SHORT REPORT

Attenuated aerobic exercise capacity in CD36 deficiency

Hidekatsu Yanai, Ichiro Watanabe, Kojiro Ishii, Mie Morimoto, Hironobu Fujiwara, Shigeru Yoshida, Shu-Ping Hui, Kazuhiko Matsuno, Hitoshi Chiba

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Background: An important role of CD36 in muscle fatty acid (FA) uptake has been shown in CD36-knockout or CD36-overexpressed mice. FA is a predominant substrate in energy production during light exercise below the anaerobic threshold (AT). We studied whether aerobic exercise capacity in humans could be affected by CD36 deficiency.

Methods: We investigated the ventilatory threshold (VT) and serum FA changes in normal participants (n=22) and participants with CD36 deficiency (n=12) during pedalling on a cycle ergometer.

Results: In participants with CD36 deficiency, FA levels were not reduced at peak work rate, whereas FA levels decreased by about 50% in normal participants. Participants with CD36 deficiency showed significantly lower VT than normal participants. A significant correlation was observed between VT and percentage changes in FA at peak work rate.

Conclusion: This study found reduced FA utilisation and an attenuated aerobic exercise capacity in CD36 deficiency, indicating that CD36-mediated FA oxidation is an important determinant for aerobic exercise capacity in humans.

CD36 is a multifunctional membrane protein expressed in various cells such as platelets, monocytes, adipocytes and muscle cells. CD36 deficiency is divided into two subgroups according to the phenotype. In type I deficiency, neither platelets nor monocytes express CD36, whereas in type II, monocyte CD36 is expressed despite the lack of platelet CD36.¹ Our previous study revealed that people who are type I deficient were homozygous or compound heterozygous for mutations and that people with type II deficiency were heterozygous for mutations.² Of the membranous proteins that can bind fatty acid (FA), CD36 has been found to have an important role in muscle FA, shown in CD36-knockout or CD36-overexpressed mice.^{3–4} We previously showed that human CD36 deficiency is associated with a significant delay in plasma FA disappearance after glucose loading, indicating an important physiological role for this protein in peripheral FA uptake in humans.⁵ FA is a predominant substrate (40–90%) in energy production during light exercise below the anaerobic threshold (AT). To understand whether aerobic exercise capacity in humans could be affected by CD36 deficiency, we investigated the ventilatory threshold (VT) and serum FA changes in normal participants and in participants with CD36 deficiency during pedalling on a cycle ergometer.

METHODS

Subjects and phenotyping

Informed consent was obtained from all participants. In total, 34 apparently healthy female students (mean (SD) age 20.0 (1.0) years; body mass index 20.6 (1.9)) comprised the study group. CD36 phenotypes were determined by flow cytometry using a monoclonal antibody against CD36.² Briefly, fasting venous blood was taken into tubes containing EDTA-2K. A

fluorescein isothiocyanate-conjugated monoclonal antibody against CD36 (FA6-152; Immunotech, Miami, Florida, USA) was used to detect CD36 in flow cytometry. The signal from the anti-CD36 was gated with a phycoerythrin-conjugated monoclonal antibody against CD42b (AN51; Dako, Copenhagen, Denmark), a specific marker for platelets, or a fluorescein isothiocyanate-conjugated monoclonal antibody against CD14 (MY4-FITC; Coulter, Miami, Florida, USA), a specific marker for monocytes.

Genotyping of CD36 deficiency

Genomic DNA was extracted from whole blood from participants with CD36 deficiency. Genotyping was performed by examining endonuclease restriction fragment length polymorphisms of PCR products as previously reported for the three common mutations in Japanese populations: a substitution of T for C at nt478 in exon 4 (nt478T), an AC deletion at nt539 in exon 5 (nt539delAC) and an A insertion at nt1159 in exon 10 (nt1159insA).² In each CD36-deficient participant without any of the known mutations, genomic DNA was subjected to sequencing for all exons including the 5' and 3' untranslated region (UTR) and the 5' flanking region.

Measurements for ventilatory threshold and serum fatty acids

Participants were studied during an incremental work test in which the initial work rate consisted of 3 min of pedalling on a 15 W-loaded cycle ergometer following which the work rates were incrementally increased by 15 W every minute. VT was calculated using computerised regression analysis of the slopes of the CO₂ production versus O₂ consumption plot (V-slope method).⁶ Blood samples were obtained at rest, peak work rate and 15 min after exercise. Serum free FA was measured by an automated enzymatic method.

RESULTS

Of the 34 participants, 12 were CD36-deficient. The two type I participants were homozygous for T at position 478 (nt478T). Of the 10 type II participants, 5 were heterozygous for nt478T and we found no mutations in the other 5. There were no significant differences between normal participants and participants with CD36 deficiency for age (normal vs participants with CD36 deficiency; 20.3 (1.1) vs 20.6 (1.4) years old) or body mass index (20.1 (1.6) vs. 20.6 (1.8) kg/m²).

In normal participants, serum FA levels decreased at peak work rate (fig 1A). In contrast, in participants with CD36 deficiency, FA levels were not decreased at peak work rate and remained at significantly higher levels than normal participants 15 min after exercise (fig 1A).

Participants with CD36 deficiency showed significantly lower VT than normal participants (fig 1B). A significant correlation

Abbreviations: AT, anaerobic threshold; CPT I, carnitine palmitoyltransferase I; FA, fatty-acid; UTR, untranslated region; VT, ventilatory threshold

Figure 1 (A) Effect of exercise on fatty-acid (FA) metabolism in normal participants ($n = 22$, open circles) and participants with CD36 deficiency ($n = 12$, filled circles). Data are serum free FA levels (mean (SE)) relative to those while resting, at peak work rate and 15 min after exercise. The asterisk and double asterisk indicate differences between normal and CD36 deficiency at $p < 0.01$ and $p < 0.001$, respectively (Mann-Whitney U test). (B) Ventilatory threshold in normal ($n = 22$, open circles) and participants with CD36 deficiency ($n = 12$, filled circles). Data indicate mean (SD). The asterisk indicates differences between normal and CD36 deficiency at $p < 0.001$, Mann-Whitney U test.

($r = 0.785$, $p < 0.001$) was observed between VT and percentage changes in FA at peak work rate, suggesting an important contribution of FA metabolism to AT (fig 2).

DISCUSSION

Five participants with type II CD36 deficiency had none of the investigated mutations. We have previously studied all exons including the 5' and 3' UTRs and the 5' flanking region of CD36 gene in 22 people with type II CD36 deficiency without any of the known mutations.² We found two unreported polymorphisms: G/T at position 53, located within potential transcription-binding sites for the activator protein 2, and G/A at position 2164 in the 3' UTR. However, our family study showed no definite effect of these polymorphisms on the phenotype

Figure 2 Correlation between ventilatory threshold and percentage changes of serum fatty acid at peak work rate. Correlation coefficient was 0.785 ($p < 0.001$ by Fisher's Z transformation). Open circles, filled triangles and filled circles indicate normal ($n = 22$), type I ($n = 2$) and type II ($n = 10$) participants with CD36 deficiency, respectively.

determination. We can suggest that there is an important role of platelet CD36 mRNA content in the establishment of type II deficiency, as platelet RNA content is known to decrease to a negligible level after reticulated platelets transform to matured platelets. It may be important to identify unknown factors that can affect the CD36 mRNA content. A mutation in intron in the β -globin gene can not only result in β -thalassaemia by affecting the mRNA but also can affect the phenotype of β -thalassaemia by interaction with a second defect.⁷ In addition, various cytokines and other soluble mediators have been reported to affect the CD36 expression.^{8–10} The possible contribution of these factors to CD36 phenotype should be studied in the future.

In participants with CD36 deficiency, FA levels were not decreased at peak work rate, whereas serum FA levels were decreased in normal participants, indicating defective FA uptake by CD36 from the circulation. CD36 is a key protein involved in regulating the uptake of FA across the plasma membrane in skeletal muscle. Within minutes of beginning muscle contraction, FA transport is increased due to the increase in contraction-induced translocation of CD36 from an intracellular depot to the cell surface.¹¹

In three participants with type II CD36 deficiency, serum FA levels were increased at peak work rate and the percentage increased FA levels were higher than those in participants with type I CD36 deficiency (fig 2). In our previous study, which investigated serum FA after glucose loading, FA disappearance was significantly reduced in type I compared with type II deficiency at 60–120 min after glucose loading; however, the rate of FA disappearance in type II deficiency did not significantly differ from that in type I deficiency at 30 min.⁵ This may suggest no difference in defective FA uptake between type I and type II CD36 deficiency during the early phase. A prompt increase in plasma catecholamine, which increases lipolysis in adipose tissue or muscle due to activation of hormone-sensitive lipase, occurs simultaneously with AT.^{12,13} These results can explain increased FA at peak work rate in some participants with type II deficiency compared with those with type I deficiency. However, further studies should be performed to clarify this discrepancy. Possible differences in CD36 expression in muscle among normal people, people with type I and people with type II CD36 deficiency have not been examined and should also be elucidated.

The progressive decline in plasma FA turnover with increasing exercise intensity is offset by progressive increases in blood glucose turnover. Shifts in energy substrate mobilisation and utilisation occur as exercise intensity increases, and these shifts are associated with AT.⁶ Excess CO₂ is generated when lactate is raised during exercise because its [H⁺] is buffered by HCO₃⁻.⁶ The V-slope method that we used is a method to detect AT, using computerised regression analysis of the slopes of the CO₂ uptake versus O₂ uptake plot, which detects the beginning of the buffering of [H⁺].⁶ An impaired energy substrate metabolism seems to attenuate AT. In CD36 deficiency, an increased glucose oxidation due to defective FA utilisation may be associated with lower VT.

As regulation of fatty acyl-CoA entry into the mitochondria by carnitine palmitoyltransferase I (CPT I) is a rate-limiting step in FA oxidation, the capacity of skeletal muscle to oxidise FA has been considered to be limited by the activity of CPT I.¹⁴ Recently, CD36 has been reported to be present on the mitochondrial membrane of skeletal muscle and to regulate FA oxidation during exercise.¹⁵ An increase in CD36 that co-immunoprecipitated with CPT I by a physiological stimulus increasing FA oxidation has also been found.¹⁶

Few specific genetic factors that strongly influence human physical exercise have been identified. Our study demonstrated reduced FA utilisation and attenuated aerobic exercise capacity in CD36 deficiency, indicating that CD36-mediated FA oxidation is an important determinant for human aerobic exercise capacity. CD36 deficiency has been reported to be associated with insulin resistance and dyslipidaemia.¹⁷ We believe that our findings may have potential applications to exercise physiology or preventive medicine.

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Authors' affiliations

Hidekatsu Yanai, Hironobu Fujiwara, Hitoshi Chiba, Department of Laboratory Medicine, Hokkaido University School of Medicine, Sapporo, Japan

Ichiro Watanabe, Department of Rehabilitation Medicine and Physical Medicine, Hokkaido University School of Medicine, Sapporo, Japan

Kojiro Ishii, Laboratory of Human Performance and Fitness, Hokkaido University Graduate School of Education, Sapporo, Japan

Mie Morimoto, Shigeru Yoshida, Kazuhiko Matsuno, Hitoshi Chiba, Department of Health Science, Hokkaido University School of Medicine, Sapporo, Japan

Shu-Ping Hui, Faculty of Pharmaceutical Sciences, Health Sciences University of Hokkaido, Hokkaido, Japan

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Correspondence to: Dr Hidekatsu Yanai, Department of Laboratory Medicine, Hokkaido University School of Medicine, Sapporo 060-8648, Japan; yanaih@jikei.ac.jp

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