Title: Nutritional, muscular and metabolic characteristics in patients with neurofibromatosis type 1

Authors: Monji KOGA[1], Yuichi YOSHIDA[2], Shinichi IMAFUKU[1]

[1] *Department of Dermatology, Faculty of Medicine, Fukuoka University, Fukuoka, Japan*

[2] *Department of Medicine of Sensory and Motor Organs, Division of Dermatology, Faculty of Medicine, Tottori University, Tottori, Japan*

Correspondence:

Monji Koga, MD, Department of Dermatology, Fukuoka University School of Medicine, 7-45-1 Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan

Tel: +81-92-801-1011; Fax: +81-92-861-7054; E-mail: mob3mob3@yahoo.co.jp

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Abstract

Neurofibromatosis type 1 (NF1) has many reported clinical characteristics. We previously found that NF1 patients (especially, men) had lower body mass index (BMI) than controls, but the reason has not been elucidated. To address this issue, a retrospectively case-control study was conducted. Anthropometric and serum chemistry data that potentially relate to BMI were collected from medical records of NF1 patients and their age- and sex-matched controls. Enrollment of 98 adult patients who underwent skin surgery with NF1 (41 men, 57 women) and 173 without NF1 (74 men, 99 women) were investigated. The median BMI in male NF1 patients was significantly lower than that of the controls. Triglycerides in male NF1 patients were significantly lower than male controls, creatine kinase and lactate dehydrogenase in NF1 patients were also lower than controls, aspartate aminotransferase and alanine aminotransferase showed a lower tendency in NF1 patients, but were significantly lower in female patients. With correlation analysis, lactate dehydrogenase was moderately correlated with BMI in male NF1 patients. Creatine kinase and creatinine showed no statistical correlation with BMI in either group. Triglycerides, alanine aminotransferase showed a positive correlation with BMI in both male and female controls, but not in NF1 patients. In conclusion, only lactate dehydrogenase was moderately correlated with BMI in male NF1 patients, although results of some nutritional and metabolic parameters suggest a specific metabolism in NF1.

Introduction

Recently, new findings about neurocutaneous syndrome including neurofibromatosis type 1 (NF1) have increased owing to the rapid development of genetics.[1-4] Therefore, it would be useful to elucidate these new clinical characterizations, even though the principal aspects of neurocutaneous syndrome have been established. However, new concepts of neurocutaneous syndrome such as Legius syndrome, which has recently been recognized as a new genetic disorder, might be determined with evidence based on new genetic and classical clinical findings, which is well known in other established neurocutaneous syndromes.

A mutation in the *NF1 gene*, which encodes the tumor- suppressing protein neurofibromin, results in various types of tumorigenesis including neurofibromas. It is also well known that patients with NF1 have numerous clinical characteristics, such as learning disability, attention-deficit hyperactivity disorder, and short stature except in patients with the 17q11 microdeletion, which manifests as higher stature.[5, 6] Clinical information is lacking, however, on body mass index (BMI) of NF1 patients and there is currently no consensus about BMI of NF1 patients. We have previously reported that NF1 patients have lower BMI (especially men) than that of other patients with dermatological diseases, although the precise mechanism is yet unclear.[7] In the literature, some reports have identified that muscle size and strength are decreased in NF1 patients[8, 9] and that they have inadequate intake for various nutrients than controls.[10] This may explain why NF1 patients are thinner than the general population. To address this question, nutritional, muscular, and metabolic characteristics of NF1 patients were assessed by comparing serum chemistry data

between NF1 patients and controls. Additionally, each serum parameter that showed a significant difference between the two groups was analyzed by correlation to BMI to estimate the contribution of lower BMI. Finally, we discussed the pathoetiology of the tendency for lower BMI in NF1 patients.

Patients and Methods

A retrospective case-control study was conducted. Diagnosis of NF1 was established according to clinical manifestations, using the diagnostic criteria set by the National Institutes of Health in 1988.[11] NF1 and age- and sex- matched control subjects were recruited from all the adult patients who underwent skin surgery to remove benign tumor(s) at the Dermatology Department at Fukuoka University Hospital (FUH) and Tottori University Hospital (TUH) between 1990 and 2014. The subjects who were age- and sex- matched with non-NF1 patients were recruited as controls. All subjects undertook serum chemistry screening before surgery. Collected data were height, weight, total cholesterol (TC), triglyceride (TG), uric acid (UA), creatine kinase (CK), creatinine (Cr), total protein (TP), albumin (Alb), hemoglobin (Hb), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH). The serum chemistry data of both NF1 and control groups were compared by the Student's *t*-test. Correlation between BMI and each parameter of the serum chemistry in both groups were analyzed by Pearson's product-moment correlation coefficient. Significant probability (p) < 0.05 was considered statistically significant. A correlation coefficient (r) > 0.4 was considered as moderately correlated

and $0.2 < r \leq 4$ as weakly correlated. All statistical analyses were performed using Statcel3 software package (OMS Publishing Co., Saitama, Japan) and SPSS statistics (IBM, Tokyo, Japan). This study was approved by the Ethics Committee of the Institutional Review Board of FUH and TUH.

Results

Height and BMI

There were 98 adult NF1 patients enrolled (41 men, 57 women, median age 36.5 years with range of 20–81 years) and 173 controls (74 men, 99 women, median age 39.0 years with range of 20–81 years).

Table 1 shows the median height in the NF1 patients for both sexes and was significantly lower than that of the controls (men 161.2 cm, $p = 8.164\times 10^{-7}$, women 151.6 cm, $p = 0.003$). Median BMI in male NF1 patients (21.7 kg/m^2) was significantly lower than that in male controls (23.7 kg/ m^2 , $p = 0.014$). There was no significant difference in median BMI between female NF1 patients (21.1kg/ m^2) and female controls (21.4 kg/ m^2, $p = 0.843$).

TG, CK, Cr, AST, ALT and LDH were lower in NF1 patients than controls

Summarized data of the serum chemistry of NF1 patients and control are shown in Table 2. The parameters that showed a significantly lower value in both sexes of NF1 patients were CK (men $p = 0.0001$, women $p = 0.0002$) and LDH (men $p = 0.026$, women $p = 2.164\times 10^{-7}$). Only male NF1

patients showed lower TG ($p = 0.028$) and Cr ($p = 7.531\times 10^{-6}$) values than those of controls. The value of Cr in female NF1 patients showed a lower tendency than that of controls (0.57 ± 0.10 and 0.60 ± 0.11, respectively), but the *p*-value did not reach significance ($p = 0.074$). In female NF1 patients, AST ($p = 0.001$) and ALT ($p = 0.049$) were significantly lower than those of female controls. This tendency was not observed in male NF1 patients. The graphic plot of ALT and BMI showed fewer outliers both in male and female NF1 patients (Fig. 1).

Serum TG and ALT have no correlation with BMI in NF1 patients

The contribution of the serum chemistry data to the difference in BMI, was assessed by analyzing the correlation between each parameter (TG, CK, Cr, AST, ALT and LDH) and BMI (Table 3). CK and Cr showed no statistical correlation with BMI in both patient and control groups. ALT had no correlation with BMI in NF1 patients of both sexes and AST showed a weakly positive correlation in female controls ($r = 0.283$, $p = 0.006$) and a moderately positive correlation with ALT in both male and female controls ($r = 0.404$, $p = 0.001$ and $r = 0.432$, $p = 0.000$, respectively). TG also showed a similar tendency. TG had no correlation with BMI in NF1 patients of both sexes. However, there was a positive correlation between TG and BMI in both male and female controls ($r = 0.574$, $p = 0.007$ and $r = 0.394$, $p = 0.042$). These findings indicate that ALT and TG in normal populations has a positive correlation with BMI, which was not observed in NF1 patients. LDH showed a moderately positive correlation in male NF1 patients ($r =0.426$, $p =0.008$), but a weakly positive correlation in female controls ($r = 0.357$, $p = 0.000$).

Discussion

The amount of subcutaneous fat mainly depends on caloric-intake, and this is the factor that most influences the BMI level. However, other factors such as metabolism and size of muscle can also be related to BMI.

The BMI level in NF1 patients is still controversial. There is no consensus among the few existing studies regarding NF1 and obesity. [12 – 14] We previously reported that adult NF1 patients, especially in men, have lower BMI than patients with other dermatological diseases.[7] In the present study, we only selected patients who underwent skin surgery for benign tumors, because such patients were thoroughly screened by blood tests. Therefore, the groups of patients and controls were different from our previous study, with subjects also recruited from another facility. Nevertheless, the tendency of the lower BMI in male NF1 patients was clearly observed in both our present and previous studies. Therefore, we believe this observation to be valid, at least in the Japanese population. For female NF1 patients, it is unclear whether they are actually thinner or not. The mean BMI in both female groups was obviously lower than the female individuals recorded in national public data (mean BMI 22.3 kg/ m^2). [15] In contrast, the male controls showed similar values to the national public data (mean BMI 23.6 kg/ m^2). [15] This difference might be explained because the mean age of the female patients in this study were relatively young. In general, women tend to gain weight from the age of around 50 years or over, while in men this tendency is seen to occur at a younger age. Therefore, it is quite possible that female NF1 patients would show lower

BMI in a large population sample that includes more elderly subjects.

In this study, we investigated the serum chemistry data of NF1 patients and age- and sex-matched controls to reveal the contribution of BMI. We found that NF1 patients tended to have lower TG, CK, Cr, AST, ALT, and LDH compared with controls.

Some interesting reports that may potentially explain BMI in NF1 patients, have been published. One concerns nutritional intake, in which de Souza ML *et al*. [10] stated that NF1 patients have a tendency to have inadequate intake for various nutrients compared with controls. Other studies have suggested that NF1 patients have reduced muscle size and strength. [8, 9]

Based on these previous studies, we discuss three categories related to BMI, 1) caloric intake, 2) muscular volume, and 3) metabolism.

1) Caloric intake

As shown in Table 2, TG in male patients with NF1 was significantly lower than that in the controls (men, $p = 0.028$, women $p = 0.148$). Additionally, BMI in male and female controls showed a positive correlation with TG, which was not seen in NF1 patients. Because TG partially represents calorie intake, it is possible that NF1 patients have less calorie intake, resulting in lower BMI. Although all blood samples may not have been taken during fasting conditions, the condition is same in both groups and there were very few elevated values only in NF1 patients when checked by the scatter plot (not shown). Our data may partially support that nutrition intake is one of the reason for lower BMI in male NF1 patients. [10]

2) Muscular volume

We also found that serum CK in both sexes of NF1 patients was significantly lower than those of controls. Additionally, Cr in male patients with NF1 was significantly lower than those of controls. Both CK and Cr are well known as myogenic deviation enzyme that generally represents volume of muscle, and they are generally higher in men than women. [16] Taken together, lower myogenic enzymes in serum chemistry may reflect the lower volume and strength of male NF1 patients, compared with male controls.[8, 9] A similar tendency was also seen in women, but the difference was less significant. This may be because of men having more muscle, and therefore, the impact of the difference is more evident in men. Although CK showed a significant difference between the NF1 and control groups, CK had no direct correlation with BMI in both groups and sexes. A study using the murine model featuring NF1 knockout in muscle exhibited no differences in overall weight. [17] Our data may suggest that NF1 patients have reduced muscle volume, but this will not have a direct relationship with BMI.

3) Metabolism

The concentration of TG increases with calorie intake, but is also strongly related with metabolic syndrome (MetS). Serum TG level usually counteracts with that of HDL-cholesterol, which is a good marker of MetS. Therefore, the lower TG may suggest that the male NF1 group contains fewer MetS subjects.

It is commonly known that fatty liver and elevated ALT (typically AST: ALT ratio < 1) is frequently seen in subjects with obesity or MetS. Elevated serum ALT is also reported as a metabolic factor related to general and abdominal obesity.[18] Indeed, our control data clearly

supports that BMI and ALT show a positive correlation (Table 3), although the mean ALT in all groups was within normal limits. However, this correlation was not seen in NF1 patients. With AST and ALT, only female NF1 patients showed significantly lower values than those of female controls (Table 2) and these parameters were correlated with BMI in female controls (Table 3). However, male NF1 patients showed no significant difference and very few outliers in contrast to the controls (Fig.1). We speculate that NF1 patients have less accumulation of lipid in the liver. This anti-MetS mechanism may also be involved in relation to BMI in NF1 patients as well as in caloric intake.

In NF1 patients, LDH was significantly lower than that in controls (Table 2). Reduced LDH was observed in both male and female NF1 subjects. The enzyme LDH reflects the inflammation of various tissues, including blood, heart, lung, liver, muscle, and skin. Although the exact tissue that caused lower LDH in the NF1 group could not be identified because the LDH isoenzymes were not examined in this study, other parameters (AST, ALT, CK, Cr) suggest that LDH in men was involved in muscle tissue. In women, LDH was involved in liver and/or muscle tissue. The finding of LDH should be reassessed in a larger sample population.

In summary, BMI in NF1 patients was lower than in controls, and there was a significant difference in male NF1 patients and the controls. NF1 patients have a tendency for lower TG, CK, Cr, ALT, and LDH, suggesting that they have less calorie intake, have less muscle volume, and have a greater anti-MetS tendency than normal subjects. However, the main reason for this phenomenon remains unclear, and we will address this issue that is mainly focused on metabolism

by another approach in the future.

This study has several limitations. First, because only Japanese patients were studied, we cannot exclude the possibility that ethnicity, life styles, and food habits could affect the studied variables. Second, blood samples were not taken in fasting conditions. Therefore, some parameters such as TG may have been affected, although the same condition affected both NF1 and controls. Third, the mean age of women in this study was relatively young for evaluating metabolism, because hyperlipidemia and other metabolic syndrome factors are frequently seen in women over the age of approximately 50 years. Further studies including elderly patients will be needed to elucidate the pathophysiology of this phenomenon.

Table legends

Table 1

Height and BMI from all subjects

Height of patients with NF1 in both sexes is lower than that of controls. BMI in male NF1 patients is lower than the controls. M: men, W: women, SD: standard deviation, BMI: body mass index, *P*: *p*-value, * represents $p<0.05$

Table 2

Summary data of serological markers from all subjects and controls

CK and LDH in both sexes of NF1 patients are significantly lower than the control groups. NF1 patients have lower values of TG and Cr in men and AST and ALT in women are statistically lower than controls. M: men, W: women, SD: standard deviation, BMI: body mass index, *P*: *p*-value, * represents $p<0.05$

Table 3

Results of Pearson's correlation between BMI and each parameters in both groups

Serum TG and ALT showed a negative correlation with BMI in NF1 patients. [a] Triglyceride, [b] Creatine kinase, [c] Creatinine, [d] Aspartate aminotransferase, [e] Alanine aminotransferase, [f] Lactate dehydrogenase, M: men, W: women, **represents significant moderate correlation ($p < 0.05$ and $r > 0.4$), and *means significant weakly correlation ($p < 0.05$ and $0.2 < r \leq 0.4$).

Figure legends

Figure 1

Graphic plot between BMI and ALT in both groups

Longitudinal axis shows values of ALT, and horizontal axis shows values of BMI. Only the controls in both sexes have outliers from the group of plots. Values of ALT are within normal limits and there are no outliers, suggesting none with fatty liver in NF1 patients.

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Table1 Height and BMI from all subjects

		NF1 median [range] (n)	**Control** median [range] (n)	***P*-value**
Age	Total	36.5 [20-81] (98)	39.0 [20-81] (173)	
Height (cm)	M	161.2 [148-180] (41)	170.2 [154-183] (74)	**8.164× 10 $^{-7}$***
	W	151.6 [137.3-167.3] (57)	156.2 [139.2-170] (99)	**0.0037***
BMI (kg/m^2)	M	21.7 [17.5-28.0] (41)	23.7 [17.8-39.1] (74)	**0.014***
	W	21.1 [17.8-31.4] (57)	21.4 [15.8-34.7] (99)	0.843

Height of patients with NF1 in both sexes is lower than that of controls. BMI in male NF1 patients is lower than the controls. M: men, W: women, SD: standard deviation, BMI: body mass index, *P*: *p*-value, * represents $p<0.05$

Table2 Summary data of serological markers from all subjects and controls

	sex	**NF1** (mean±SD)	**Control** (mean±SD)	*p*-value
Total cholesterol (mg/dl)	M	195.4±27.8 (n=34)	191.5±36.9 (n=56)	0.595
	W	209.1±32.5 (n=42)	201.2±38.9 (n=71)	0.268
Triglyceride (mg/dl)	M	98.1±45.9 (n=13)	176.4±117.5 (n=23)	**0.028***
	W	75.2±26.1 (n=16)	105±78.3 (n=28)	0.148
Uric acid (mg/dl)	M	5.69±0.87 (n=12)	5.70±0.96 (n=28)	0.979
	W	4.12±1.06 (n=31)	4.13±1.10 (n=28)	0.943
Creatine kinase (IU/L)	M	81.6±46.8 (n=33)	139.8±72.7 (n=49)	**0.0001 ***
	W	52.6±35.6 (n=49)	76.1±30.4 (n=63)	**0.0002***
Creatinine (mg/dl)	M	0.69±0.13 (n=41)	0.84±0.18 (n=73)	**7.531×10^{-6}***
	W	0.57±0.10 (n=57)	0.60±0.11(n=97)	0.074
Total protein (g/dl)	M	7.35±0.47 (n=41)	7.26± 0.40(n=72)	0.289
	W	7.28±0.44 (n=56)	7.29±0.46 (n=98)	0.862
Albumin (g/dl)	M	4.46±0.45 (n=41)	4.56±0.28 (n=72)	0.139
	W	4.45±0.33 (n=56)	4.41±0.31 (n=95)	0.522
Hemoglobin (g/dl)	M	15.2± 1.95(n=41)	15.1± 1.36(n=74)	0.760
	W	13.0± 1.58(n=57)	12.8± 1.42(n=99)	0.722
Aspartate aminotransferase (IU/L)	M	20.3±5.69 (n=41)	22.0± 7.23(n=74)	0.211
	W	16.9±4.27 (n=57)	20.1±6.22 (n=99)	**0.001***
Alanine aminotransferase (IU/L)	M	24.0±11.0 (n=41)	27.5±17.8 (n=74)	0.255
	W	15.3±7.02 (n=57)	18.7±12.0 (n=99)	**0.049***
Lactate dehydrogenase (IU/L)	M	165.4±32.0 (n=40)	180.4± 34.8(n=74)	**0.026***
	W	153.6±22.0 (n=57)	182.3± 36.2(n=98)	**2.164×10^{-7}***

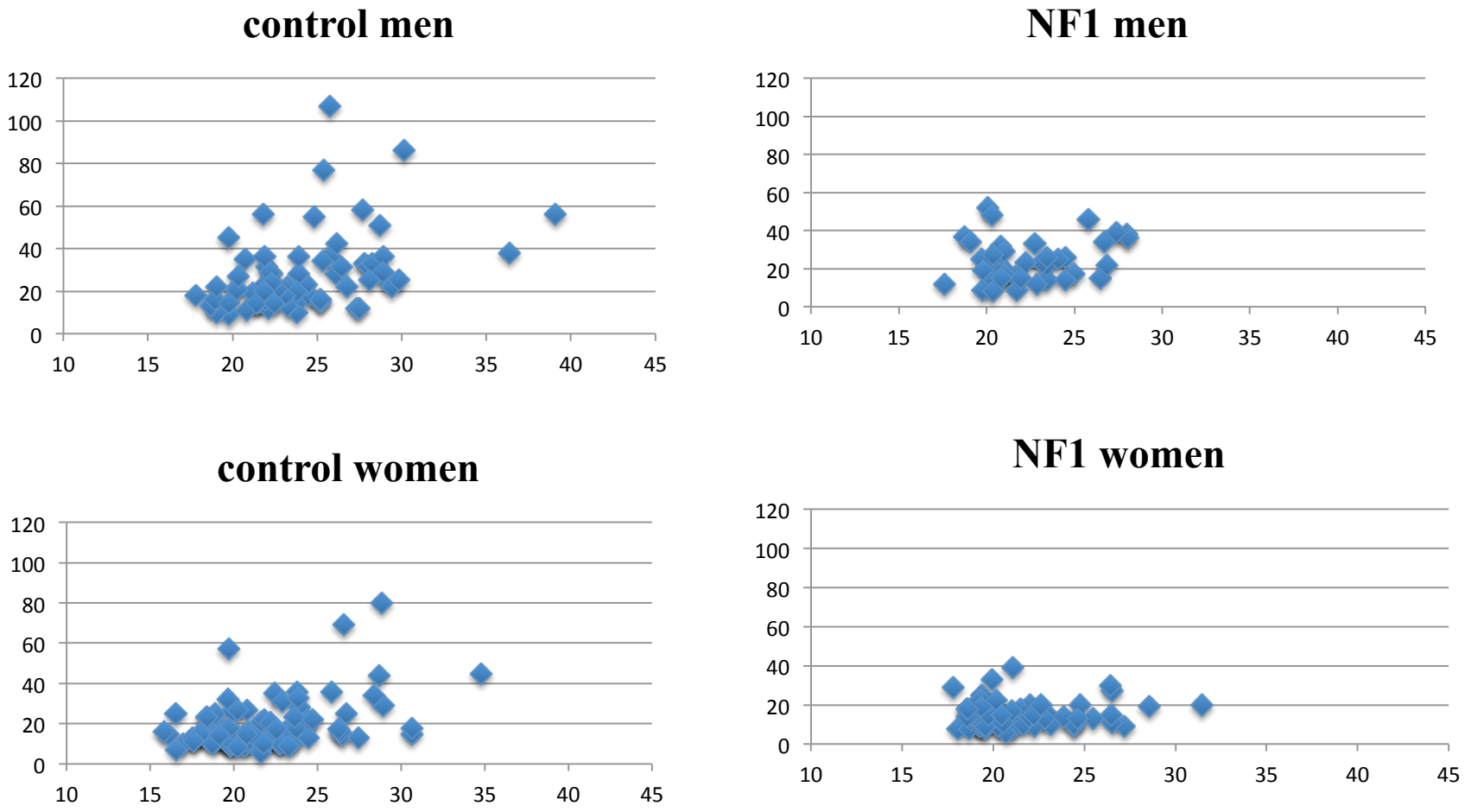
CK and LDH in both sexes of NF1 patients are significantly lower than the control groups. NF1 patients have lower values of TG and Cr in men and AST and ALT in women are statistically lower than controls. M: men, W: women, SD: standard deviation, BMI: body mass index, *P*: *p*-value, * represents $p<0.05$

Table 3 Results of Pearson's correlation between BMI and each parameters in both groups

	vs TG [a]	vs CK [b]	vs Cr [c]	vs AST [d]	vs ALT [e]	vs LDH [f]
NF1 M	r=-0.236 p=0.461	r=0.141 p=0.441	r=0.178 p=0.279	r=0.146 p=0.377	r=0.216 p=0.186	****r=0.426** **p=0.008**
NF1 W	r=-0.262 p=0.388	r=-0.020 p=0.893	r=-0.129 p=0.356	r=0.053 p=0.706	r=0.089 p=0.526	r=0.190 p=0.172
Control M	****r=0.574** **p=0.007**	r=0.175 p=0.255	r=0.130 p=0.295	r=0.183 p=0.135	****r=0.404** **p=0.001**	r=-0.030 p=0.805
Control W	*** r=0.394** **p=0.042**	r=0.241 p=0.064	r=0.142 p=0.171	***r=0.283** **p=0.006**	****r=0.432** **p=0.000**	***r=0.357** **p=0.000**

Serum TG and ALT showed a negative correlation with BMI in NF1 patients. [a] Triglyceride, [b] Creatine kinase, [c] Creatinine, [d] Aspartate aminotransferase, [e] Alanine aminotransferase, [f] Lactate dehydrogenase, M: men, W: women, **represents significant moderate correlation ($p < 0.05$ and $r > 0.4$), and *means significant weakly correlation ($p < 0.05$ and $0.2 < r \leq 0.4$).

Figure 1 Graphic plot between BMI and ALT in both groups



Longitudinal axis shows values of ALT, and horizontal axis shows values of BMI. Only the controls in both sexes have outliers from the group of plots. Values of ALT are within normal limits and there are no outliers, suggesting none with fatty liver in NF1 patients.