5) 厚生労働省編：『厚生労働白書（平成19年版)』，ぎょうせい，2007.
6) 内閣府編：『平成19年版食育白書』，社団法人時事画報社，2007.

参考文献

・厚生労働省編：『厚生労働白書（平成18年版)』，ぎょうせい，2006.
・文部科学省編：『文部科学白書（平成18年度)』，国立印刷局，2007.
・厚生統計協会編：国民衛生の動向・厚生の指標臨時増刊，54(9)，2007.
・日本学校保健会編：『学校保健の動向（平成18年度版)』，勝美印刷，2007.

第５章　地域住民の健康づくり

山津幸司

１．北海道民の健康状態

本章では、地域住民を北方圏の中でも特に北海道民に限定して論を展開したい。さて、北海道民の健康状態はどうだろうか。いくつかの統計データからは、北海道民の健康状態は全国平均と比べて良好とはいいがたい。例えば、平成18年度の平均寿命は男性78.45歳、女性85.63歳で全国平均（男性79.0歳、女性85.81歳）を下回っている（北海道保健統計年報）。また、同統計によると、日本人の三大死因のうち、悪性新生物（ガン）と心疾患の死亡率が全国平均を大幅に上回っている。さらに、高血圧症、糖尿病、心疾患、脳卒中などの生活習慣病の通院有病者率も年々上昇傾向にあり、集団としての北海道民の健康状態をより好ましい方向に向かわせるためのアプローチが不可欠といえる。

次に、北海道民の生活習慣はどうだろうか。「21世紀における国民健康づくり運動（健康日本21）」の北海道版である「すこやか北海道21」[1]の中間評価結果にそれを見ることができる。例えば、食習慣（脂肪エネルギー比率、食塩摂取量、緑黄色野菜の摂取量、朝食摂取）が計画策定時の平成11年度より改善しているものの、カリウムやカルシウム摂取は悪化し、肥満者の割合は横ばいか若干悪化傾向である。睡眠不良や高い自殺率などのメンタルヘルスの悪化傾向、さらに喫煙率の高さも北海道が抱える問題である。身体活動・運動行動に目を向けると、運動習慣者（週２回以上30分以上の持続運動で、１年以上継続している人）の割合が増加している。しかし、道民の約半数は運動をしていないし、そのうち約３割（男性29.1%、女性27.5%）が運動を全くしたことがないというのである（平成11年度健康づくり道民調査）。運動習慣のない者は、身体活動量の少しの増加でも大きな健康上の効果を享受できる集団である。この層の運動関心度を高める方法論の確立が望まれる。

以上のように、北海道民の健康状態は一部に改善傾向が認められるものの、全国平均との比較では不良な状況にあり、高齢化や医療費急増の抑制の観点からも道民の健康づくり対策が急務である。

２．集団としての北海道民の健康づくりを効果的に支援するには

北海道における最大の運動阻害要因のひとつは、冬季における厳しい積雪寒冷環境であろう。積雪寒冷下では外出が制限され運動不足に陥りやすい。我々が最近取組んだ積雪寒

冷下での身体活動介入の試みを紹介する。

1）集団と個別化によるアプローチ

身体活動介入の代表的な形式は、施設などに集まり行う集団（Group-based）介入と自宅中心での実践をサポートする個別（Home-based）介入の2つである[2)]。集団介入は、管理下で運動量を確保できる、支援者は対象者の情報を即時に獲得できるなどの利点をもつ。一方、個別介入は、支援者と参加者の時間的制約が少なく、一般的に抵コストで提供可能である。それぞれ両介入の特徴を踏まえ、北海道富良野市と我々は、国保ヘルスアップ事業の中で、上記2形式のどちらが北海道民に適しているかの検証[2)]を行った。その概要は以下の通りである。

対象は富良野市在住でかつメタボリックシンドローム（MS）またはその予備軍であった中高年者73名であり、集団運動群（40名）と個別運動群（33名）に分けた。全参加者に提供したのは保健師による個別面談とスポーツ科学の専門家が作成した運動処方（身体活動量の目標）である。その後、集団運動群の参加者は運動施設に集まりインストラクターのもとで運動やレクリエーションを実施し、個別運動群の参加者は初回面談で設定した運動目標を自宅中心で実践した。プログラム終了率は集団運動群95%、個別運動群97%と共に良好であり、歩数は両群とも増加し群間差は認められなかった（集団運動群1901歩／日増、個別運動群735歩／日増）。歩数とは対照的に、3ヵ月後の集団運動群の体重減少率は個別運動群を大幅に上回っており（図5-1）、集団運動群の減量効果が優れているという結果であった。

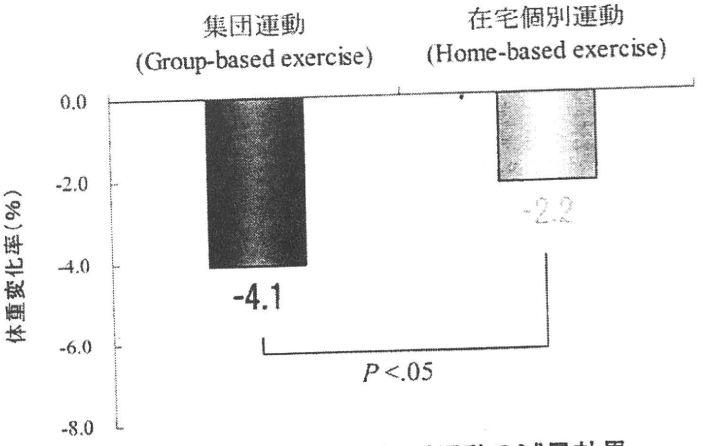

図5-1　集団運動と在宅個別運動の減量効果

集団運動の減量効果は有意に大きい

減量効果を介入参加時期（季節）に分けて分析してみると、さらに興味深い成績が得られた。冬季のみの参加者での分析では集団運動群の介入成績が明らかに良好であるが、夏季のみではその差が認められなかった（図5-2）。

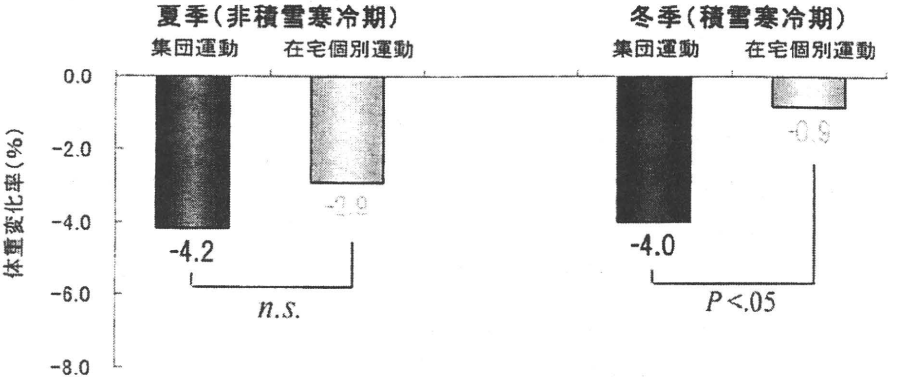

図5-2　集団運動と在宅個別運動の減量効果の季節差
集団運動の減量効果は冬季では有意に大きいが、夏季では差がない

すなわち、介入効果には季節差が認められるのだ。この結果の解釈は、複数の交絡因子の影響が予想されるため簡単ではないが、北海道における夏季と冬季の運動量の確保の容易さが影響した可能性が大きいと考えられた。すなわち、夏季では個別運動群も自宅内外である程度運動量確保が可能であるため集団運動群との効果の差は顕著ではないが、自宅外での運動が制限される冬季では一定強度以上の運動量を確実に確保できる集団運動群の成績が良好であったと推測できよう。

以上の結果から、北海道民に対し集団運動も在宅個別運動も歩数の増加や減量に有効であるが、少なくとも3ヵ月という短期では集団運動の方がより効果的である可能性が高い。ただし、その効果には季節差も認められ、特に自宅近辺での運動が制限される冬季では集団運動がより有効となると考えられた。

2）在宅運動機器の活用

先述のとおり、冬季（積雪寒冷下）では施設で行う集団運動が優れている。しかし、近隣に運動施設がない場合、北海道民は健康増進や身体活動の習慣化をあきらめなければならないのだろうか？冬季の個別運動の効果を高める方法はないのだろうか？その解決策のひとつを紹介する。

結論からいうと、冬季の個別介入の効果を高める方法のひとつは『在宅での運動機器の活用』である[3)]。我々は、札幌市民を対象に、ステップエクササイズと歩行を促進するための身体活動介入を行った。参加者は、自宅でのステップエクササイズと自宅近辺での歩

行数の増加に挑戦し、モチベーション維持のために週1回の集団運動教室に参加した。ステップ台と歩数計を無償貸与した。この研究の時期は春季から夏季にかけてではあったが、ステップエクササイズは在宅で年中実施可能であり、積雪寒冷下の道民の運動習慣保持に有効である。きっかけ作りとして、ステップ台以外でも例えば近年普及が目覚しい運動のできるテレビゲームなどの活用も有効性が確認できれば活用可能となりうるだろう。

3）通信手法の活用の可能性

北海道には積雪寒冷以外にも運動阻害要因がある。それは広大な面積を誇るため特に人口の少ない地域での運動指導者の確保が難しい、という点である。それを解決する手段の一つが『通信手法の活用』である。

通信指導は、対面型の指導と対極をなす指導形態であり、"郵便、電話、ファクシミリ、双方向性のテレビ、コンピュータ端末、インターネット、電子メール、および携帯電話などの通信媒体を用いて、指導者が対象者と直接会うことなしに介入に要する情報を交換する指導形態[4]" である。その利点は、a）指導時間や場所の制約が少なく、b）多数例に適用可能で、c）費用効果が高い、などである[5]。

通信指導による身体活動介入の成功例は、北海道民を対象とした報告[5]もあり、今後有用性の高い方法である。対面指導と比べて、通信指導の参加者一人当たりの介入効果は小さいとの指摘もあるが、『テーラーメード手法』や『コンピュータ技術』の活用により、通信指導の効果をさらに高める試みも進んでいる[6,7,8]。テーラーメード手法とは、優れた服職人が客の体型に応じた見事なテーラーメードの服を作成するように、健康づくり支援者もクライエントの特性に応じた助言や支援を提供するというものである。また、コンピュータ技術を利用すれば、支援者一人でも数万人規模の参加者を対象とすることも可能となりうる。現実的には、介入においてより時間を要する問診や情報提供を可能な範囲で自動化し、支援者は個別フィードバックなどの人にしかできない部分に集中できる環境をつくることが有用である。

3．心の健康に対するアプローチ

北海道における自殺率は平成18年度人口10万対で26.4であり全国平均の23.7を大幅に上回り、心の健康に対するアプローチの重要性も高まっている。中でも、不眠などの睡眠障害は日本成人の約5人に1人が有するとされている。そこで、我々は、北海道江別市の協力を受けて、生活習慣介入を主体とした睡眠介入を行った[9]。その概要と主な結果は以下の通りである。

対象は、不眠に関する講演に参加し研究への参加に同意した江別市民37名であった。

この37名のうち、18名は身体活動以外の生活改善目標に1ヵ月間取組む対照群に、残り19名はそれに加えて身体活動量の増加に取組む介入群とした。研究対象者全員に著者が提案した26項目の生活改善目標の中から2～3個選んだ目標を1ヵ月間続けるよう指示した。介入群には加速度計を貸与し、最初の3日間の平均歩数より1000歩以上増加させるという身体活動目標の実施を指示した。介入群の歩行運動量は約2.8倍に増加したが、対照群ではほぼ横ばいであった。睡眠指標も両群で改善傾向を示したが、一部の指標では身体活動介入群でより大きく改善した。また、相関分析の結果からは、総身体活動量や中等度の身体活動が睡眠指標の良好さと関連するだけでなく、平日や休日の不活動時間と睡眠指標の改善にも明らかな関連が認められた。以上の結果から、身体活動介入は生活習慣病の予防以外に不眠軽減などのメンタルヘルス対策としても重要な役割を担うことが可能と思われた。

4．まとめ

集団としてみた北海道民の心身の健康状態や生活習慣は決して良好とはいえない。北海道民の運動習慣が低い理由として、積雪寒冷をあげる専門家は少なくない。積雪寒冷という運動阻害要因は人為的に変えることは現時点で不可能に近いため、我々は以下のような提案を行いたい。まず、健康づくりに携わる専門家は、財政が許す範囲で集団運動教室を第一優先とし北海道民の参加を促すべきだが、財政的に困難な場合や夏季には自宅を中心に行う個別運動を勧めてもよいだろう。また、冬季に個別運動を勧めなければならない場合は、在宅で運動できる機器を準備するか、同等の方法を考案する必要があろう。北海道民の健康状態は、北海道民自らの知恵と実践でしか向上できない。

引用文献

1) 北海道：第2章，道民の健康と生活習慣の現状，『すこやか北海道21』，http://www.hokkaidohealth-net.or.jp/sukoyaka/index.html
2) Koji Yamatsu, Atsuko Hanai：Comparison of Group-and Home-based physical activity intervention in Japanese subjects with metabolic syndrome. Archivos de medicina del desporte, 128(6), 542, 2008.
3) Koji Yamatsu, Yasuko Azuma, Satoshi Nakae, Hitoshi Chiba, Kojiro Ishii：Efficacy of Group- and Home- based physical activity intervention on cerebrovascular risk factors and fall-related physical fitness, 10th International Congress of Behavioral Medicine Abstract book, p237, 2008.

4) 山津幸司，足達淑子，熊谷秋三：非対面による行動的体重コントロールプログラムの開発・評価とその意義，健康科学，27，13-25，2005.
5) Ryo Miyazaki, Yasuko Azuma, Nao Koyama, Koji Yamatsu, Koichiro Hayashi, Hitoshi Chiba, Kojiro Ishii : Effects of a walking program using pedometers and newsletters for preventing lifestyle-related diseases of the elderly men and women, Journal of Aging and Physical Activity, 16(Suppl), S170, 2008.
6) 山津幸司：行動科学的アプローチとその実践，日本臨床2009年増刊，身体活動・運動と生活習慣病，運動生理学と最新の予防・治療，日本臨床.
7) 山津幸司，足達淑子：男性に対する非対面の行動的減量プログラムを用いた無作為介入試験，肥満研究，11(3)，311-316，2005.
8) Yoshiko Adachi, Chifumi Sato, Koji Yamatsu, Sakurako Ito, Kyo Adachi, Toshiko Yamagami : A randomized controlled trial on the long-term effects of a one-month behavioral weight control program assisted by computer tailored advice, Behaviour Research and Therapy, 45, 459-470, 2007.
9) Koji Yamatsu : Moderate-intensity physical activity and sleep disturbances in Japanese adults:, A controlled trial, Annals of Behavioral Medicine, 35(Suppl), s153, 2008.

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SHORT COMMUNICATION

Brain-Derived Neurotrophic Factor Treatment Increases the Skeletal Muscle Glucose Transporter 4 Protein Expression in Mice

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Summary

The purpose of the present study was to investigate whether peripheral brain-derived neurotrophic factor (BDNF) treatment induced metabolic adaptations in mouse skeletal muscle. BDNF (20 mg/kg/day) was injected subcutaneously for successive 14 days. BDNF treatment significantly reduced the total food intake and inhibited the weight gain in comparison to the control group. The glucose transporter 4 (GLUT4) protein expression in the gastrocnemius muscle was significantly increased by BDNF treatment in comparison to the control and pair-fed groups. Neither the oxidative nor the glycolytic enzyme activities in the gastrocnemius muscle changed after the BDNF treatment. These results suggest that the peripheral BDNF treatment promotes the skeletal muscle GLUT4 protein expression as well as hypophagia.

Key words

BDNF • GLUT4 • Hypophagia • Skeletal muscle

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Brain-derived neurotrophic factor (BDNF) is a part of the neurotrophin family and is produced in the nervous system and periphery. The BDNF controls the food consumption (Xu *et al.* 2003), lipid and glucose metabolism (Nakagawa *et al.* 2000, Tsuchida *et al.* 2002), and insulin resistance (Kuroda *et al.* 2003). Recent human studies have shown that circulating BDNF is associated with eating disorders (Nakazato *et al.* 2003, Monteleone *et al.* 2004), obesity (Monteleone *et al.* 2004, Suwa *et al.* 2006), glucose and lipid metabolism (Suwa *et al.* 2006, Levinger *et al.* 2008), type II diabetes mellitus (Suwa *et al.* 2006) and metabolic syndrome (Chaldakov *et al.* 2003, 2004). Based on these metabolic contributions, BDNF is considered to be a "metabotrophins" (Chaldakov *et al.* 2007).

Skeletal muscle metabolic characteristics such as glucose transporter 4 (GLUT4) expression and mitochondrial oxidative capacity are associated with skeletal muscle insulin-stimulated glucose uptake, whole body insulin sensitivity and prevalence of type II diabetes mellitus (He *et al.* 2001, Bruce *et al.* 2003, Doehner *et al.* 2010). Chronic BDNF treatment to diabetic mice significantly improves the glucose uptake in skeletal muscle (Yamanaka *et al.* 2007). Based on these results, the BDNF is hypothesized to regulate the skeletal muscle metabolism. This study examined whether chronic BDNF treatment to mice affects skeletal muscle metabolic characteristics such as GLUT4 protein expression and glycolytic and oxidative enzyme activities.

Sixty-nine- to 72-day old female ICR mice were

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used for the current study. All mice were fed a standard rodent chow (CE-2, CLEA Japan, Inc., Tokyo, Japan). All experimental procedures were approved by the Nakamura Gakuen University Animal Experiment Committee.

Because BDNF treatment reduces food intake (Nakagawa *et al.* 2003), the effects of BDNF treatment was studied in comparison with both *ad libitum*-fed control and pair-fed mice. The mice were divided into an *ad libitum*-fed (AL, n=8), a pair-fed (PF, n=8), or a BDNF-treated (BDNF, n=8) group. The mice of the BDNF group were subcutaneously administered daily with 20 mg/kg body mass BDNF (Dainippon Sumitomo Pharma, Osaka, Japan) in saline for 14 successive days. This dose of BDNF has been shown to enhance the skeletal muscle glucose uptake (Yamanaka *et al.* 2007). In the AL and PF groups, a comparable volume of saline was administered subcutaneously.

About 24 h after the last administration, the mice were fasted for 4 h and anesthetized with pentobarbital sodium (60 mg/kg body weight i.p.). The gastrocnemius muscle was rapidly dissected, frozen in liquid nitrogen and stored at −80 °C until the analyses were performed.

The GLUT4 protein expression was determined by Western blotting and the enzyme activities including citrate synthase (CS), malate dehydrogenase (MDH), β–hydroxyacylCoA dehydrogenase (βHAD), hexokinase (HK), and lactate dehydrogenase (LDH) were measured spectrophotometrically as described previously (Suwa *et al.* 2008).

To compare the findings among the three groups, a one-way analysis of variance (ANOVA) was used. Fisher's PLSD was conducted if the ANOVA indicated a significant difference. A value of $P<0.05$ was considered to be significant.

The body mass prior to the treatment was similar in all three groups (AL; 27.5±0.5 g, PF; 27.9±0.6 g, BDNF; 27.6±0.2 g). The changes in the body mass in the PF (0.0±0.7 g) and BDNF (-0.3±0.4 g) groups were significantly lower than AL group (1.7±0.4 g) (Fig. 1A, $P<0.05$). Total food intake in the PF (49.7±2.1 g) and BDNF (49.7±2.1 g) groups were significantly lower than in AL group (58.7±1.9 g) (Fig. 1B, $P<0.01$). These results suggest that BDNF treatment inhibits the body mass increase because of reducing food intake.

The GLUT4 protein expression in the BDNF group was significantly higher by +37 % and +35 % than in the AL and PF groups, respectively (Fig. 2, $P<0.05$). Oxidative (CS, MDH and βHAD) and glycolytic (HK and LDH) enzyme activities were measured, and no differences were observed among the groups in any enzymes (data not shown).

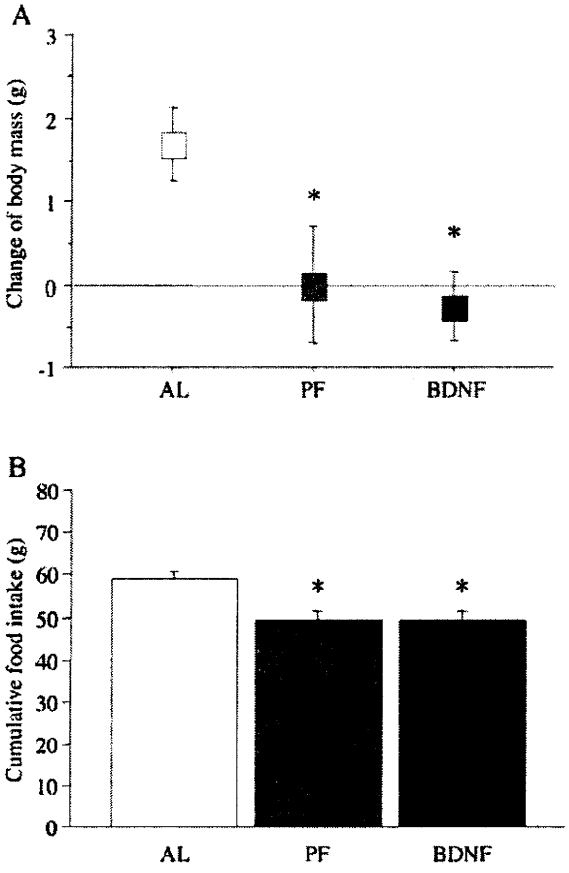

Fig. 1. A: Change of body mass during treatment. **B:** Cumulative food intake during the treatment. N=8 per group. Data are expressed as the mean ± S.E.M. *; P<0.05 vs. AL.

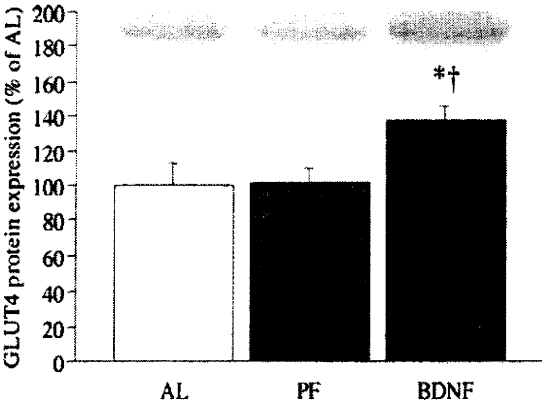

Fig. 2. GLUT4 protein expression of the gastrocnemius muscle in the 3 groups. N=8 muscles per group. Data are expressed as the mean ± S.E.M. *; P<0.05 vs. AL. †; P<0.05 vs. PF.

The current study demonstrated that the subcutaneous BDNF injection to mice significantly decreased the food intake in agreement with the previous study (Nakagawa *et al.* 2003). In humans, the serum BDNF level may demonstrate a possible link with such eating disorders as *bulimia nervosa* and *anorexia nervosa* (Nakazato *et al.* 2003, Monteleone *et al.* 2004). Circulating BDNF may therefore play a role in suppressing food intake. BDNF expressed in ventromedial hypothalamus neurons has been shown to apparently suppress food consumption downstream of the melanocortin-4 receptor (Xu *et al.* 2003). Because BDNF can cross the blood-brain barrier (Pan *et al.* 1998), the subcutaneous injection of BDNF reduces the food intake possibly *via* hypothalamus neurons.

The most important finding in the current study is that the BDNF treatment increases the GLUT4 expression. GLUT4 plays an important role in skeletal muscle glucose uptake (Röckl *et al.* 2008). GLUT4 protein abundance is strongly associated with capacity of skeletal muscle glucose uptake (Doehner *et al.* 2010), suggesting that skeletal muscle GLUT4 abundance is a potential limiting factor of whole body and skeletal muscle glucose metabolism. The increasing GLUT4 protein expression in the current study is thus considered to improve the glucose metabolism.

Although it has been generally accepted that the neurotrophins act by either paracrine or autocrine mechanisms (Davies 1996), BDNF also exists in the blood (Radka *et al.* 1996). More than 90 % of blood BDNF is stored in platelets, and platelets can release the BDNF (Fujimura *et al.* 2002). Platelets are assumed to release BDNF at nerves or other tissues expressing BDNF receptor tyrosine kinase B (Fujimura *et al.* 2002). In addition, circulating BDNF level is associated with eating behavior (Monteleone *et al.* 2004), metabolic disorders (Chaldakov *et al.* 2003, 2004, Suwa *et al.* 2006), physical activity (Nofuji *et al.* 2008), depression (Brunoni *et al.* 2008), Alzheimer's disease (Laske *et al.* 2006), and cognitive function (Gunstad *et al.* 2008). We therefore presume that circulating BDNF might possess several of physiological functions including GLUT4 biogenesis and thereby mimic the endocrine mechanism.

Although this is only a preliminary study, the results presented herein raise the possibility that BDNF treatment may potentially contribute to the therapy of obesity and type II diabetes mellitus, while also helping to treat related cardiometabolic diseases. Further studies are necessary to identify the therapeutic effects of BDNF for such diseases and to clarify the mechanism underlying the effects of BDNF for GLUT4 expression and hypophagia.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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References

BRUCE CR, ANDERSON MJ, CAREY AL, NEWMAN DG, BONEN A, KRIKETOS AD, COONEY GJ, HAWLEY JA: Muscle oxidative capacity is a better predictor of insulin sensitivity than lipid status. *J Clin Endocrinol Metab* **88**: 5444-5451, 2003.

BRUNONI AR, LOPES M, FREGNI F: A systematic review and meta-analysis of clinical studies on major depression and BDNF levels: implications for the role of neuroplasticity in depression. *Int J Neuropsychopharmacol* **11**: 1169-1180, 2008.

CHALDAKOV GN, FIORE M, HRISTOVA MG, ALOE L: Metabotrophic potential of neurotrophins: implication in obesity and related diseases? *Med Sci Monit* **9**: HY19-21, 2003.

CHALDAKOV GN, FIORE M, STANKULOV IS, MANNI L, HRISTOVA MG, ANOTONELLI A, GHENEV PI, ALOE L: Neurotrophin presence in human coronary atherosclerosis and metabolic syndrome: a role for NGF and BDNF in cardiovascular disease? *Prog Brain Res* **146**: 279-289, 2004.

CHALDAKOV GN, FIORE M, TONCHEV AB, DIMITROV D, PANCHEVA R, RANCIC G, ALOE L: Homo obesus: a metabotrophin-deficient species. Pharmacology and nutrition insight. *Curr Pharm Des* **13**: 2176-2179, 2007.

DAVIES AM: Paracrine and autocrine actions of neurotrophic factors. *Neurochem Res* **21**: 749-753, 1996.

DOEHNER W, GATHERCOLE D, CICOIRA M, KRACK A, COATS AJ, CAMICI PG, ANKER SD: Reduced glucose transporter GLUT4 in skeletal muscle predicts insulin resistance in non-diabetic chronic heart failure patients independently of body composition. *Int J Cardiol* **138**: 19-24, 2010.

FUJIMURA H, ALTAR CA, CHEN R, NAKAMURA T, NAKAHASHI T, KAMBAYASHI J, SUN B, TANDON NN: Brain-derived neurotrophic factor is stored in human platelets and released by agonist stimulation. *Thromb Haemost* **87**: 728-734, 2002.

GUNSTAD J, BENTEZ A, SMITH J, GLICKMAN E, SPTZNAGEL MB, ALEXANDER T, JUVANCIC-HELTZEL J, MURRAY L: Serum brain-derived neurotrophic factor is associated with cognitive function in healthy older adults. *J Geriatr Psychiatry Neurol* **21**: 166-170, 2008.

HE J, WATKINS S, KELLEY DE: Skeletal muscle lipid content and oxidative enzyme activity in relation to muscle fiber type in type 2 diabetes and obesity. *Diabetes* **50**: 817-823, 2001.

KURODA A, YAMASAKI Y, MATSUHISA M, KUBOTA M, NAKAHARA I, NAKATANI Y, HOSHI A, GOROGAWA S, UMAYAHARA Y, ITAKURA Y, NAKAGAWA T, TAIJI M, KAJIMOTO Y, HORI M: Brain-derived neurotrophic factor ameliorates hepatic insulin resistance in Zucker fatty rats. *Metabolism* **52**: 203-208, 2003.

LASKE C, STRANSKY E, LEYHE T, ESCHWEILER GW, WITTORF A, RICHARTZ E, BARTELS M, BUCHKREMER G, SCHOTT K: Stage-dependent BDNF serum concentrations in Alzheimer's disease. *J Neural Transm* **113**: 1217-1224, 2006.

LEVINGER I, GOODMAN C, MATTHEWS V, HARE DL, JERUMS G, GARNHAM A, SELIG S: BDNF, metabolic risk factors, and resistance training in middle-aged individuals. *Med Sci Sports Exerc* **40**: 535-541, 2008.

MONTELEONE P, TORTORELLA A, MARTIADIS V, SERRITELLA C, FUSCHINO A, MAJ M: Opposite changes in the serum brain-derived neurotrophic factor in anorexia nervosa and obesity. *Psychosom Med* **66**: 744-748, 2004.

NAKAGAWA T, TSUCHIDA A, ITAKURA Y, NONOMURA T, ONO M, HIROTA F, INOUE T, NAKAYAMA C, TAIJI M, NOGUCHI H: Brain-derived neurotrophic factor regulates glucose metabolism by modulating energy balance in diabetic mice. *Diabetes* **49**: 436-444, 2000.

NAKAGAWA T, OGAWA Y, EBIHARA K, YAMANAKA M, TSUCHIDA A, TAIJI M, NOGUCHI H, NAKAO K: Anti-obesity and anti-diabetic effects of brain-derived neurotrophic factor in rodent models of leptin resistance. *Int J Obes Relat Metab Disord* **27**: 557-565, 2003.

NAKAZATO M, HASHIMOTO K, SHIMIZU E, KUMAKIRI C, KOIZUMI H, OKAMURA N, MITSUMORI M, KOMATSU N, IYO M: Decreased levels of serum brain-derived neurotrophic factor in female patients with eating disorders. *Biol Psychiatry* **54**: 485-490, 2003.

NOFUJI Y, SUWA M, MORIYAMA Y, NAKANO H, ICHIMIYA A, NISHICHI R, SASAKI H, RADAK Z, KUMAGAI S: Decreased serum brain-derived neurotrophic factor in trained men. *Neurosci Lett* **437**: 29-32, 2008.

PAN W, BANKS WA, FASOLD MB, BLUTH J, KASTIN A: Transport of brain-derived neurotrophic factor across the blood-brain barrier. *Neuropharmacology* **37**: 1553-1561, 1998.

RADKA SF, HOLST PA, FRITSCHE M, ALTAR CA: Presence of brain-derived neurotrophic factor in brain and human and rat but not mouse serum detected by a sensitive and specific immunoassay. *Brain Res* **709**: 122-130, 1996.

RÖCKL KS, WITCZAK CA, GOODYEAR LJ: Signaling mechanisms in skeletal muscle: acute responses and chronic adaptations to exercise. *IUBMB Life* **60**: 145-153, 2008.

SUWA M, KISHIMOTO H, NOFUJI Y, NAKANO H, SASAKI H, RADAK Z, KUMAGAI S: Serum brain-derived neurotrophic factor level is increased and associated with obesity in newly diagnosed female patients with type 2 diabetes mellitus. *Metabolism* **55**: 852-857, 2006.

SUWA M, NAKANO H, RADAK Z, KUMAGAI S: Endurance exercise increases the SIRT1 and peroxisome proliferator-activated receptor γ coactivator-1α protein expressions in rat skeletal muscle. *Metabolism* **57**: 986-998, 2008.

TSUCHIDA A, NONOMURA T, NAKAGAWA T, ITAKURA Y, ONO-KISHINO M, YAMANAKA M, SUGARU E, TAIJI M, NOGUCHI H: Brain-derived neurotrophic factor ameliorates lipid metabolism in diabetic mice. *Diabetes Obes Metab* **4**: 262-269, 2002.

XU B, GOULDING EH, ZANG K, CEPOI D, CONE RD, JONES KR, TECOTT LH, REICHARDT LF: Brain-derived neurotrophic factor regulates energy balance downstream of melanocortin-4 receptor. *Nat Neurosci* **6**: 736-742, 2003.

YAMANAKA M, TSUCHIDA A, NAKAGAWA T, NONOMURA T, ONO-KISHINO M, SUGARU E, NOGUCHI H, TAIJI M: Brain-derived neurotrophic factor enhances glucose utilization in peripheral tissues of diabetic mice. *Diabetes Obes Metab* **9**: 59-64, 2007.

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Research article

Association of cardiorespiratory fitness with elevated hepatic enzyme and liver fat in Japanese patients with impaired glucose tolerance and type 2 diabetes mellitus

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Abstract
No study has so far determined whether a favorable level of cardiorespiratory fitness (CF) contributes to a reduced risk of elevated hepatic enzymes and a high degree of liver fat in patients having various metabolic risks. This study investigated the association between the maximal oxygen uptake (VO_2max) and the prevalence of elevated liver enzymes and high liver fat, while considering such factors as abdominal obesity, hyperinsulinemia and the other metabolic risks. The study enrolled newly diagnosed Japanese patients (n = 84; 52 males and 32 females; aged 25-69 years) with impaired glucose tolerance (IGT) and type 2 diabetes mellitus (Type2DM) who did not receive any intervention or pharmacological therapy. The subjects were divided into 3 groups according to the distribution of the VO_2max for each sex. The odds ratios (ORs) for the prevalence of elevated aspartate and alanine aminotransferase (AST and ALT) and high degree of liver fat adjusted for age, sex, disease type, daily ethanol intake, and current smoking were significantly lower in the moderate- and high CF groups in comparison to the low CF group. In addition, a significant OR for AST was maintained in the moderate and high CF group after adjusting for abdominal obesity and/or hyperinsulinemia. The significant ORs for the prevalence of elevated ALT and a high degree of liver fat were attenuated after adjusting for abdominal obesity and/or hyperinsulinemia. No significant OR for the prevalence of elevated gamma-glutamyl transferase (GGT) was recognized in all logistic models. These results indicated that CF was negatively and independently associated with the prevalence of elevated AST even in Japanese diabetic patients having various metabolic risks. It was concluded that the AST level might be useful as a simple marker reflecting physical inactivity in such subjects.

Key words: Cardiorespiratory fitness, hepatic enzyme, non-alcoholic fatty liver, abdominal obesity, insulin resistance.

Introduction

Hepatic enzymes are primary indices for the diagnosis of non-alcoholic fatty liver disease (NAFLD), which is noticed as one of phenotypes of metabolic syndrome (André et al., 2007). Furthermore, elevated hepatic enzymes have been noted as a predictor of metabolic syndrome, type 2 diabetes mellitus (Type2DM) and cardiovascular disease (André et al., 2006; Cho et al., 2007; Doi et al., 2007, Monami et al., 2008; Nakanishi et al., 2004; Rector et al., 2008; Sattar et al., 2004). Hepatic enzymes might therefore be a general marker reflecting the pathology of these diseases.

On the other hand, cardiorespiratory fitness (CF), which is a direct index of physical activity, plays a role of suppressing the onset of type 2 DM, metabolic syndrome, cardiovascular diseases and mortality (LaMonte et al., 2005; Lakka et al., 2002; Sawada et al., 2003; Sui et al., 2007; Lyerly et al., 2009). In addition, recent cross-sectional studies reported an inverse association between CF and NAFLD (Church et al., 2006; Lawlor et al., 2005; Nguyen-Duy et al., 2003; Perseghin et al., 2007). It is therefore naturally expected that a favorable level of CF might be related not only with a low prevalence of NAFLD, but also elevated levels of hepatic enzymes.

A recent study (Messier et al., 2010) has demonstrated that metabolically healthy but obese women who were in the upper quartile of insulin sensitivity values had significantly lower concentrations of ALT, AST, and GGT as well as a lower fatty liver index in comparison to individuals in the lower 3 quartiles. However, this study did not evaluate either the physical activity or CF. A survey performed on adults aged 17 yrs of age or older in US (n = 15676) (Clark et al., 2003) reported unexplained aminotransferase elevation, which was significantly associated with a higher body mass index, waist circumference, triglyceride levels, fasting insulin, and lower HDL. It is well-known that these indices are strongly influenced by physical activity; however, no description regarding lifestyle was made in that report. Furthermore, the most of those studies are conducted in normal populations, and no study has yet investigated the impact of the maximal oxygen uptake on both liver fat and liver enzymes while taking other metabolic risks into consideration in specific subjects having a number of metabolic abnormalities.

The current study therefore investigated whether the prevalence of high degree of liver fat and elevated liver enzymes could be associated with low level of CF in newly diagnosed impaired glucose tolerance (IGT) and Type2DM patients with various metabolic risks but not consuming excessive amounts of alcohol.

Methods

Subjects

One hundred fifty-seven Japanese outpatients (114 males and 43 females, aged 25 to 81 years) who were newly-diagnosed to have IGT and Type2DM based on a 75g oral glucose tolerance test (75g OGTT) participated in the present study. The pathological state was classified based

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on the diagnostic criteria of the Committee of Japan Diabetes Society (Kuzuya et al., 2002). Though 2-24 months passed from the time that the patients were noted to have an elevated blood glucose level at a group medical checkup, none of the subjects had received pharmacological therapy or intervention until the diagnosis.

The patients answered a questionnaire to assess their alcohol consumption and current smoking habits. The type, amount, and frequency of alcohol consumption were assessed, from which the total amount of alcohol consumption was calculated and converted to the daily ethanol intake. Sixty-five subjects whose daily ethanol intake was more than 20g in males and 10g in females (Hashimoto, 2004), were excluded from the analysis. In addition, any cases including missing data needed for an analysis (n = 8) were also excluded. Finally, the data of 84 patients (52 male and 32 female, aged 25 to 69 years) were used for the analysis of the present study. Informed consent was obtained from each patient and the study was approved by The Ethics Committee of Institute of Health Science in Kyushu University.

Anthropometric measurement and protocol for computed tomography

The BMI was calculated as the weight (kilograms) divided by height (meters) squared. The waist circumference was measured at the level of the umbilicus. The visceral (VFA) and subcutaneous fat areas (SFA) were assessed by computed tomography (CT; VIGOR LAU DATOR, Toshiba, Japan). The subjects were examined following overnight fasting and in the supine position. Scanning was performed using the usual clinical assessment settings, i.e., 120kV and 200mA, 400mm field of view, 5mm thickness, and 2sec scanning time. The regions of interest were determined by the clinical specialists by tracing an outline of the adipose tissue on the CT image at the umbilical level. The whole abdominal and visceral fat areas were computed automatically based on the pixels for the X-ray attenuation range of these areas (Tokunaga et al., 1983). The SFA values were derived by subtracting the VFA from the whole abdominal fat area. In addition, liver fat deposition was evaluated using a CT image including both the liver and spleen derived from the twelfth thoracic vertebra level to the second lumbar vertebra level. The analysis of the mean CT attenuation values derived for the liver and spleen were performed by clinical specialists in diagnostic imaging. The ratio of the liver/spleen attenuation value (L/S ratio) was defined as an index of liver fat (Church et al., 2006).

Measurements of clinical data

Following overnight fasting of at least 9 hrs, blood samples were drawn from antecubital vein for the analysis as below; sampling tubes of EDTA 2K-NaF and plain were used. A 75g OGTT was performed on the subjects' blood samples obtained at 30, 60, 120, and 180 minutes. The fasting insulin and fasting blood glucose concentrations were measured using a radioimmunoassay and an enzymatic method, respectively. The levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transferase (GGT) were determined as indices of the hepatic function, using a method recommended by the Japanese Society of Clinical Chemistry for determining the catalytic amounts of enzymes. Tests for hepatitis B or C virus and other liver diseases were performed on the subjects whose AST and/or ALT were over 100 IU/L. The levels of fasting triglyceride, total cholesterol, and high-density lipoprotein cholesterol were assessed using an enzymatic method. The resting systolic (SBP) and diastolic blood pressure (DBP) were determined 3 times following a 30-minute rest period using a mercury sphygmomanometer, with the lowest values used as the resting blood pressure. The subjects newly diagnosed to have IGT or Type2DM were instructed to undergo an anthropometric evaluation and a fitness test within 2 to 3 weeks following the diagnostic tests.

Criteria for abnormalities of parameters

The definition of elevated liver enzymes based on a statement by The Ministry of Health, Labour and Welfare in Japan, 2007. The abnormal criteria for each enzyme were as follows; elevated AST: AST > 30U/L, elevated ALT: ALT > 30U/L, and elevated GGT: GGT > 50U/L. Furthermore, a patient with an L/S ratio less than 0.9, which is a cutoff value usually adopted in domestic medical institutions (Hashimoto, 2006), was regarded as having high liver fat.

Patients whose VFA levels were more than 100 cm^2 were defined as having excess visceral fat (The Examination Committee of Criteria for "Obesity Disease" in Japan, 2002). The fasting insulin equivalent was determined to be 7μU/mL, a 75th percentile value of fasting insulin in Japanese male workers (Tamakoshi et al., 2003) as the basic criteria for hyperinsulinemia in this study.

Evaluation of cardiorespiratory fitness

Graded exercise tests were performed by a skilled examiner using a cycle ergometer (Monark, Stockholm, Sweden) to evaluate the CF. The heart rate, electrocardiogram, and blood pressure were monitored and recorded during the test. The exercise intensity was increased 3 or 4 times every 4 minutes until the heart rate reached 70% of the maximum or higher. Maximal oxygen uptake (VO_2max), which is regarded as an index of CF, was determined according to the nomogram of Åstrand & Rhyming (1954), a modality that is generally used to predict the VO_2max.

The distributions of VO_2max were divided into tertiles in each sex. The details regarding the range in each group were as follows; the lowest tertile (Low-CF group): $VO_2max \leq 31.8$ml/kg/min in males and $VO_2max \leq 26.2$ in females; the intermediate tertile (Moderate-CF group): $31.8 < VO_2max \leq 35.6$ in males and $26.2 < VO_2max \leq 30.2$ in females; and the highest tertile (High-CF group): $VO_2max > 35.6$ in males and $VO_2max > 30.2$ in females.

Statistical analysis

An analysis of variance (ANOVA) was performed to compare continuous variables of the subjects classified by CF level. TG, fasting glucose and insulin, AST, ALT, and GGT had a skewed distribution and were therefore analyzed following log-transformation. A comparison of categorical variables was analyzed using chi-square analysis. The odds-ratio (OR) and 95% confidence inter-

val (95%CI) for the prevalence of any abnormalities in each group were calculated using 4 logistic regression models. First, ORs adjusted for age, sex, disease type, daily ethanol intake, and smoking as basic confounding factors for the prevalence of these abnormalities were calculated (Model-1). After the analysis using Model-1, the ORs were adjusted for abdominal obesity or hyperinsulinemia (Model-2 and 3), finally, adjustments for both abdominal obesity and hyperinsulinemia were added (Model-4). All statistical analyses were performed using the SPSS version 14.0 software program (SPSS Japan Inc.). Statistical significance was set at a value of $p < 0.05$.

Results

Characteristics of the subjects divided by the CF level

Characteristics of all subjects and those classified by CF levels are indicated in Table 1. The distribution of the subjects' VO_2max was observed to have shifted slightly to a lower level and the whole range was narrower than that in the Japanese healthy population.

The mean value of the VFA in all the subjects ($160.4 \pm 63.2 cm^2$) was substantially higher than the Japanese criteria for abdominal obesity ($\geq 100 cm^2$). The mean value of the fasting insulin level ($7.4 \pm 4.7 \mu U/ml$) was as high as the mean value of the top quartile in Japanese male workers (Tamakoshi et al., 2003). Prevalence of elevated AST, ALT and GGT in all subjects was 23, 49 and 31%, respectively. The subjects having elevated AST accounted for 48, 14 and 7% in the high, moderate and low CF group, respectively. The elevated ALT in each group accounted for 74, 41 and 32%, in addition, the elevated GGT was accounted for 37, 35 and 21%, respectively. Further, prevalence of high liver fat in all subjects was 21%, and 41, 14 and 11% in each fitness level, respectively. The Abdominal and liver fatness, fasting insulin, AST and ALT levels showed a gradual decrease according to the increase of CF level.

Analysis of the prevalence of abnormalities in the groups classified by CF level

As indicated in Table 2, The ORs for the prevalence of elevated AST in the moderate- and high CF group were significantly low in all models in comparison to the low CF group; the ORs ranged from 0.06 to 0.14. The ORs for an elevated ALT in the moderate- and high CF group were also significantly low in model 1, which ranged from 0.15 to 0.25. Model 2 showed a significant OR for elevated ALT only in high CF group. However, the significant ORs were attenuated after adjusting for only hyperinsulinemia (model 3), and after adjusting for both abdominal obesity and hyperinsulinemia (model 4). The ORs for an elevated GGT showed no significance in any group. The OR for high liver fat in the high CF group was significantly low in comparison to the low CF group (OR: 0.21) in model 1; however, the ORs in the other models adjusted for abdominal obesity and/or hyperinsulinemia showed no significance in any group.

Discussion

The main finding in the current study was that a favorable level of CF contributed to the attenuation of the elevated

Table 1. Comparison of characteristics of subjects classified by fitness level.

Continuous variables	All subject (M=52, F=32)		Fitness level Low (M=18, F=9)		Moderate (M=17, F=12)		High (M=17, F=11)		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	p
Age (yrs)	50.9	10.7	47.4	11.5	53.6	10.4	51.4	9.6	N.S.
BMI ($kg \cdot m^{-2}$)	25.1	4.1	27.7	4.4	24.8	3.2	22.9	3.2	< .001
Waist girth (cm)	88.4	10.1	94.3	10.8	88.0	7.5	83.0	8.8	< .001
Daily ethanol intake (g)	3.0	5.2	1.7	3.6	3.9	5.5	3.4	6.1	N.S.
Type 2 DM (%)	60.0 (71.4)		20.0 (74.1)		21.0 (72.4)		19.0 (67.9)		
Current smoking (%)	26.0 (31.0)		10.0 (37.0)		6.0 (20.7)		10.0 (35.7)		N.S.
Visceral fat area (cm^2)	160.4	63.2	197.6	70.4	155.2	44.9	129.8	55.2	< .001
Subcutaneous fat area (cm^2)	172.1	86.0	202.1	104.1	165.7	78.6	149.8	66.7	N.S.
L / S ratio †	1.03	0.26	0.90	0.28	1.09	0.17	1.08	0.26	< .005
AST (U/L)	26.3	12.5	33.9	14.3	22.6	8.9	22.8	10.6	< .001
ALT (U/L)	38.8	31.0	57.6	39.3	29.3	17.3	30.5	25.3	< .001
GGT (U/L)	43.7	26.8	53.0	31.9	42.2	25.7	36.3	19.7	N.S.
VO_2max ($ml \cdot kg^{-1} \cdot min^{-1}$)	32.1	5.7	27.5	3.8	31.1	3.0	37.5	4.9	< .001
Total cholesterol (mg/dL)	217.6	35.9	211.0	39.1	226.6	31.2	214.7	36.8	N.S.
Triglyceride (mg/dL)	134.4	78.3	140.1	77.8	127.9	67.2	135.5	90.8	N.S.
HDL-C (mg/dL)	51.4	12.5	47.1	11.0	55.3	14.9	51.6	10.1	N.S.
Fasting glucose (mg/dL)	136.1	33.5	136.3	41.8	140.9	32.8	131.0	24.3	N.S.
Fasting insulin (μU/mL)	7.4	4.7	10.3	5.8	6.3	3.3	5.6	3.3	< .001
Systolic blood pressure (mmHg)	127	17	133	16	124	18	124	17	N.S.
Diastolic blood pressure (mmHg)	80	11	84	11	79	11	76	9	< .05

Abbreviations are denoted in text. Data are expressed as means±S.D. or number of patients. The percentage in each group is shown in parenthesis. One-way ANOVA or Chi-square test was performed for statistical analysis. N.S. not significant.

Table 2. Odds ratios for the elevated hepatic enzymes and NAFL in the groups classified by fitness level (n = 84).

	Model 1 [a]			Model 2 [b]			Model 3 [c]			Model 4 [d]		
	OR	95%CI	p	OR	95%CI	p	OR	95%CI	p	OR	95%CI	p
Elevated AST												
Moderate CF	**.11**	**.02-.55**	**.007**	**.12**	**.02-.58**	**.009**	**.13**	**.02-.78**	**.025**	**.14**	**.02-.85**	**.033**
High CF	**.06**	**.01-.36**	**.002**	**.06**	**.01-.42**	**.004**	**.07**	**.01-.49**	**.008**	**.07**	**.01-.58**	**.013**
Elevated ALT												
Moderate CF	**.25**	**.06-.94**	**.041**	.28	.07-1.07	.063	.48	.11-2.02	.314	.52	.12-2.29	.390
High CF	**.15**	**.04-.58**	**.006**	**.20**	**.05-.83**	**.027**	.29	.07-1.25	.096	.39	.09-1.79	.226
Elevated GGT												
Moderate CF	.99	.28-3.47	.981	1.08	.31-3.81	.906	1.15	.29-4.65	.842	1.30	.32-5.25	.714
High CF	.52	.14-1.90	.320	.66	.17-2.53	.545	.60	.14-2.52	.488	.78	.18-3.37	.740
High liver fat												
Moderate CF	.35	.08-1.49	.155	.37	.09-1.63	.191	1.04	.18--5.86	.963	1.06	.19-5.92	.950
High CF	**.21**	**.05-.99**	**.048**	.28	.06-1.33	.109	.62	.10-3.63	.592	.77	.12-4.77	.778

These odds ratios are referring for that in the low CF group. Abbreviations are denoted in text. [a]: Adjusted for age, sex, disease type, daily ethanol intake and current smoking. [b]: Added adjusting for abdominal obesity to the Model 1. [c]: Added adjusting for hyperinsulinemia to the Model 1. [d] Added adjusting for abdominal obesity and hyperinsulinemia to the Model 1. CI: confidence interval.

AST, independent of the pathology frequently observed in the diabetic subjects. The prevalence of elevated AST was below one fourth of all subjects, whereas one half of them were included in the low CF group. On the other hand, the association of elevated ALT or high liver fat with CF depended on the presence of abdominal obesity and/or hyperinsulinemia in diabetic subjects. No association found between CF level and elevated GGT.

It is highly important to identify the difference in the strength of association with CF among these enzymes. This remains a matter for speculation, but might be due to a difference in the location of these enzymes. While ALT and GGT exist mainly in hepatic cells, AST exists not only in the hepatic cells, but also in cardiac and muscle cells. In the current study, the prevalence of elevated AST among the subjects with a high degree of liver fat was 50%, which was obviously lower than that in the subjects demonstrating both high liver fat and elevated ALT or GGT (88.9 and 72.2%, respectively). It is speculated that AST might therefore reflect either cell injury or inflammation beside hepatic tissue in such subjects having various metabolic abnormalities. At this point, the robust inverse relationship between CF and elevated AST can be attributed to the findings of recent studies reporting an inverse association of directly measured CF and such inflammation markers as C-reactive protein, fibrinogen and cytokine, etc (Kullo et al., 2007; Jae et al., 2008). In addition, a recent clinincal study showed a significant correlation between the carotid intimal media thickness and hepatic enzymes, including AST (Abdou et al., 2009). However, these explanations remain mere speculation. Further accumulation of evidence is thus needed to clarify the association between the CF and AST levels in the future.

On the other hand, ALT which mainly exists in the hepatic cells might be directly affected by higher levels of liver fat, which is related to both abdominal fat and insulin resistance (Messier et al., 2010). Results from recent animal experiments, which examined the effect of daily aerobic exercise (Rector et al., 2008), the cessation of exercise (Rector et al., 2008) and a genetically low aerobic capacity (Thyfault et al., 2009) to the hepatic oxidative capacity, are all consistent with the hypothesis that regular aerobic exercise or a favorable CF improve the hepatic oxidative capacity. Such evidence could therefore help us to explain both the low prevalence of high liver fat and the elevated ALT levels observed in the high CF group. However, the prevalence of both abnormalities was dependent on abdominal obesity and/or hyperinsulinemia rather than on the CF level in diabetic subjects; the result in the current study agreed with that in the prior-mentioned study (Messier et al., 2010).

No association between CF and elevated GGT found in the logistic model adjusted for basic confounders including disease type. Considerable number of prospective studies reported elevated GGT was a strong predictor of Type 2 DM (André et al., 2005, André et al., 2006, André et al., 2007, Doi et al, 2007, Lee et al., 2003, Nakanishi et al., 2004). The GGT level was closely correlated with the insulin level in the present study ($r = 0.452$, $p < 0.0001$, data not shown). Taking these evidences into consideration, it was speculated that GGT level in diabetic subjects was affected by insulin resistance rather than aerobic capacity strongly reflecting muscle oxidative capacity and cardiac function.

The present study has some limitations. The design of the study was cross-sectional and thus unable to identify causality between CF and elevated hepatic enzymes or high liver fat. In addition, the results of the current study were derived from diabetic patients; it should not be regarded as phenomena in healthy population. The VO_2max data was calculated using heart rate during exercise, thus few errors in the values of VO_2max might occur, though VO_2max measurements were performed by a skilled examiner. The daily ethanol intake was self-reported, and may therefore be biased or inaccurate. Tests for hepatitis B or C virus were only performed for the patients who were suspected of having these viruses. At least a 3-year treatment regimen by the subjects' primary doctor and at least a 1-year follow-up of lifestyle modification was performed for almost all subjects after the assessment of the present study; however, no onset of hepatitis B or C was recognized.

Conclusion

The current study is thus considered to demonstrate, for the first time, a favorable level of cardiorespiratory fitness could contribute to a reduced risk of elevated aminotransferase and high liver fat in Japanese patients newly diagnosed as IGT or type 2 DM. An independent and inverse association between the CF level and the prevalence of an elevated AST level was observed, the possibility that AST may potentially be useful as a simple marker concerning physical inactivity should therefore be assessed. Prospective cohort studies in the general population, exercise-intervention for high-risk populations, and a biochemical approach are required to address the effect of physical activity on both the hepatic enzyme levels and liver fat levels in the future.

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References

Abdou, A.S., Magour, G.M. and Mahmoud, M.M. (2009) Evaluation of some markers of subclinical atherosclerosis in Egyptian young adult males with abdominal obesity. *British Journal of Biomedical Science* **66**, 143-147.

André, P., Balkau, B., Vol, S., Charles, M.A. and Eschwège, E. (2007) Gamma-glutamyltransferase activity and development of the metabolic syndrome (International Diabetes Federation Definition) in middle-aged men and women: Data from the Epidemiological Study on the Insulin Resistance Syndrome (DESIR) cohort. *Diabetes Care* **30**, 2355-2361.

André, P., Balkau, B., Born, C., Charles, M.A. and Eschwège, E. (2006) Three-year increase of gamma-glutamyltransferase level and development of type 2 diabetes in middle-aged men and women: the D.E.S.I.R. cohort. *Diabetologia* **49**, 2599-2603.

André, P., Balkau, B., Born, C., Royer, B., Wilpart, E., Charles, M.A. and Eschwège, E. (2005) Hepatic markers and development of type 2 diabetes in middle aged men and women: a three-year follow-up study. The D.E.S.I.R. Study (Data from an Epidemiological Study on the Insulin Resistance syndrome). *Diabetes & Metabolism* **31**, 542-550.

Åstrand, P.O. and Rhyming, I. (1954) A nomogram for calculation of the aerobic capacity (physical fitness) from pulse rate during submaximal work. *Journal of Applied Physiology* 7, 218-221.

Cho, N.H., Jang, H.C., Choi, S.H., Kim, H.R., Lee, H.K., Chan, J.C. and Lim, S. (2007) Abnormal liver function test predicts type 2 diabetes: a community- based prospective study. *Diabetes Care* **30**, 2566-2568.

Church, T.S., Kuk, J.L., Ross, R., Priest, E.L., Biltoft, E. and Blair, S.N. (2006) Association of cardiorespiratory fitness, body mass index, and waist circumference to NAFLD. *Gastroenterology* **30**, 2023-2030.

Clark, J.M., Brancati, F.L. and Diehl, A.M.(2003) The prevalence and etiology of elevated aminotransferase levels in the United States. *American Journal of Gastroenterology* **98,** 960-967.

Crandall, D.L., Feirer, R.P., Griffith, D.R. and Beitz, D.C. (1981) Relative role of caloric restriction and exercise training upon susceptibility to isoproterenol-induced myocardial infarction in male rats. *American Journal of Clinical Nutrition* **34,** 841-847.

Doi, Y., Kubo, M., Yonemoto, K., Ninomiya, T., Iwase, M., Tanizaki, Y., Shikata, K., Iida, M. and Kiyohara, Y. (2007) Liver enzymes as a predictor for incident diabetes in a Japanese population: the Hisayama study. *Obesity* **15**, 1841-1850.

Hashimoto, E. (2004) NASH: Clinical course and prognosis. *Acta Hepatologica Japonica* **45,** 66-76.

Hashimoto, E. (2006) Diagnostic criteria for non-alcoholic steatohepatitis. *Nippon Rinsho* **64,** 1025-1032. (In Japanese)

Jae, S.Y., Heffernan, K.S., Lee, M.K., Fernhall, B. and Park, W.H. (2008) Relation of cardiorespiratory fitness to inflammatory markers, fibrinolytic factors, and lipoprotein(a) in patients with type 2 diabetes mellitus. *American Journal of Cardiology* **102,** 700-703.

Kullo, I.J., Khaleghi, M. and Hensrud, D.D. (2007) Markers of inflammation are inversely associated with VO_2 max in asymptomatic men. *Journal of Applied Physiology* **102,** 1374-1379.

Kuzuya, T., Nakagawa, S., Satoh, J., Kanazawa, Y., Iwamoto, Y., Kobayashi, M., Nanjo, K., Sasaki, A., Seino, Y., Ito, C., Shima, K., Nonaka, K. and Kadowaki, T. (2002) Report of the committee on the classification and diagnostic criteria of diabetes mellitus. *Diabetes Research and Clinical Practice* **55**, 65-85.

Lakka H.M., Laakssonen D.E., Lakka T.A., Niskanen, L.K., Kumpusalo, E., Tuomilehto, J. and Salonen, J.T. (2002) The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. *JAMA* **288**, 2709-2716.

LaMonte, M.J., Barlow, C.E., Jurca, R., Kampert, J.B., Church, T.S. and Blair, S.N. (2005) Cardiorespiratory fitness is inversely associated with the incidence of metabolic syndrome: a prospective study of men and women. *Circulation* **26**, 112, 505-512.

Lawlor, D.A., Sattar, N., Smith, G.D. and Ebrahim, S. (2005) The Associations of physical activity and adiposity with alanine aminotransferase and gamma- glutamyltransferase. *American Journal of Epidemiology* **161**, 1081-1088.

Lee, D.H., Jacobs, D.R. Jr, Gross, M., Kiefe, C.I., Roseman, J., Lewis, C.E. and Steffes, M. (2003) Gamma-glutamyltransferase is a predictor of incident diabetes and hypertension: the Coronary Artery Risk Development in Young Adults (CARDIA) Study. *Clinical Chemistry* **49**, 1358-1366.

Ling, P.R., Smith, R.J. and Bistrian, B.R.(2007) Acute effects of hyperglycemia and hyper- insulinemia on hepatic oxidative stress and the systemic inflammatory response in rats. *Critical Care Medicine* **35**, 555-560.

Lyerly, G.W., Sui, X., Lavie, C.J., Church, T.S., Hand, G.A. and Blair, S.N. (2009) The association between cardiorespiratory fitness and risk of all-cause mortality among women with impaired fasting glucose or undiagnosed diabetes mellitus. *Mayo Clinic Proceedings* **84**,780-786.

Messier, V., Karelis, A.D., Robillard, M.E., Bellefeuille, P., Brochu, M., Lavoie, J.M. and Rabasa-Lhoret, R. (2010) Metabolically healthy but obese individuals: relationship with hepatic enzymes. *Metabolism* **59**, 20-24.

Monami, M., Bardini, G., Lamanna, C., Pala, L., Cresci, B., Francesconi, P., Buiatti, E., Rotella, C.M. and Mannucci, E. (2008) Liver enzymes and risk of diabetes and cardiovascular disease: results of the Firenze Bagno a Ripoli (FIBAR) study. *Metabolism* **57**, 387-392.

Nakanishi, N., Suzuki, K. and Tatara, K. (2004) Serum gamma-glutamyltransferase and risk of metabolic syndrome and type 2 diabetes in middle-aged Japanese men. *Diabetes Care* **27**, 1427-1432.

Nguyen-Duy, T.B., Nichaman, M.Z., Church, T.S., Blair, S.N. and Ross, R. (2003) Visceral fat and liver fat are independent predictors of metabolic risk factors in men. *American Journal of Physiology-Endocrinology and Metabolism* **84**, E1065-1071.

Perseghin, G., Lattuada, G., De Cobelli, F., Ragogna, F., Ntali, G., Esposito, A., Belloni, E., Canu, T., Terruzzi, I., Scifo, P., Del Maschio, A. and Luzi, L. (2007) Habitual physical activity is associated with intrahepatic fat content in humans. *Diabetes Care* **30**, 683-688.

Rector, R.S., Thyfault, J.P., Wei, Y. and Ibdah, J.A. (2008) Non-alcoholic fatty liver disease and the metabolic syndrome: An update. *World Journal of Gastroenterology* **14**, 185-192.

Rector, R.S., Thyfault, J.P., Morris, R.T., Laye, M.J., Borengasser, S.J., Booth, F.W. and Ibdah, J.A. (2008) Daily exercise increases hepatic fatty acid oxidation and prevents steatosis in Otsuka Long-Evans Tokushima Fatty rats. *American Journal of Physiology - Gastrointestinal and Liver Physiology* **294**, G619-626.

Rector, R.S., Thyfault, J.P., Laye, M.J., Morris, R.T., Borengasser, S.J., Uptergrove, G.M., Chakravarthy, M.V., Booth, F.W. and Ibdah, J.A. (2008) Cessation of daily exercise dramatically alters precursors of hepatic steatosis in Otsuka Long-Evans Tokushima Fatty (OLETF) rats. *Journal of Physiology* **586**, 4241-4249.

Sattar, N., Scherbakova, O., Ford, I., O'Reilly, D.S., Stanley, A., Forrest, E., Macfarlane, P.W., Packard, C.J., Cobbe, S.M. and Shepherd, J. (2004) Elevated alanine aminotransferase predicts new-onset

type 2 diabetes independently of classical risk factors, metabolic syndrome, and C-reactive protein in the west of Scotland coronary prevention study. *Diabetes* **53**, 2855-2860.

Sawada, S., Lee, I.M., Muto, T., Matsuzaki, K. and Blair, S.N. (2003) Cardiorespiratory fitness and the incidence of type 2 diabetes: prospective study of Japanese men. *Diabetes Care* **26**, 2918-2922.

Sui, X., LaMonte, M.J. and Blair, S.N. (2007) Cardiorespiratory fitness as a predictor of nonfatal cardiovascular events in asymptomatic women and men. *American Journal of Epidemiology* **165**, 1413-1423.

Tamakoshi, K., Yatsuya, H., Kondo, T., Hori, Y., Ishikawa, M., Zhang, H., Murata, C., Otsuka, R., Zhu, S. and Toyoshima, H. (2003) The metabolic syndrome is associated with elevated circulating C-reactive protein in healthy reference range, a systemic low-grade inflammatory state. *International Journal of Obesity and Related Metabolic Disorders* **27**, 443-449.

The Examination Committee of Criteria for "Obesity Disease" in Japan, Japan Society for the Study of Obesity. (2002) New Criteria for "Obesity Disease" in Japan. *Circulation Journal* **66**, 987-992.

Thyfault, J.P., Rector, R.S., Uptergrove, G.M., Borengasser, S.J., Morris, E.M., Wei, Y., Laye, M.J., Burant, C.F., Qi, N.R., Ridenhour, S.E., Koch, L.G., Britton, S.L. and Ibdah, J.A. (2009) Rats selectively bred for low aerobic capacity have reduced hepatic mitochondrial oxidative capacity and susceptibility to hepatic steatosis and injury. *Journal of Physiology* **587**, 1805-1816.

Tokunaga, K., Matsuzawa, Y., Ishikawa, K. and Tarui, S. (1983) A novel technique for the determination of body fat by computed tomography. *International Journal of Obesity* **7**, 437-445.

Key points

- The prevalence of elevated AST was negatively, and strongly associated with the CF level independent of abdominal obesity, hyperinsulinemia, and the other confounders in the subjects with glucose intolerance.
- The association between the CF level and both an elevated ALT level and a high degree of liver fat, as defined by the L/S ratio of CT images depended on abdominal fat and/or hyperinsulinemia in the subjects with glucose intolerance.
- No association was recognized between CF and elevated GGT in the subjects with glucose intolerance in the subjects with glucose intolerance.
- Having a favorable level of CF could lead to a reduced risk of hepatic-related abnormalities even in diabetic patients having the other metabolic risks.

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通信制生活習慣改善法が睡眠改善に及ぼす効果とその関連要因

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目的　研究目的は，1）睡眠と睡眠に関連する生活習慣における1か月間の通信制習慣改善法の効果を例数を増やして確認すること，および2）その改善効果に影響を与える要因を検討することの2点であった。

方法　対象者は，職域で通信制睡眠習慣改善プログラムに参加した，入眠潜時（就眠時刻−就床時刻）と睡眠効率（睡眠時間/就床時間）に問題がある睡眠困難者178人であった。介入法は，小冊子の自己学習と自己設定した目標行動のセルフモニタリングという最小限の行動技法からなる簡便な方法であった。期間は1か月間で，やりとりは全て郵送で行われた。介入前後に自己記入式の質問票調査を実施した。介入前後の睡眠指標と睡眠に関係の深い生活習慣の変化を検討した。また，本法の効果に影響する要因を検討するために，対象者を入眠潜時および睡眠効率の平均改善値をカットオフ値として有効群63人と比較群115人に2分し，介入前の基本特性，睡眠指標，生活習慣および介入による変化を群間で比較した上で，さらにロジスティック回帰分析を行った。

結果　介入後に，全体で睡眠時間が5.71時間から6.05時間に増加，入眠潜時は18分短縮し，睡眠効率は5.6ポイント向上するなど，先行研究と同等の短期効果が確認された。習慣については9項目中8項目で望ましくない行動を選択する人の割合が減少した。目標行動としての選択や達成率で群間の差はなかったが，習慣改善個数は有効群2.63個，比較群2.06個と改善群が有意に多かった。ロジスティック回帰分析により，「ベースライン時の入眠潜時」が大きい者，および「定期的な運動の改善」をした者の2要因が睡眠改善に影響していることが明らかとなった。

結論　本法における短期効果が確認された。また，本法は介入前に入眠潜時が長く入眠困難を持つ者に対してより有用であること，睡眠指標の改善には特に定期的な運動習慣が重要な役割を持つことが示唆された。本研究の結果から，行動療法による睡眠改善教育は簡便な形であっても実施可能であり，その効果が期待できると考えられた。

Key words：睡眠改善，通信制，生活習慣改善法，行動療法

I　緒　　言

厚生労働省の調査によると，20歳から59歳の約4割は睡眠時間が6時間未満であり[1]，睡眠による休養が十分でない人の割合は21.2%にのぼることが報告されている[2]。健康日本21では，休養・こころの健康づくりのために睡眠改善が目標の一つとされ，平成15年には「健康づくりのための睡眠指針〜快適な睡眠のための7箇条〜」[3]が発表された。また，睡眠に関連した事故の発生[4]からマスメディアでも睡眠時無呼吸症候群を中心に睡眠の問題が多く取り上げられるなど社会の関心も高まっている。さらに近年，睡眠時間と血圧[5]，糖代謝[6〜9]，脂質代謝[10]など生活習慣病の危険因子との関連[11,12]が明らかになってきたことから，睡眠状態の改善は公衆衛生上重要な課題となっている。

睡眠障害の治療としては一般的には薬物療法が用いられているが，薬を用いない治療法として，慢性の不眠に対する行動療法がある。欧米においては1960年代より積極的にその治療研究が行われ，1990年代末にアメリカ睡眠学会[13]やNIH[14]（米国国立

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衛生研究所）は，行動療法について薬物療法よりも優れていると総括した。行動療法は行動科学を応用した心理療法であり，不適切な習慣行動の変容を目的としている。不眠の場合は，それを維持・強化していると思われる不適切な生活習慣や的外れの努力を修正することで，睡眠を改善させようとする[15]。最近のレビューでは，本法は性，年齢に無関係に有効であるだけではなく，慢性疼痛や癌など他疾患の合併の有無に関係なく改善効果があることも明らかになってきた[16,17]。

このように睡眠改善に対する行動療法の有効性についてはほぼ評価が確定している。しかし，一般的にその普及は難しいと考えられており，その理由としては行動療法の実施に時間や専門的知識と熟練を要する点があげられている[15]。これらの課題に対して，行動療法の治療構造の明確さを生かし，自己マニュアルや通信指導，コンピュータによる簡素化した指導法の研究が多くの問題において行われてきた[18~21]。足達らは自己学習と自己設定した目標行動のセルフモニタリングという最小限の行動技法から成る1か月間の通信指導プログラム（以下通信制習慣改善法）を作成し，職域においてメニュー式通信プログラムとして実施してきた[22]。睡眠については，この方法による介入によって，睡眠指標と睡眠に関連した生活習慣（以下習慣とする）が改善し[24]，1年後も効果が維持されたことを報告した[25]。さらに，入眠潜時（就眠時刻—就床時刻）と睡眠効率（睡眠時間/就床時間×100）に問題がある者（以下，睡眠困難者とする）47人では，睡眠改善効果が大きく習慣改善個数が睡眠効率，起床に要する時間との関連があることを報告した[26]。しかし，対象者が少数であり，通信制習慣改善法がどのような集団に効果的であるか，またどの習慣の改善が睡眠改善に影響を及ぼすかという検討は行っていなかった。

そこで今研究では例数を増やし，先行研究で得られた睡眠指標の改善と同等の結果が得られるかどうかを確認するとともに，その改善効果に関連する要因の検討を行った。

Ⅱ　研究方法

1.　対象と方法

対象者の選別は次のように行った。朝日新聞健康保険組合が2001–2004年の毎年11月に実施した通信制習慣改善法の睡眠コースの参加者は計371人で，終了者は324人（87.3%）であった。その中で開始時と終了時の就床時刻，入眠時刻，覚醒時刻，起床時刻の4つのデータが得られたのは250人であり，そこから睡眠困難者を選別した。睡眠困難者とは，開始時における入眠潜時が30分以上，または睡眠効率が85%未満のいずれかを満たしたものであった。重複参加者については初回参加時のデータのみを採用した。解析対象者は，睡眠困難者178人（男性67人，女性111人，平均年齢35.5±9.2歳）であった。なお，これらの中には先行研究[26]の対象者47人も含まれる。

次に本プログラムの睡眠指標の改善に影響を与える要因を検討するために，対象者の入眠潜時および睡眠効率の改善の平均値で対象者を2分した。すなわち，最終分析対象者178人のうち，入眠潜時18分以上短縮および睡眠効率が5.6%以上改善した63人を有効群，それ以外の115人を比較群とし，両群の基本特性，前後の睡眠指標と生活習慣，気分の変化を検討した。

プログラムの流れは図1に示した通りである。参加者には，習慣の自己評価および目標設定を行わせた。教材「ぐっすり眠る」とセルフモニタリングシートを送付し，自己学習後，1か月間選んだ目標をセルフモニタリングさせた。1か月後に質問票とセルフモニタリングシートを管理者へ返送させた。継続の強化子として開始2週間後には，記録を続けるように励ましの手紙を送付し，終了者には1,000～2,000円の記念品を贈呈することを事前に提示した。参加者とのやりとりはすべて郵送で行われ，管理者は1人であった。

2.　調査項目と解析法

睡眠指標については，就床時刻，入眠時刻，入眠潜時，覚醒時刻，起床時刻，起床に要する時間（起床時刻—覚醒時刻），睡眠時間，就床時間，睡眠効率（睡眠時間/就床時間×100）の9項目を調査した。習慣は9項目，睡眠に関連する気分（以下気分とす

図1　睡眠改善プログラムの流れ