelmann et al. indicated no pronounced effect of plant-based diets on adipokines levels, but they added that the analysis was restricted by number and quality of available studies suggesting necessity for more research [29]. Some other clinical trials indicated that specific nutritional components such as soups, vegetables, vegetable oils or dietary fiber might have an increasing effect on leptin concentration in weight-stable healthy participants [26,30–32]. Leptin is considered a pro-inflammatory cytokine being increased in patients with rheumatic diseases [7,33]. Consumption of meat is hypothesized to be one of the major contributors to promote inflammation in rheumatic diseases being supported by clinically observed improvement of disease activity by plant-based diets [16,34,35]. The increase of meat intake in our male MD participants led to an increase of leptin that supports a relationship between higher meat intake and inflammation. It remains unclear whether an increase in leptin leads to pathological increased inflammation of healthy participants as none of the participants showed adverse effects clinically or in laboratory tests [19]. Nevertheless, numerous influencing factors such as sex, age and sexual hormone status have to be taken into account and may bias results of studies with adipokines. Significance of our age-related analyses is limited due to the younger age of our participants (median 27 years of age). Most of other publications

reported age-related effects on adipokine levels in participants older than 60 years [36]. Furthermore, affection by sexual hormone status and menstrual cycle was not captured in our trial, which might imply a bias of our results concerning especially female participants [37]. The relatively short duration of our trial, 4-weeks, may be a factor contributing to the apparent diffuse association between diet and adipokine profiles. Interestingly, leptin responds quickly to changes in dietary composition. More than 20 years ago Havel et al. performed a trial comparing a just 24-hours-lasting high fat/low carb vs. a low fat/high carb diet in healthy women, and found a decrease of the physiologically circadian rhythmic leptin concentration in participants with high fat/low carb diet [38]. This finding of leptin is supported by other studies indicating possibility of rapid modulation of human adipokine levels [39,40]. Furthermore, Havel et al. hypothesized that greater intake of fat might lower leptin concentration whereas low fat/high carb diet induces higher levels of leptin. Comparability of those earlier studies with our results is limited as the composition of VD and MD were not comparable to low fat/high carb and high fat/low carb diet [18]. Although, intake of cholesterol and saturated fatty acids differed significantly between VD and MD in our cohort, we found no correlation of these parameters with leptin, which might be attributable to a similar intake of carbohydrates in VD and MD not-inducing a smaller insulin glucose response as seen in high fat/low carb diets. Leptin and insulin are known to be able to affect each other's serum concentration [41,42]. Furthermore, we found that adiponectin was negatively correlated with cholesterol and saturated fatty acids intake. Rodent models have shown an effect of dietary fatty acids on serum adiponectin indicating lower levels of adiponectin in high fat diet supporting our results, but clinical data in humans are lacking [43]. In our study, the type of diet affected the correlation of cholesterol and adiponectin as correlation was only found in VD, which is nearly free of dietary cholesterol. The observed correlation might, therefore, not be caused by the amount of cholesterol intake, but by other elements of the diet itself.

In a recent publication we reported about an association of lower levels of BCAA with lower inflammatory parameters in VD [19]. The previously described immunomodulatory potential of adipokines motivated us to investigate possible associations between lower levels of BCAA and lower levels of leptin and higher levels of adiponectin, respectively. The concentration of BCAA correlated inversely with the concentration of adiponectin in VD group. Regardless of diet, a correlation between BCAA and adiponectin was also reported by others [44,45]. Interestingly, we found a correlation of BCAA and adiponectin only in VD group, potentially indicating the pre-described anti-inflammatory effect of VD. However, we found no correlation of adiponectin with immune parameters underlining the controversial immunomodulatory potential of adiponectin. Recent literature suggests pro- and anti-inflammatory effects of adiponectin depending on pre-existing diseases [46]. In participants not suffering from inflammatory diseases, adiponectin appears to have anti-inflammatory potential being an insulin-sensitizing, vascular-protective and anti-inflammatory protein [7,46,47]. Similar effects are described for VD underlining a potential relation between higher levels of adiponectin in VD [17,48]. Finally, while leptin and adiponectin are considered 'key cytokines' that have dedicated cellular receptors and well-described roles in human physiology [49], recent studies suggest that novel cytokines such as GDF15, CXCL14, S100A4 and Meteorin-like may also be important in the regulation of glucose and lipid metabolism and immune cell function [50–53]. Further

research incorporating comprehensive profiling of cytokines in clinical trials is necessary and desirable to uncover whether the concentration of these cytokines is modifiable by dietary intervention.

Conclusions

In summary, serum levels of the most commonly known adipokines, leptin and adiponectin, do not fully explain the immunomodulatory potential of VD in healthy participants. However, the results of our trial suggests that the effect of VD and MD on adipokines levels might depend on participants' sex, as male and female participants showed different response on the nutritional change. Elucidating whether the observed sex-specific differences emerge from the inflammatory potential of diets requires further investigation, ideally in long-term trials.

Abbreviations

BCAA
Branched-chain amino acids, BMI:Body mass index, CRP:C-reactive protein, DGE:German Nutrition Association (Deutsche Gesellschaft für Ernährung), MD:meat-rich diet, RA:rheumatoid arthritis, VD:vegan diet

Declarations

Ethics and Consent to Participate

Study was approved by local ethical committee (ethical committee of the University Medical Center of Freiburg, Germany (EK Freiburg 38/17)).

Consent for publication

Not applicable

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

None. The authors have no conflicts of interest or financial ties to disclose.

Funding for research

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector. The Ministry of Science, Research and Arts of the state Baden-Württemberg, Germany,

financed the position of Dr. Lederer within the Academic Center for Complementary and Intergrative Medicine (AZKIM), otherwise the study was financed by institutional resources.

Authors' contributions

Dr. Ann-Kathrin Lederer, Manuel Hettich and Prof. Dr. Roman Huber are responsible for conception and design. Acquisition of data was performed by Dr. Ann-Kathrin Lederer, Manuel Hettich, Dr. Luciana Hannibal and PD Dr. Elena Neumann. Data was analyzed by all authors. Statistical analysis was performed by Dr. Ann-Kathrin Lederer and Dr. Luciana Hannibal. Dr. Ann-Kathrin Lederer wrote the manuscript with help of Dr. Luciana Hannibal and PD Dr. Elena Neumann. Prof. Dr. Roman Huber and Manuel Hettich revised the article.
All Authors have seen and approved the manuscript.

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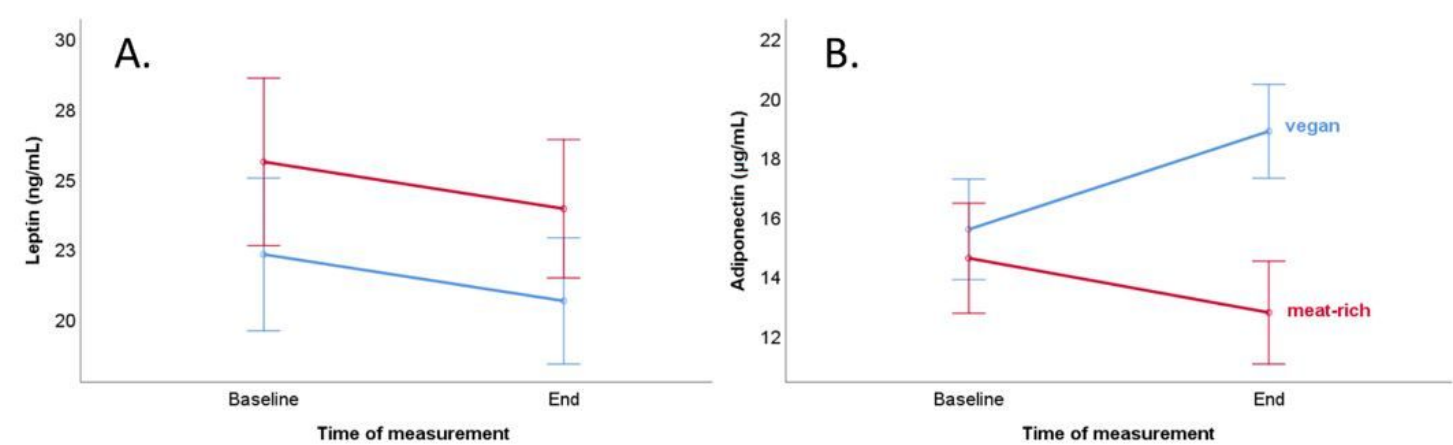
Figures

Figure 1

Course of adipokine level of the vegan group (blue line) and the meat-rich group (red line) during the trial with consideration of potential confounders (BMI and age) in female participants. Bars show standard error ± 1. A. Course of serum leptin concentration (ng/mL). B. Course of serum adiponectin concentration (µg/mL).

Figure 2

Correlation of the end concentration of adipokines with the end BMI (kg/m2) in female participants. A. Correlation between leptin and BMI (Spearman's r = 0.742, p < 0.001). B. Correlation between adiponectin and BMI (r = -0.240, p = 0.178).
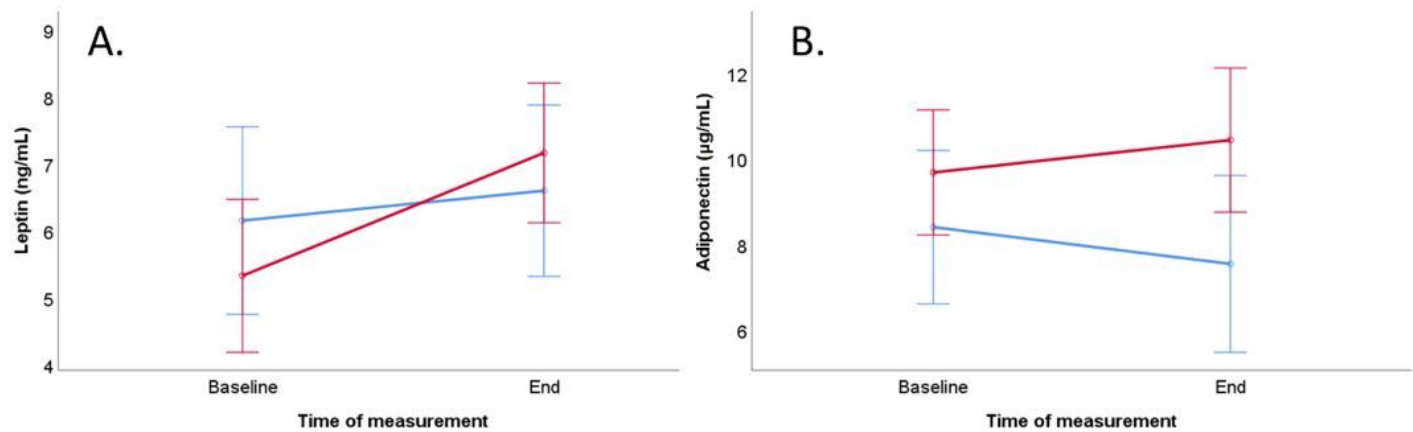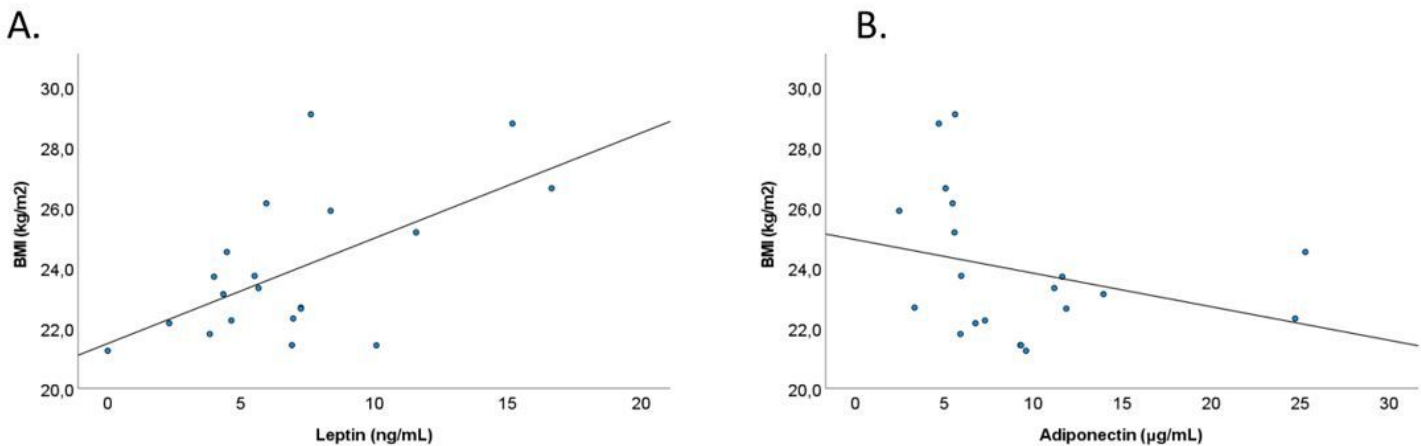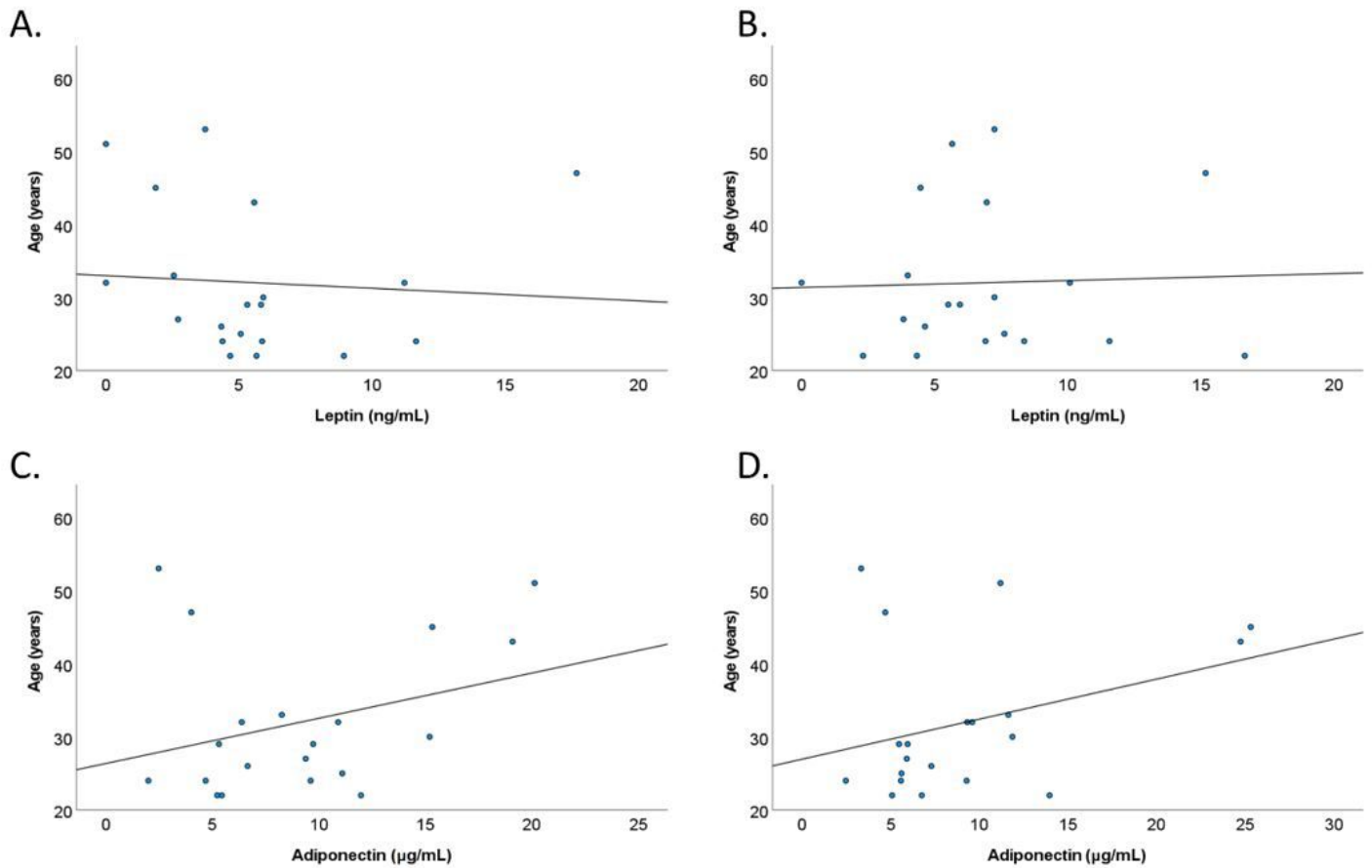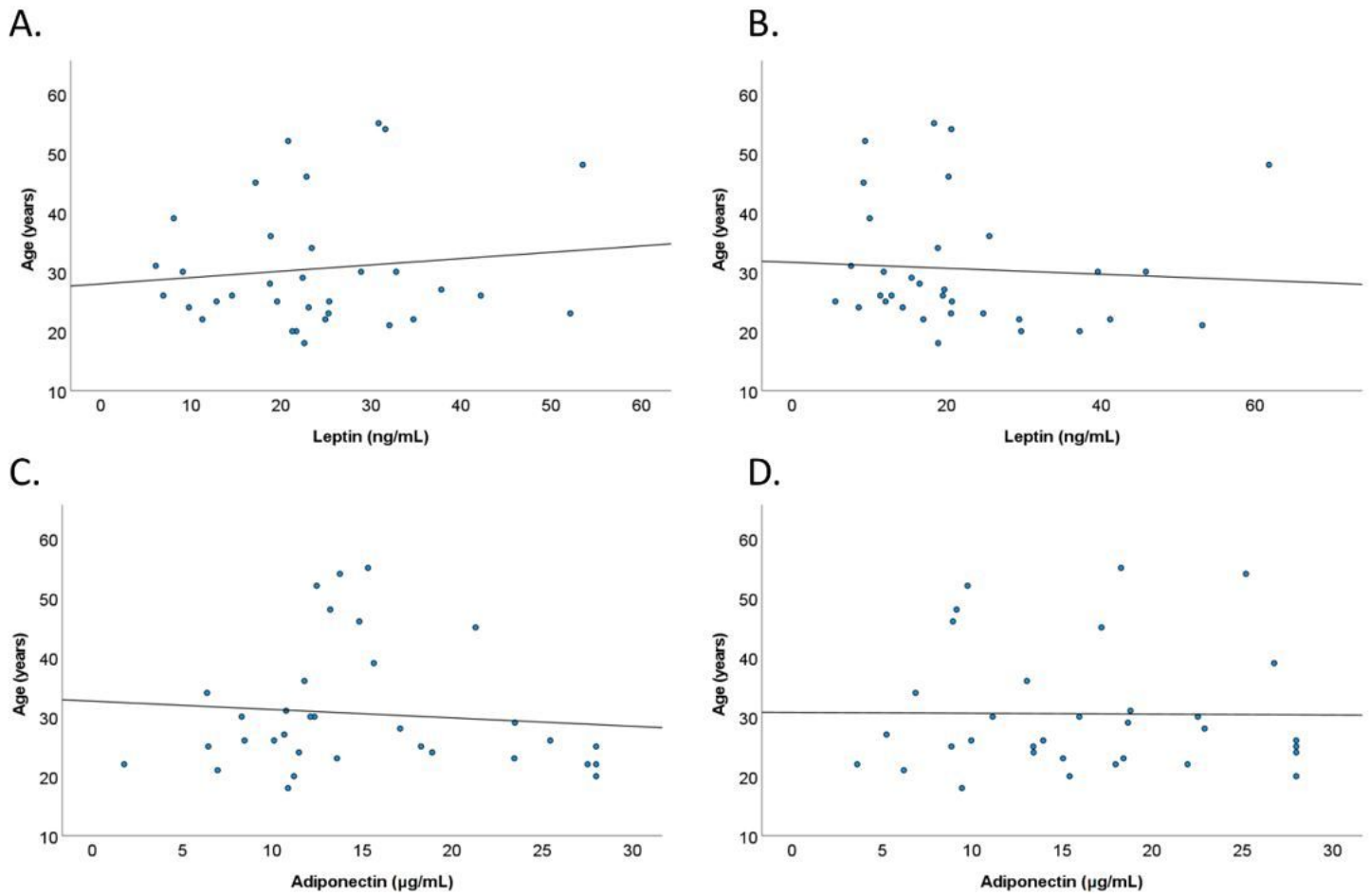

Figure 3

Course of adipokine levels of the vegan group (blue line) and meat-rich group (red line) during the trial with consideration of potential confounders (BMI and age) in male participants. Bars show standard error ± 1. A. Course of serum leptin concentration (ng/mL). B. Course of serum adiponectin concentration (μg/mL).

Figure 4

Correlation of the end concentration of adipokines with the end BMI (kg/m2) in male participants. A. Correlation between leptin and BMI (Spearman's r = 0.596, p = 0.006). B. Correlation between adiponectin and BMI (r = -0.293, p = 0.211).
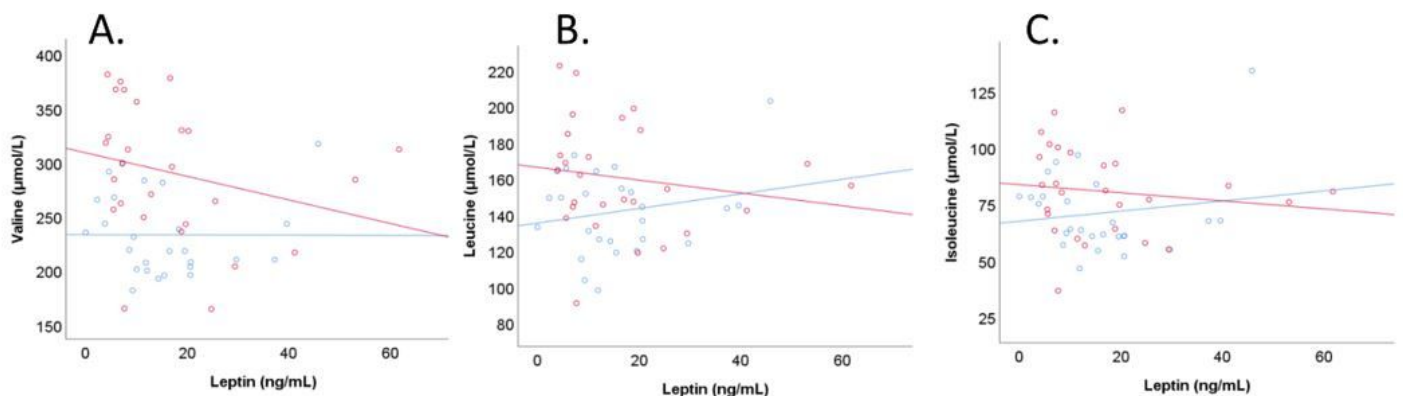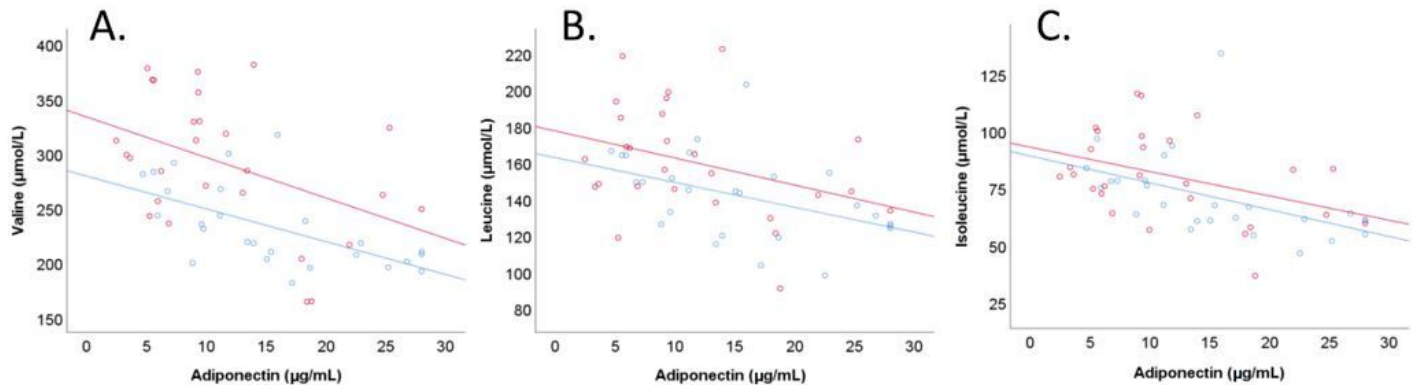
Figure 5

Correlation of adipokine concentration with male participants' age. A. Correlation between baseline leptin and age (Spearman's r = -0.071, p = 0.767). B. Correlation between end leptin and age (r = 0.038, p = 0.874). C. Correlation between baseline adiponectin and age (r = 0.317, p = 0.174). D. Correlation between end adiponectin and age (r = 0.335, p = 0.149).

Figure 6

Correlation of adipokine concentration with female participants' age. A. Correlation between baseline leptin and age (Spearman's r = 0.118, p = 0.512). B. Correlation between end leptin and age (r = -0.064, p = 0.721). C. Correlation between baseline adiponectin and age (r = -0.095, p = 0.600). D. Correlation between end adiponectin and age (r = -0.010, p = 0.958).

Figure 7

Correlation of the end concentration of leptin (ng/mL) with the concentration of serum branched-chain amino acids (µmol/L) in the VD group (blue regression line and blue dots) and MD group (red regression line and red dots) at the end of the trial. A. Correlation between leptin and valine (VD: Spearman's r = -0.244, p = 0.230; MD: r = -0.408, p = 0.034). B. Correlation between leptin and leucine (VD: r = -0.336, p = 0.093; MD: r = -0.276, p = 0.164). C. Correlation between leptin and isoleucine (VD: r = -0.077, p = 0.709; MD: r = -0.309, p = 0.117).

Figure 8

Correlation of end concentration of adiponectin (µg/mL) with the concentration of serum branched-chain amino acids (µmol/L) in VD group (blue regression line and blue dots) and MD group (red regression line and red dots). A. Correlation between adiponectin and valine (VD: Spearman's r = -0.569, p < 0.001; MD: r = -0.358, p = 0.066). B. Correlation between adiponectin and leucine (VD: r = -0.478, p < 0.001; MD: r = -0.330, p = 0.092). C. Correlation between adiponectin and isoleucine (VD: r = -0.575, p < 0.001; MD: r = -0.353, p = 0.071).

Supplementary Files

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- CONSORT2010Checklist.doc