



Changes in Gene Expression in Responders and Nonresponders to a Low-Intensity Walking Intervention

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OBJECTIVE

Daily physical activity remains an effective strategy to prevent obesity and type 2 diabetes. However, the metabolic response to exercise training is variable, and the precise clinical and molecular determinants that mark the metabolic improvements remain unknown. We tested the hypothesis that clinical improvements in glucose control after low-intensity exercise in individuals with impaired glucose tolerance (IGT) are coupled to alterations in skeletal muscle gene expression.

RESEARCH DESIGN AND METHODS

We investigated 14 overweight individuals with IGT before and after a 4-month low-intensity unsupervised walking exercise intervention. Clinical and anthropometric measurements and glucose tolerance were determined before and after the intervention. Skeletal muscle biopsy specimens were obtained for mRNA expression analysis.

RESULTS

Waist circumference and work capacity during cycle ergometry were improved in individuals who achieved normal glucose tolerance (NGT) after exercise training (IGT-NGT; $n = 9$) but in not individuals who remained IGT (IGT-IGT; $n = 5$). Pretraining glycemic control was better in IGT-NGT compared with IGT-IGT. mRNA expression of mitochondrial markers and transcription factors was increased in IGT-NGT after exercise intervention and normalized to levels measured in a separate cohort of nonexercised individuals with NGT. Conversely, these markers were unaltered after exercise intervention in IGT-IGT.

CONCLUSIONS

Normalization of metabolic control can be achieved after low-intensity exercise in individuals with IGT. This can be tracked with increased mRNA expression of mitochondrial and metabolic genes in skeletal muscle. However, for individuals presenting with a greater derangement in glycemia, the potential for clinical and metabolic improvements after this low-intensity unsupervised exercise protocol appears to be limited.

Physical exercise is a highly potent lifestyle intervention that improves whole-body glucose metabolism by increasing insulin sensitivity and reducing body weight (1). Prospective randomized controlled trials underscore the effectiveness of physical activity to enhance skeletal muscle insulin sensitivity, normalize glycemia, and slow

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or prevent metabolic decline (2,3). This is particularly apparent in patients with impaired glucose tolerance (IGT) or prediabetes who have not progressed to overt type 2 diabetes. Although complete reversal of type 2 diabetes by lifestyle modifications alone appears less successful (4), persistent daily exercise in people with IGT is estimated to reduce the diabetes risk by 30% or more (5). Thus, a focused examination of the overall health benefits of exercise intervention in high-risk target groups of people with prediabetes is warranted.

One goal of health care professionals in the prevention of metabolic disease is to prescribe an achievable intervention program that combines appropriate education and support to encourage and promote permanent lifestyle modifications (6,7). Yet, the current public health recommendations of a minimum of 30 min of aerobic-based exercise daily (8–10) may be unrealistic and unattainable for most of the populace. Factors such as compliance (11) and exercise responsiveness (12,13) complicate the development and implementation of clear physical activity recommendations and/or guidelines for people with IGT or overt type 2 diabetes. Furthermore, older individuals may be restricted or limited in their exercise modality because of prior illness, reduced mobility, or other complications (14). In addition, controlled, supervised exercise trials do not necessarily reflect the actual adherence to an exercise prescription offered by many primary care professionals. Thus, the health-promoting benefits of achievable exercise programs designed for individuals who are overweight or those with declining metabolic control are incompletely defined.

The pathogenesis of type 2 diabetes is often accompanied by reduced metabolic function and mitochondrial content (15,16) and by a concomitant decrease in mass and strength of skeletal muscle (17,18). The discovery of molecular markers for early metabolic disease and the identification of individuals most likely to benefit from exercise interventions to improve both insulin sensitivity and the functional properties of skeletal muscle could aid in the detection of future diabetes risk in a population with prediabetes. The notion that people differ in their individual response to defined exercise training

protocols (12,13) has received increasing attention. Molecular analysis of the heterogeneity of the response to endurance exercise (19), even in an aging population (20), has provided insight into the adaptive response of skeletal muscle to exercise training. Thus, an effort to identify the molecular “fingerprint” that defines not only metabolic disease but also the adaptive response to exercise intervention is warranted. Molecules involved in fuel utilization or mitochondrial biogenesis could serve as molecular end points that define the optimal exercise intervention to preserve glucose homeostasis and skeletal muscle mass.

In this study we tested the hypothesis that clinical improvements in glucose control after unsupervised daily exercise in individuals with IGT are coupled to alterations in skeletal muscle gene expression. This unique clinical material can provide insight into the molecular mechanisms by which low-intensity unsupervised exercise improves glucose homeostasis and thus influences the progression from IGT to type 2 diabetes. We reveal that practical, time-efficient exercise interventions can be easily incorporated into daily living to induce clinically beneficial health outcomes. Waist circumference, exercise capacity, and skeletal muscle gene expression were improved after an exercise intervention in individuals with IGT who reverted to normal glucose tolerance (NGT [IGT-NGT]), but not in individuals who remained IGT (IGT-IGT). Our results also highlight the need for approaches to probe for clinical and molecular signatures associated with the early indications of IGT and the adaptive response to exercise.

RESEARCH DESIGN AND METHODS

Study Participants

The IGT cohort ($n = 14$) in the present investigation is a subgroup of a larger population of overweight, sedentary male and female volunteers who were recruited from Gustavsberg, a suburb of Stockholm, Sweden, to participate in a low-cost randomized controlled exercise intervention study with Nordic walking poles (21,22). Comprehensive details describing the entire study cohort have been published elsewhere (21,22). Participants in the entire cohort were categorized as NGT, IGT, or having type 2 diabetes based on 2-h glucose

values determined during an oral glucose tolerance test (OGTT). A subset of individuals from the entire cohort who participated in the exercise intervention volunteered to donate muscle biopsy specimens before and after the study period. Gene expression values from a separate group of nonexercised individuals with NGT ($n = 54$) were included for comparative purposes (NGT-nonexercised). The NGT-nonexercised cohort was assembled from our previously described material (21,22) and constitutes a subset of participants from whom skeletal muscle biopsy specimens were available. Study protocols were approved by the Karolinska Institutet Ethics Committee.

Exercise Test and Intervention Protocol

Details of the exercise test and intervention protocol were published by our group earlier (21). VO_2max was determined on a Rodby RE 820/830 bicycle ergometer for all participants before and after the 20-week exercise intervention. The participants cycled at a 50-W load, which was increased by 10 W every min. Gas exchange was measured by a Vmax Encore ergospirometer (Sensor-Medics). Absolute VO_2max (L/min) was determined at the point of exhaustion, and VO_2 uptake (mL/kg/min) was calculated using the mean values of the last 30 s of the final workload. The highest workload (W) achieved was recorded. For the walking intervention program, participants were instructed to engage in 5 h/week of unsupervised Nordic walking with poles, at a moderate intensity for 20 weeks (between the months of May to August) in addition to maintaining daily habitual activity. Although exercise intensity was not monitored, the participants logged the duration (h/week) and subjective intensity of weekly activity in a diary.

Clinical Parameters

Anthropometric measurements, including weight, height, and waist circumference, were recorded before and after the 20-week exercise intervention program. Seated systolic and diastolic blood pressure was determined using a Speidel and Keller tonometer (Jungingen, Germany). Baseline measurements were recorded in the morning in conjunction with an OGTT to measure fasting and 2-h blood glucose. Blood glucose was measured using a HemoCue B-glucose analyzer

(HemoCue AB, Ängelholm, Sweden). Fasting insulin was determined by an assay kit from Linco Research (Electra-Box Diagnostica AB, Tyresö, Sweden) and quantified in the Lumines Bio-Plex 200 system (Bio-Rad, Stockholm, Sweden). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated by fasting glucose (mmol/L) \times fasting insulin (mU/L)/22.5. HbA_{1c} values were obtained from the Laboratory of Clinical Chemistry, Karolinska University Hospital, Stockholm, Sweden. Clinical data are presented in Table 1.

IGT Participant Stratification

Individuals classified as IGT ($n = 14$), based on the World Health Organization diagnostic criteria for diabetes of 2-h blood glucose (<7.0 mmol/L) values during an OGTT (cohort mean 9.8 ± 0.2 mmol/L) were the focus of the present analyses. After the exercise intervention, 9 of the 14 subjects achieved normal 2-h blood glucose values (IGT-NGT), but 5 did not (IGT-IGT). Comparisons of clinical profiles and skeletal muscle gene expression were performed between these two groups.

Skeletal Muscle Biopsies

Skeletal muscle biopsy specimens were obtained from volunteers before and after the 20-week exercise intervention. A muscle specimen (40–100 mg) was obtained from the vastus lateralis with a Weil-Blakesley conchotome (allgaier instrumente GmbH, Frittlingen/Tuttlingen, Germany). Specimens were immediately frozen and stored in liquid nitrogen until further analysis.

Muscle mRNA Expression Analysis

mRNA was prepared from vastus lateralis muscle specimens for gene expression analysis. A standard TRIzol (Invitrogen) extraction method was used to extract total mRNA from 25–30 mg frozen tissue. The purity and concentration of mRNA were determined spectrophotometrically. cDNA was generated from 1 μ g purified mRNA using a high-capacity cDNA RT kit (Applied Biosystems, Stockholm, Sweden). Gene expression analysis was performed using a custom design TaqMan-based microfluidic card gene expression assay (Applied Biosystems, Foster City, CA). Because the biopsy material was limited, gene expression assays were designed to maximize the number of targets analyzed.

The following assays from Applied Biosystems were lyophilized in the microfluidic card wells: ATP5J (Hs00365888_m1), COX4I1 (Hs00266371_m1), CYC1 (Hs00357717_m1), UQCRC2 (Hs00996395_m1), NDUFS1 (Hs00192297_m1), SDHB (Hs01042482_m1), DGKD (Hs00177552_m1), DGKZ (Hs01632414_s1), DNMT1 (Hs00945899_m1), DNMT3A (Hs01027166_m1), DNMT3B (Hs01003405_m1), NRF1 (Hs00602161_m1), NRF2 (Hs01022023_m1), PPARGC1A (Hs00173304_m1), PPARGC1B (Hs00370186_m1), PPARA (Hs00231882_m1), PPARD (Hs00602622_m1), GAPDH (Hs99999905_m1), and B2M (Hs99999907_m1). cDNA samples were diluted (10 ng/50 μ L/well) and applied to the microfluidic card. Real-time PCR amplification was performed using a Prism 7900HT sequence detection system (Applied Biosystems, Stockholm, Sweden). Relative expression of mRNA was calculated using a Δ -critical threshold method and normalized to the housekeeping gene B2M, selected by NormFinder as an appropriate reference gene.

Statistical Analysis

Data are presented as mean \pm SEM. Significant differences between clinical

Table 1—Clinical and morphometric profile of IGT participants before and after 4 months of exercise intervention

	IGT-IGT ($n = 5$)			IGT-NGT ($n = 9$)		
	Pretraining	Posttraining	<i>P</i>	Pretraining	Posttraining	<i>P</i>
Sex, <i>n</i>						
Male	2			6		
Female	3			3		
Age, years	57.6 \pm 4.3			62.2 \pm 1.4		
Reported walking, h/week		4.6 \pm 0.7			4.8 \pm 0.6	
Weight, kg	97.8 \pm 8.3	97.4 \pm 8.9	NS	89.9 \pm 4.3	88.1 \pm 4.0	NS
BMI, kg/m ²	34.8 \pm 2.8	34.6 \pm 2.8	NS	28.4 \pm 1.0*	28.1 \pm 1.1*	NS
Waist, cm	105.2 \pm 6.5	103.8 \pm 7.2	NS	100.4 \pm 2.6	97.4 \pm 2.7	0.017
Blood pressure, mmHg						
Systolic	144 \pm 8	139 \pm 4	NS	137 \pm 5	131 \pm 5	NS
Diastolic	82 \pm 4	82 \pm 4	NS	82 \pm 3	78 \pm 3	NS
Plasma glucose, mmol/L						
Fasting	6.3 \pm 0.3	6.3 \pm 0.4	NS	5.5 \pm 0.2*	5.3 \pm 0.2*	NS
2-h	10.5 \pm 0.2	10.7 \pm 0.5	NS	9.4 \pm 0.2*	7.5 \pm 0.4*	0.008
HbA _{1c} , %	5.2 \pm 0.2	5.2 \pm 0.2	NS	4.7 \pm 0.1*	4.7 \pm 0.1*	NS
HbA _{1c} , mmol/mol	34 \pm 2	34 \pm 2	NS	28 \pm 1*	28 \pm 1*	NS
Fasting insulin, pmol/L	82.7 \pm 25.0	70.5 \pm 18.9	NS	52.5 \pm 16.5	37.9 \pm 7.0*	NS
HOMA-IR	3.4 \pm 1.0	2.9 \pm 0.8	NS	1.9 \pm 0.6	1.3 \pm 0.2	NS
Work, W	150 \pm 18	162 \pm 21	NS	174 \pm 15	190 \pm 15	0.016
VO ₂ , L/min	2.0 \pm 0.3	2.3 \pm 0.3	NS	2.1 \pm 0.3	2.3 \pm 0.1	NS
VO ₂ uptake, mL/kg/min	20.7 \pm 1.7	23.0 \pm 2.6	NS	23.0 \pm 2.5	26.2 \pm 1.3	0.096

Groups were stratified based on normalization of 2-h blood glucose after exercise training. Results are mean \pm SEM. The boldface *P* values indicate a significant difference between pre- and posttraining for IGT-IGT and IGT-NGT groups, respectively, as determined by a Wilcoxon signed rank test. NS, not significant. *Represents statistical difference between IGT-IGT and IGT-NGT groups at pre- or posttraining using a Mann-Whitney *U* test.

parameters of the IGT-NGT and IGT-IGT subgroups at baseline or postintervention were conducted using a nonparametric Mann-Whitney *U* test. Changes (Δ values) in clinical parameters after exercise intervention in the IGT-NGT and the IGT-IGT subgroups were determined using a nonparametric Kruskal-Wallis paired-samples test. Postintervention gene expression of the IGT subgroups was evaluated using a repeated-measures ANOVA. Gene expression values between IGT subgroups postintervention were compared with values from the NGT-nonexercised cohort using an ANOVA with Bonferroni post hoc tests. Natural log transformation was applied when data were not normally distributed. Comparatives analyses and correlation analysis were performed using SPSS 22.0 software (IBM Corp., Armonk, NY). The level of significance for the analyses was established at $P < 0.05$.

RESULTS

Baseline characteristics of study participants have been previously reported (21,22). We have also reported changes in the clinical characteristics for this IGT cohort after 4 months of unsupervised Nordic walking (21,22). At the onset of the study, all individuals were IGT based on OGTT measurements (Table 1). Glucose tolerance was improved in a subset of individuals (Table 1). Therefore, the IGT subjects ($n = 14$) were stratified into two separate groups. The IGT-NGT individuals ($n = 9$) who achieved improved 2-h blood glucose values during an OGTT after 4 months of exercise (9.4 ± 0.2 vs. 7.5 ± 0.4 mmol/L; pre- vs. postexercise, respectively; $P = 0.008$) were compared with the IGT-IGT individuals ($n = 5$) who showed no improvement (10.5 ± 0.2 vs. 10.7 ± 0.5 mmol/L; pre- vs. postexercise, respectively).

Low-Intensity Exercise Improves Clinical Characteristics in IGT-NGT Participants

Low-intensity unsupervised exercise decreased waist circumference ($P = 0.017$) and increased work capacity ($VO_2\max$) during a cycle ergometry test ($P = 0.016$) in the IGT-NGT group. Oxygen uptake also tended to increase but did not reach statistical significance in the IGT-NGT group. Baseline BMI, blood glucose profiles (fasting and 2-h glucose), and HbA_{1c} were lower in the IGT-NGT versus

the IGT-IGT group. The exercise-induced improvement in 2-h blood glucose was inversely correlated with the baseline preexercise insulin level ($r = -0.0685$, $P = 0.042$) and HOMA-IR ($r = -0.070$, $P = 0.036$) in the IGT-NGT group but not in the IGT-IGT group. The IGT-NGT group demonstrated normal postexercise fasting blood glucose, HbA_{1c} , and HOMA-IR values. The volume of exercise (hours walking per week) was similar between groups (4.6 ± 0.7 h/week for IGT-IGT vs. 4.8 ± 0.6 h/week for IGT-NGT).

Gene Expression Changes in IGT-NGT Mirrored Improvements in Clinical Characteristics

The candidate genes examined in this study are involved in mitochondrial biogenesis, lipid metabolism, and transcription. We observed an increase in mRNA expression of many of the genes studied after exercise intervention in the IGT-NGT versus IGT-IGT groups (Fig. 1). Because improved exercise capacity has been associated with an upregulation of mitochondrial genes, skeletal muscle mRNA expression of selected mitochondrial electron transport chain (ETC) components was determined before and after the exercise intervention. Expression of these genes before the exercise intervention was not significantly altered between the IGT-IGT and IGT-NGT groups. Exercise training increased skeletal muscle mRNA expression of ATP5J ($P = 0.001$), COX4I1 ($P = 0.029$), CYC1 ($P = 0.022$), and NDUFS1 ($P = 0.05$) in IGT-NGT but not in IGT-IGT subjects. Two

genes involved in lipid metabolism, namely DGK δ ($P = 0.058$) and DGK ζ ($P = 0.068$), showed a trend for exercise-induced increases in mRNA in IGT-NGT subjects. mRNA expression of the de novo DNA methyltransferase DNMT3A ($P = 0.012$) and several transcription factors, including NRF-1 ($P = 0.017$), NRF-2 ($P = 0.024$), PPAR δ ($P = 0.002$), and the transcriptional coactivator PGC1 β ($P = 0.037$), increased with exercise training in IGT-NGT but not in IGT-IGT subjects, whereas TFAM ($P = 0.07$, not shown) did not reach statistical significance.

Exercise-Induced Gene Expression in IGT-NGT Is Normalized to NGT Levels

To determine whether the changes in mRNA induced by the exercise intervention reflect a normalization of gene expression, the responses of the IGT-NGT group were compared with the separate NGT-nonexercised control group ($n = 54$) matched for age, weight, and sex (Fig. 2). The genes that were significantly changed in the IGT-NGT group after exercise training were compared against the mRNA expression level in the NGT-nonexercised control group. These included mitochondrial complex components (ATP5J, COX4I1, CYC1, UQCRC2, and NDUFS1), a de novo DNA methyltransferase (DNMT3A), and key coactivators and transcription factors (NRF-1, NRF-2, PPAR δ , and PGC1 β). Although the skeletal muscle expression of these genes was significantly lower in the IGT-IGT versus IGT-NGT group after the 4 months of exercise intervention,

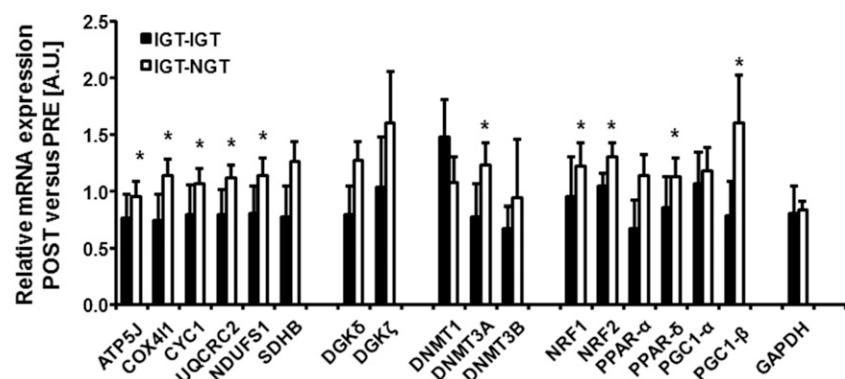


Figure 1—Relative skeletal muscle mRNA expression post- vs. preexercise values in individuals with IGT. mRNA expression of mitochondrial markers, enzymes, coactivators, and transcription factors was measured in skeletal muscle obtained from individuals who remained IGT (IGT-IGT; $n = 5$) or reverted to NGT (IGT-NGT; $n = 9$) after participating in a 4-month Nordic walking exercise intervention. Pre- (baseline) and postexercise values were normalized to the B2M gene and are reported relative to the preexercise baseline level for each respective gene. Data are mean \pm SEM. * $P < 0.05$ for IGT-IGT vs. IGT-NGT.

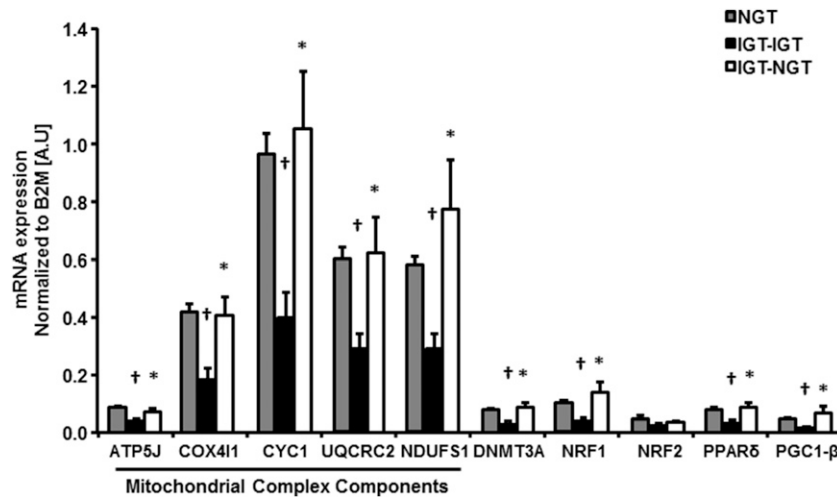


Figure 2—Skeletal muscle mRNA expression in IGT-IGT and IGT-NGT participants postexercise compared with the NGT-nonexercised reference group. mRNA expression of selected targets that differed between IGT-IGT ($n = 9$) and IGT-NGT ($n = 5$) postexercise (Fig. 1) were compared with baseline values from the NGT-nonexercised group ($n = 54$). Postexercise (IGT-IGT and IGT-NGT) and control (NGT-nonexercised) values were normalized to the B2M gene. Data are mean \pm SEM. * $P < 0.05$ for IGT-IGT vs. IGT-NGT, † $P < 0.05$ for NGT-nonexercised vs. IGT-IGT.

mRNA expression level between the IGT-NGT versus the NGT-nonexercised group was comparable, reflecting positive shifts in gene expression in the IGT-NGT group after exercise training.

CONCLUSIONS

People with IGT are at higher risk for developing type 2 diabetes, and consequently, these individuals should be encouraged to engage in lifestyle intervention programs, such as exercise training, to maintain insulin sensitivity. In a larger study including 212 overweight, older people classified as NGT, IGT, or as having type 2 diabetes, we previously observed improvements in cardiovascular risk factors after 4 months of walking, but these changes were most profound in people with NGT (21). In this present study, we focused on the individuals with IGT ($n = 14$). Our analysis revealed that an unsupervised exercise intervention improved blood glucose profiles, waist circumference, and work capacity in 64% of the participants. This subgroup ($n = 9$) reverted to NGT after 4 months of low-intensity walking (4.8 ± 0.6 h/week). Remarkably, in the remaining subgroup ($n = 5$), glucose profiles, waist circumference, and work capacity were unaltered, and these individuals remained IGT, despite reporting a similar amount of walking (4.6 ± 0.7 h/week). This discovery of interindividual response

to low-intensity exercise prompted us to further examine skeletal muscle gene expression after stratification of the IGT cohort based on the normalization of blood glucose levels during an OGTT after the exercise intervention.

Although the effects of various exercise training regimens on gene expression profiles in skeletal muscle have been assessed (19,23–28), comparatively limited information is available on the adaptive response to exercise training in people at risk for developing type 2 diabetes (29). We have taken a focused approach to gain insight into the molecular “fingerprint” associated with the early indications of IGT and the adaptive response to low-intensity unsupervised exercise. We determine genes with key roles in glucose and lipid metabolism in skeletal muscle. We provide evidence that the clinical improvements observed in the IGT-NGT group occur in concert with positive shifts in gene expression of key metabolic regulatory markers in skeletal muscle. Our observations support the notion that a habitual low-intensity lifestyle intervention for people with prediabetes/IGT is beneficial to reduce blood glucose and prevent or delay the development of type 2 diabetes. However, we noted that the molecular and clinical profiles were unaltered in a subgroup of the participants (36%) who remained IGT after exercise intervention, despite reporting

the same volume of walking. This indicates that for some individuals alternate strategies with increased volume or intensity of exercise may be needed. Our findings advance efforts to identify valid, reliable, and sensitive biological markers for the early detection of individuals at greatest risk for type 2 diabetes and to highlight that a range of interindividual responses to exercise exists.

Physical activity is an effective strategy to prevent the development and/or advancement of type 2 diabetes and related complications (30). In particular, the greatest success is observed in people with prediabetes or IGT (2,3). Several intervention programs to improve fasting and 2-h glucose levels have been developed for high-risk IGT individuals, including a pedometer-monitored walking activity (31). However, interindividual differences have been observed in the adaptive response to exercise, which may depend on molecular signatures of skeletal muscle (32). In our subanalysis of individuals with IGT, the exercise intervention reduced waist circumference, improved work capacity during a cycle ergometry test, and lowered 2-h glucose measurements in 9 of 14 participants (IGT-NGT). These changes did not appear to be coupled to any greater metabolic derangement at the start of the intervention, which could presumably allow for a greater scope for improvement. Rather BMI, fasting glucose, and 2-h glucose were lower in the IGT-NGT versus the IGT-IGT group.

The number of IGT participants in this study was relatively low ($n = 14$), which is a limitation of the study. However, the IGT participants were their own control for the shift in glucose tolerance, which was carefully monitored, as well as the gene expression changes. Clearly, the next step is to perform a randomized trial with a larger sample size to fully evaluate the effect of exercise intervention on skeletal muscle remodeling and glucose homeostasis in people with IGT. Future studies should also evaluate the effect of low-intensity versus more strenuous physical activity on insulin sensitivity, given that a greater exercise stimulus may be required for some individuals.

An earlier study of patients with type 2 diabetes enrolled in a Nordic walking program of 30 min daily, 2 days a week for 4 months provided evidence for a

decrease in fat mass but not HbA_{1c} or other clinical parameters after training (33). Although the subjects in our study reported nearly five times more walking per week, we still identified a subgroup of individuals that did not show improvements in blood glucose profiles. From this perspective, we realize we cannot predict who will respond to exercise, but we have attempted to “work backwards” per se to unravel molecular signaling cascades associated with metabolic change. However, our study is limited because the exercise was unsupervised and compensatory behavior changes were not evaluated. Thus, we cannot distinguish the extent to which the exercise intervention or changes in other physical activity behavior influenced the clinical and gene expression in the IGT-NGT cohort. We have not studied behavioral modification, which we believe will be important to address in future studies.

Nevertheless, our study design has some merit. Even with this unsupervised design, 64% of the IGT subjects had NGT after 4 months of low-intensity exercise intervention, whereas 36% were non-responders to the exercise intervention. Moreover, gene expression levels in the IGT-NGT group after the exercise intervention were comparable to a well-matched NGT-nonexercised control group ($n = 54$). We included data from the NGT-nonexercised subjects as a reference for the expression level of these genes in healthy people.

From a public health perspective, focusing on identifying people with IGT is important because if they can be encouraged to participate in low-intensity exercise programs, slowing or preventing metabolic decline and type 2 diabetes progression may be possible. Future randomized controlled exercise intervention studies in IGT subjects are needed to address this point. Efforts to identify biomarkers will also be required to predict the responders and nonresponders to exercise intervention. There may be a window of opportunity during which a low-intensity exercise may be sufficient to elicit improvements in glucose homeostasis in overweight, aging individuals. Taken together, individuals with IGT who show greater metabolic derangements may require more intense exercise or a different modality of exercise intervention to induce health benefits.

Transcriptome analysis of skeletal muscle after exercise training has bridged the link between cardiovascular and metabolic responses with molecular adaptations in the working muscle (19,23). Oxidative capacity is reduced with age (18,34) and metabolic disease (16,35), whereas positive effects on mitochondrial biogenesis and oxidative capacity in skeletal muscle are often seen after physical training (36). Still, the heterogeneity of human genomics and the variable response to exercise training poses a challenge to prescribing exercise as personalized medicine. Few studies have examined gene alterations in skeletal muscle concomitant with clinical changes from unsupervised exercise programs. In the IGT-NGT group, we observed a greater fold change in mRNA expression of several genes, including mitochondrial ETC complex subunits and key transcription factors regulating mitochondrial or cellular fuel substrate utilization in response to low-intensity exercise intervention. PGC1 α/β - and NRF-1-dependent genes, key factors in the induction of mitochondrial biogenesis and oxidative metabolism, are downregulated in skeletal muscle from patients with type 2 diabetes (37,38). Here we report gene expression of mitochondrial ETC genes as well as transcription factors NRF-1, NRF-2, PPAR δ , and PGC1 β were increased in IGT-NGT compared with the IGT-IGT group after exercise intervention, with levels comparable to those of the NGT-nonexercised cohort. Thus, skeletal muscle from the IGT-NGT group demonstrated greater plasticity in the gene regulatory response to exercise. This indicates that a low-intensity Nordic walking program elicits shifts in the mitochondrial profile in individuals who manifest a positive exercise response through a reduction in blood glucose values. These alterations in mRNA expression mirror the positive clinical improvements, but whether these gene changes occur as a direct response to the exercise training or as a consequence of the blood glucose normalization remains unknown. Individuals with IGT who have impaired fasting glucose also have lower muscle glycogen and elevated fat oxidation during exercise compared with those with isolated IGT, indicating that mild fasting hyperglycemia may shift fuel reliance toward fat during exercise in these individuals (39). Thus, the elevated glucose levels in the IGT-IGT group

may alter fuel utilization and/or gene expression.

A major challenge in treatment of type 2 diabetes and related metabolic disease pertains to adherence. Our findings support the notion that even an unsupervised low-intensity activity program can be sufficient to promote positive health benefits (40). Structured, supervised physical activity programs have had ~80% retention rates (30), with similar success achieved with supervised and unsupervised programs (40). Further randomized trials with a larger sample size, a control group, and measures of behavioral and metabolic compensation are warranted to fully evaluate the effects of exercise intervention on skeletal muscle remodeling and glucose homeostasis in people with IGT.

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