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Title: A longitudinal analysis of the relationships of physical activity and body fat with nerve growth factor (NGF) and brain-derived neural factor (BDNF) in children

Running title: Physical activity, NGF and BDNF in children

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Abstract

Background: Nerve growth factor (NGF) and brain-derived neural factor (BDNF) are important for brain function and detectable in the blood. This study explored the longitudinal associations of physical activity and body fat with serum NGF and BDNF in children.

Methods: Two waves of measurements two years apart were performed in 8-11 years old children, including physical activity using the ActiGraph model 7164, body composition by dual x-ray absorptiometry, and serum NGF and BDNF determined by multiplex immunoassay. The first wave included 248 children. Full Information Maximum Likelihood estimation with robust standard errors was applied in structural equation modeling.

Results: Vigorous physical activity showed a direct positive longitudinal relationship with NGF (standardized coefficient Beta=0.30, p=0.006) but not with BDNF (Beta=0.04, p=0.84). At the same time, body fat % was positively related to both NGF (Beta=0.59, p<0.001) and BDNF (Beta=0.17, p=0.04). There was an indication of an indirect relationship of vigorous physical activity with NGF (product of unstandardized coefficients B=-0.18, p=0.02) and BDNF (B=-0.07, p=0.053) through the negative relationship with body fat % (Beta=-0.36, p<0.001).

Conclusions: Vigorous physical activity is directly related to serum NGF and indirectly through level of body fat. The relationships with serum BDNF are more complex.

Introduction

There is strong evidence of the favorable effects of physical activity on obesity and cardiometabolic risk in children, while the evidence of the effect on brain function is less clear [1]. Nerve growth factor (NGF) and brain-derived neural factor (BDNF) are important for neural development and function [2] and have been targeted in physical activity research. These neurotrophins increase in different brain regions (e.g. hippocampus) after exercise in rats [3]. In addition to neural cells, NGF and BDNF are also produced by other cell types like inflammatory cells, structural cells and muscle cells [4-6]. In humans, NGF and BDNF have been studied by measuring their levels in the blood (serum or plasma). Exercise increase the permeability of the blood-brain barrier [7], which suggests that the levels of NGF and BDNF in the brain and in the blood may be interrelated.

Acute bouts of exercise increase serum levels of NGF [7] and BDNF [7-9]. However, well-trained individuals show higher serum levels of NGF [4,10], but lower serum levels of BDNF [8-10]. The latter is supported by a study in adolescents, where objectively assessed physical activity was negatively associated with serum levels of BDNF [11]. One suggested explanation to this contrasting results between NGF and BDNF is based on the fact that, in contrast to NGF, most of the BDNF in blood is stored in the platelets [9,12]. Changes in blood levels of BDNF may therefore reflect changes in platelet activation as a response and adaption to regular exercise for continuous local release of BDNF for tissue restoration and control of energy balance [10,13,14]. The consequence of regular exercise would then be reduced levels of BDNF in the platelets and, hence, in the blood. Both serum and plasma BDNF levels as well as platelet BDNF concentration have been negatively correlated with VO_2max/kg, while no correlation has been seen between platelet concentration and VO_2max/kg [9].

NGF and BDNF are interconnected in metabolic control and in the development of obesity and metabolic syndrome [15]. Metabolic dysfunction may alter their production, access to and effect at the body tissues, but to a degree depending on the individual metabolic state. For example, overweight women with higher plasma levels of glucose, insulin and lipids than normal-weight women showed higher level of NGF but similar level of BDNF [16]. However, regular exercise may counteract these detrimental developments. Hence, the interrelationship between physical activity, obesity and expression of NGF and BDNF in the blood versus their expression in the brain is complex. Serum levels in the normal population reflects the concomitant influence of physical activity and body fat, and their relationship.

Childhood is a period in life characterized by rapid development of the neural system supported by a physically active lifestyle. Few studies have investigated the relationship of physical activity with the expression of NGF and BDNF in children. Further, the studies described above had a cross-sectional design. Therefore, the aim of the present study was to investigate the longitudinal association of physical activity and body fat with NGF and BDNF in children.

Methods

Design

Two waves of measurements were performed two years apart. The main target was to explore whether first wave physical activity predicted second wave levels of NGF and BDNF. Structural equation modelling was performed, with the application of full information maximum likelihood estimation with robust standard error for more efficient handling of missing data and skew distributions. This study is part of the Bunkeflo project, a longitudinal investigation of physical activity in children [17]. The institutional ethics committee of Lund University approved the study.

Participants

The children were recruited from four schools in a socio-economically middle-class area in Malmö in the southern region of Sweden, with inhabitants of mainly non-immigrant origin. All 477 children (54% boys) in grade 3 and 4, 8-11 years old, were invited and 248 accepted to participate (56% boys). Written informed consent was obtained from the parents of participating children. There was no significant difference in body weight, height or body mass index (BMI) between participants and non-participants according to anthropometric data received from the school nurses [18]. Consent blood samples was received from 172 children. Table 1 presents the characteristics and the number of participants providing data of the individual variables at each wave, as well as wave 1 characteristics of those providing wave 2 data. There was a loss of data, especially blood data, from the first wave to the second wave. Participants with complete data at wave 2 did not, however, deviate from the characteristics of the full sample at wave 1.

Physical activity

Physical activity was assessed using the ActiGraph model 7164 (ActiGraph, Pensacola, FL, USA). This is a uniaxial accelerometer recording acceleration signals along the vertical axis to generate activity counts corresponding to activity intensity [19]. Recordings of physical activity in the present study were performed during the whole autumn school term. The children were instructed to wear the monitor for 4 consecutive days, including both weekdays and weekends, over the right hip in an elastic belt around the waist, during the entire day and only to remove it during activities that could damage the hardware (e.g. swimming, showering). The monitors were set to record and store data in 10-seconds epochs. Missing data defined as a continuous sequence of at least 10 minutes of zero counts were denoted non-wear time and excluded from analysis. The criteria for a valid physical activity recording were a minimum of 3 days of at least 8 hours

wear time per day, which was a compromise between having sufficient amount of recorded time and to include as many participants as possible in the analyses. The physical activity variables investigated were time spent in moderate-and-vigorous physical activity (MVPA, ≥ 3500 counts per minute) and vigorous physical activity (VPA, ≥ 6000 counts per minute). Several cut-points for physical activity intensities have been proposed, but there has been no consensus of which one to use [20]. The cut-points were therefore estimated weighed averages from previous calibration studies [21,22]. Data cleaning, wear time validation and data scoring were performed in an SAS-based software (SAS Institute Inc., Cary, North Carolina, USA).

NGF and BDNF

Blood samples were taken in a non-fasting condition and stored as serum in freezers at a temperature of -70 degrees Celsius until being analyzed. The biomarkers investigated herein are not sensitive to food intake [23]. Therefore, the non-fasting condition may have minor influence on the outcome. The frozen serum samples were sent to and analyzed at the nationally supported laboratory SciLifeLab in Uppsala, Sweden (www.scilifelab.se) using the Proseek Multiplex CVD, Inflammation and Oncology biomarker panels (Olink Bioscience, Uppsala, Sweden). The method is a multiplex immunoassay based on a Proximity Extension Assay [24]. The precision of the blood analyses was assessed by the intra-assay coefficient of variation (CV) and inter-assay CV, and they were 6% and 14% for NGF and 6% and 10% for BDNF. Data are presented as arbitrary units (au). The values can be used for relative comparison but are not measures of the absolute quantity. An approximation of the quantity can be achieved from the general calibration curve presented at the webpage of Olink Bioscience (www.olink.se).

Anthropometry

Body mass and height were measured dressed in light clothes and without shoes. Total body fat (TBF, kg) was determined from Dual-energy X-ray Absorptionmetry (DXA; DPX-L version 1.3z; Lunar, madison, WI, USA) [25]. Body fat percentage (%) was calculated as the quotient of body fat and total body mass and used in the statistical analyses.

Statistics

Structural equation modeling, with observed variables, was applied in order to test the longitudinal interrelationships between physical activity, NGF and BDNF, as well as the zero-order correlations at each measurement wave. An autoregressive cross-lagged model within a structural equation modeling framework was fit to data [26] using Mplus 7.4 (Muthén & Muthén, Los Angeles, CA, USA). As body fat % and sex may have influence on biomarker expression, these variables were considered in the modeling. Physical activity, NGF, BDNF and body fat % were investigated as endogenous parameters in order to take a potential change into account between the two measurement waves. In the estimation, a full information maximum likelihood (FIML) estimator with robust standard errors (MLR) was applied. MLR can effectively estimate unbiased parameters in the presence of data which are missing at random or missing completely at random, since it employs all the available data [27]. In addition, the MLR estimator is appropriate to analyze small and medium samples with non-normal distributions [28]. A Shapiro-Wilks test indicated that all continuous variables were associated with a non-normal distribution. Body fat can be considered an independent predictor of NGF and BDNF as well as mediator of the effect of physical activity. A mediation analysis was therefore performed where the product of regression coefficients of the relationships between physical activity and body fat %, and between body fat % and NGF/BDNF constitutes the indirect effect through body fat % [29].

Model fit was assessed by using standard index, i.e. the comparative fit index (CFI), and the misfit measure known as the root mean square error of approximation (RMSEA). While the CFI assess the adequacy of the specified model in relation to the baseline model and it ranges between 0 and 1, the RMSEA measures the approximation error in the model. Good fit is indicated by CFI values ≥0.95, and RMSEA values ≤0.06 [30]. Bayesian Information Criterion value (BIC) was also used to determine model fit. All models presented fulfilled the above criteria.

Results

Time spent in MVPA activity did not show any significant relationships with NGF or BDNF, and was therefore excluded from subsequent analysis. Consequently, only time spent in VPA was further investigated.

In the zero-order cross-sectional analyses, at wave 1, neither VPA or body fat % were significantly correlated with NGF or BDNF, but at wave 2, VPA showed a significant negative correlation with BDNF while body fat % was positively correlated with NGF. VPA and body fat % showed a significant negative inter-correlation at both waves, and being a female was negatively correlated with VPA but positively correlated with body fat % at both waves. These correlations were the starting-point for our longitudinal modeling.

In this section only the significant relationships are presented. All relationships investigated can be found in the supplement table. In the first model (Figure 1), body fat % was not included. In this case, VPA at wave 1 showed no significant relationship with NGF or BDNF at wave 2. However, when body fat % was included, VPA showed a significant positive relationship with NGF but was not significantly related with BDNF (Figure 2). Further, body fat % at wave 1 had a

significant positive relationship with both NGF and BDNF at wave 2. In a development of the second model, body fat % (wave 1) was considered a mediating variable (Figure 3). In this model, VPA had a significant direct positive relationship with NGF (path a, p=0.009). In addition, VPA had a significant negative relationship with body fat % (path b, p>0.001), which in turn had a significant positive relationship with both NGF (path c_1, p<0.006) and BDNF (path c_2, p=0.02). The indirect effect (product of unstandardized coefficients) of VPA via body fat % was negative and significant for NGF (b·c_1, B=-0.18, p=0.02) and borderline significant for BDNF (b·c_2, B=-0.07, p=0.053).

Discussion

In this longitudinal observation study in Swedish 8-11 years old children we found that vigorous physical activity was directly related to the serum levels of NGF but not to BDNF; the more time spent in vigorous physical activity the higher level of NGF. At the same time, body fat was related to the serum levels of NGF and BDNF; the higher body fat the higher levels of NGF and BDNF. The results further indicated an indirect relationship of vigorous physical activity with NGF and BDNF via body fat. Finally, vigorous physical activity showed a negative correlation with BDNF at the second measurement wave.

Altogether, our findings together with results from previous research indicate the complexity of investigating blood levels of NGF and BDNF. At the same time that physical activity may increase serum levels of NGF, there may be an opposing effect through the reduction of body fat. The same phenomenon may occur for BDNF, although we could not demonstrate a direct negative relationship with physical activity in our longitudinal analyses. In the previous cross-sectional study in adolescents, a negative relationship between MVPA and serum BDNF was

found, and this relationship seemed unaffected when adjusted for body mass index [11]. We found the relationship between VPA and BDNF in the cross-sectional analyses of the second measurement wave, when the children were closer in age compared to that study. The problem with the negative relationship between physical activity and serum BDNF is that it may reflect another mechanism not related to neural development, and one should therefore be cautious with its interpretation. This relationship can be explained by that regular exercise activates the platelets to release their stored BDNF to be used in peripheral tissue restoration and control of energy balance, and thereby lowering the blood levels of BDNF [9,10,12-14]. The platelet activation and the indirect effect through reduction of body fat may both contribute to the negative relationship between physical activity and BDNF.

We found relationships for VPA but not for MVPA in the present study. There may be several explanations to this outcome. VPA may be required for a more pronounced increase in the production of neurotrophins, as higher serum levels of NGF has been observed in athletes compared to non-athlete controls [4], and that the intensity of the acute bout of exercise may determine the change in serum levels of neurotrophins [7]. Consequently, as the majority of time in MVPA is attributed to moderate physical activity, the variable MVPA may dilute or hide the associations of VPA with the level of neurotrophins.

The role of NGF and BDNF in relation to body fat has started to be delineated [15]. Both are involved in metabolic control and the regulation of glucose, lipids and energy balance. It has been shown that muscle cells produce and use BDNF as a response to muscle contraction enhancing fat oxidation [5]. NGF on the other hand may have a general alerting effect on the body and thereby stimulates increase in metabolism [6]. Platelet concentration and serum BDNF increase with age

and growth during childhood, and overweight individuals demonstrate more advanced pubertal development and higher platelet concentration than normal-weight [31]. One might therefore expect more BDNF as well as NGF in overweight children due to larger growth and body size. However, in the present study the children with more body fat were neither taller or had more lean body mass than children with less body fat (data not shown). Therefore, body size may not explain differences in serum BDNF and NGF herein. Instead, the relationships observed (the mediator analysis) may reflect the complex mechanisms relating physical activity and body fat to serum levels. More body fat reduces metabolic and hormonal control with increasing serum levels of NGF and BDNF not entering and utilized by local tissue [15]. Higher plasma levels of NGF have been observed in overweight women with reduced metabolic control [16]. BDNF is stored in the platelets and an increased platelet production may therefore be required with higher blood concentration as has been observed in overweight children [31]. Physical activity increases production and release of NGF and BDNF and improves their access to local tissue [4,7-10]. At the same time, physical activity reduces body fat and improves metabolic control, and may thereby reduce the chronic serum levels of NGF and BDNF.

NGF and BDNF are important for neural growth and development. However, their multiple sources of origin and target cells as well as their involvement in a complex interaction between different cell types, make it difficult to understand how blood levels of NGF and BDNF are related to their levels in the neural system. A general limitation for the understanding is that it is not possible to measure brain levels of NGF and BDNF in humans. Future research assesses the relationship between serum and plasma BDNF and further investigate how NGF and BDNF are transported through the blood-brain barrier, as well as targeting longitudinal designs for causal relationships, involving also body fat in the analyses.

Strengths and limitations

Strengths of this study is the longitudinal design together with structural equation modeling with endogenous parameters to demonstrate how physical is related to NGF and BDNF. The use of objective measures of physical activity is another strength. Further, by including measurement of body composition using Dual-energy X-ray Absorptiometry, we could discriminate the effect of physical activity from body fat. A limitation is the considerable amount of missing data, especially from the blood analyses. By applying full information maximum likelihood estimation with robust standard errors in the analyses, a more efficient use of existing data in the analyses was possible. Another limitation of this study is the lack of concurrent measurement of plasma BDNF, to be able to assess the relationship between plasma and serum BDNF. Although, a previous study found that both serum and plasma BDNF were negatively related with VO_2max [9]. In the mediation analyses we used body fat % assessed at the first of two measurement waves, which is common practice in research. Optimally, three measurement waves would be required to perform a true mediation analysis including body fat % assessed at the middle wave.

Conclusions

In a longitudinal study of 8-11 years old children, more vigorous physical activity assessed from accelerometry was directly related to higher serum levels of NGF but not to BDNF. At the same time, higher body fat % assessed by DXA was related to higher serum levels of both NGF and BDNF. The influence of more vigorous physical activity on the serum levels of NGF and BDNF may be exerted indirectly through the associated lower level of body fat %. Serum levels of NGF and BDNF are influenced by mechanisms involving other cell types than neural cells and cannot easily be translated to levels in the neural system. Interpretations need therefore to be done cautiously.

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Tables

Table 1. Characteristics of participants at wave 1 and 2. Wave 1 characteristics of participants with wave 2 data are presented for comparison.

	Wave 1		**Wave 2**		*Wave 1 values*
Variable	Median (IQR)	N	Median (IQR)	N	*Median (IQR)*
Sex, n (%) female	108 (44)	248	102 (44)	231	-
Age, yrs	9.8 (1.0)	248	11.7 (1.0)	228	*9.7 (1.0)*
Weight, kg	33 (10)	248	42 (14)	231	*34 (10)*
Height, cm	141 (9)	248	153 (12)	231	*141 (9)*
BMI, kg·m^{-2}	17 (3)	248	18 (4)	231	*17 (3)*
Body fat, %	17 (13)	246	17 (13)	225	*17 (13)*
MVPA, min·d^{-1}	39 (22)	228	37 (24)	176	*38 (23)*
VPA, min·d^{-1}	12 (10)	228	9 (9)	176	*11 (10)*
Acc wear, hrs	12 (2)	228	12 (2)	176	*12 (1)*
Acc wear, days	4 (0)	228	4 (1)	176	*4 (0)*
NGF, au	0.9 (0.3)	123	0.9 (0.6)	60	*0.8 (0.5)*
BDNF, au	6.5 (6.0)	155	6.7 (5.6)	101	*7.6 (5.7)*

Acc, accelerometer; au, arbritary unit; BDNF, brain-derived neural factor; BMI, body mass index; MHPA, moderate-and-high physical activit; NGF, nerve growth factor; VPA, vigorous physical activity. Wave 2 measurements were performed 2 years after wave 1.

Table 2. Zero-order correlations at each measurement wave in the structural equation modeling. The lower diagonal shows wave 1 correlations and the upper diagonal wave 2 correlations.

	Wave 2				
Wave 1	NGF	BDNF	VPA	Body fat %	Sex female
NGF	----	0.32*	-0.14	0.42*	-0.09
BDNF	0.19*	----	-0.29*	0.22	-0.01
VPA	-0.10	-0.04	----	-0.28*	0.03
Body fat %	-0.06	0.14	-0.42*	----	0.33*
Sex, female	-0.03	-0.003	-0.18*	0.36*	----

*= statistical significance (p<0.05).

Figure caption

Figure 1. Model without body fat %. Cross-loaded standardized path coefficients from wave 1 to wave 2 two years later. Only statistically significant coefficients are shown.

Figure 2. Model including body fat %. Cross-loaded standardized path coefficients from wave 1 to wave 2 two years later. Only statistically significant coefficients are shown.

Figure 3. Model considering body fat % as mediator. Cross-loaded standardized path coefficients from wave 1 to wave 2 two years later. Only statistically significant coefficients are shown. Path *a* is the direct relationship between VPA and NGF, path $b \cdot c_1$ and $b \cdot c_2$ are the indirect relationships of VPA with NGF and BDNF through body fat %.
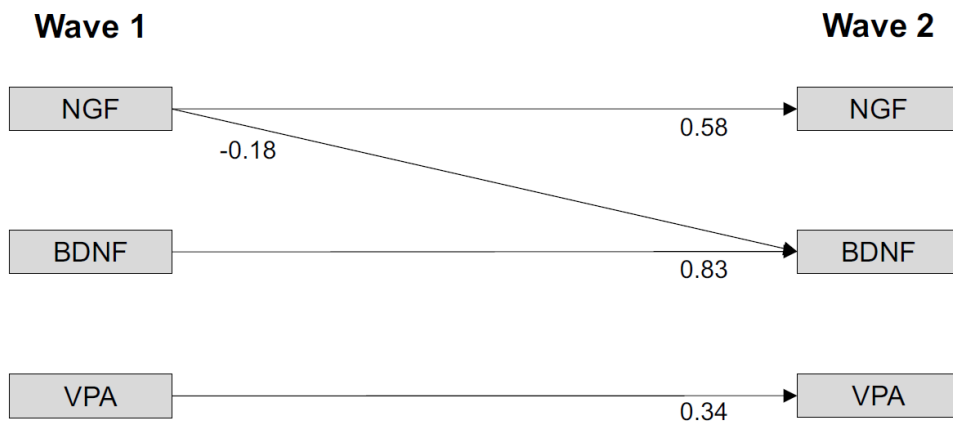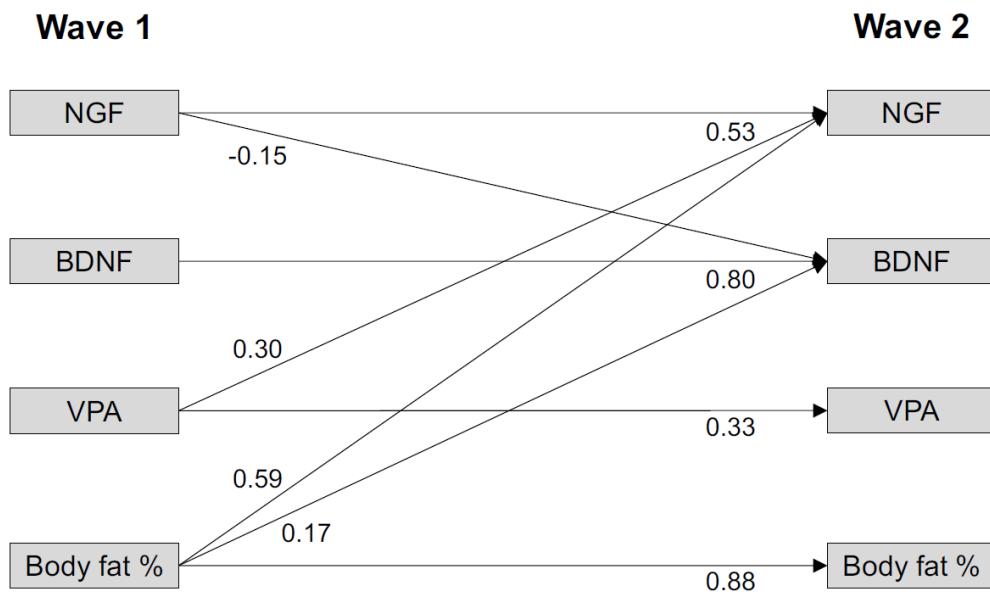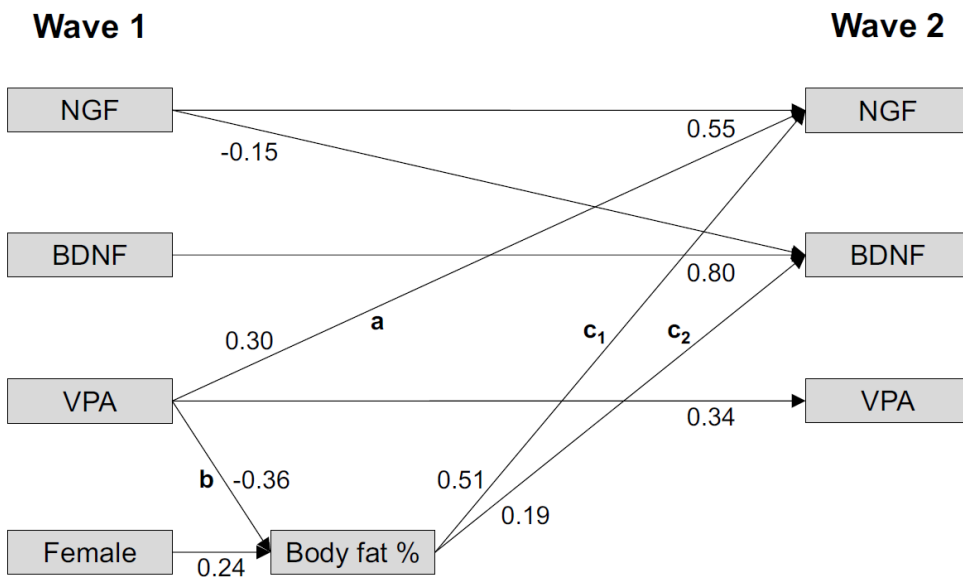
Figure 1

Figure 2

Figure 3

Supplement table. Model not including (Model 1) and including (Model 2) body fat %. Standardized cross-loaded path coefficients within structural equation modeling, to determine whether a wave 1 variable is related to a wave 2 variable, controlling for the other variables.

Wave 2 variable	Model 1			Model 2		
Wave 1 variable	Estimate	Est./SE	p-value	Estimate	Est./SE	p-value
NGF						
NGF	0.58	3.45	**0.001**	0.53	2.93	**0.003**
BDNF	-0.02	-0.15	0.87	0.04	0.28	0.78
VPA	0.16	1.23	0.22	0.30	2.73	**0.006**
Body fat %	-	-	-	0.59	4.02	**<0.001**
Sex female	0.12	0.64	0.52	-0.23	-1.46	0.15
BDNF						
BDNF	0.83	10.45	**<0.001**	0.80	9.85	**<0.001**
NGF	-0.18	-2.41	**0.02**	-0.15	-2.25	**0.02**
VPA	-0.04	-0.33	0.74	0.02	0.20	0.84
Body fat %	-	-	-	0.17	2.07	**0.04**
Sex female	-0.03	-0.34	0.73	-0.07	-0.84	0.15
VPA						
VPA	0.34	3.26	**0.001**	0.33	3.24	**0.001**
NGF	-0.10	-0.61	0.54	-0.07	-0.40	0.69
BDNF	-0.17	-1.61	0.11	-0.17	-1.52	0.13
Body fat %	-	-	-	-0.07	-0.63	0.53
Sex female	0.09	0.84	0.40	0.11	1.10	0.27
Body fat %						
Body fat %	-	-	-	0.88	21.46	**<0.001**
NGF	-	-	-	-0.02	-0.80	0.42
BDNF	-	-	-	-0.005	-0.10	0.92
VPA	-	-	-	-0.03	-0.57	0.57
Sex female	-	-	-	0.008	0.14	0.89