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SHORT COMMUNICATION

The effect of a single 2 h bout of aerobic exercise on ectopic lipids in skeletal muscle, liver and the myocardium

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Abstract
Aims/hypothesis Ectopic lipids are fuel stores in non-adipose tissues (skeletal muscle [intramyocellular lipids; IMCL], liver [intrahepatocellular lipids; IHCL] and heart [intracardiomyocellular lipids; ICCL]). IMCL can be depleted by physical activity. Preliminary data suggest that aerobic exercise increases IHCL. Data on exercise-induced changes on ICCL is scarce. Increased IMCL and IHCL have been related to insulin resistance in skeletal muscles and liver, whereas this has not been documented in the heart. The aim of this study was to assess the acute effect of aerobic exercise on the flexibility of IMCL, IHCL and ICCL in insulin-sensitive participants in relation to fat availability, insulin sensitivity and exercise capacity.
Methods Healthy physically active men were included. $\dot{V}O_{2max}$ was assessed by spiroergometry and insulin sensitivity was calculated using the HOMA index. Visceral and subcutaneous fat were separately quantified by MRI. Following a standardised dietary fat load over 3 days, IMCL, IHCL and ICCL were measured using MR spectroscopy before and after a 2 h exercise session at 50–60% of $\dot{V}O_{2max}$. Metabolites were measured during exercise.
Results Ten men (age 28.9±6.4 years, mean ± SD; $\dot{V}O_{2max}$ 56.3±6.4 ml kg^{-1} min^{-1}; BMI 22.75±1.4 kg/m^2) were recruited. A 2 h exercise session resulted in a significant decrease in IMCL (−17±22%, p=0.008) and ICCL (−17±14%, p=0.002) and increase in IHCL (42±29%, p=0.004). No significant correlations were found between the relative changes in ectopic lipids, fat availability, insulin sensitivity, exercise capacity or changes of metabolites during exercise.
Conclusions/interpretation In this group, physical exercise decreased ICCL and IMCL but increased IHCL. Fat availability, insulin sensitivity, exercise capacity and metabolites during exercise are not the only factors affecting ectopic lipids during exercise.

Keywords Ectopic lipids · Exercise capacity · Insulin sensitivity · Intracardiomyocellular lipids · Intrahepatocellular lipids · Intramyocellular lipids · Visceral and subcutaneous fat mass

Abbreviations

ΔGlucose	Difference between basal and peak glucose concentrations during exercise
ΔICCL	Exercise-related difference in ICCL, calculated as pre-exercise minus post-exercise levels
ΔIHCL	Exercise-related difference in IHCL, calculated as pre-exercise minus post-exercise levels
ΔIMCL	Exercise-related difference in IMCL, calculated as pre-exercise minus post-exercise levels
ΔNEFA	Difference between basal and peak NEFA concentrations during exercise, calculated as peak minus pre-exercise levels
ICCL	Intracardiomyocellular lipids
IHCL	Intrahepatocellular lipids

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IMCL	Intramyocellular lipids
SCAT	Subcutaneous adipose tissue
VAT	Visceral adipose tissue

Introduction

Ectopic lipids are lipids that are stored in non-adipose tissues such as skeletal muscle (intramyocellular lipids; IMCL), liver (intrahepatocellular lipids; IHCL) and myocardium (intracardiomyocellular lipids; ICCL) [1]. Ectopic lipids are used as fuel during exercise (depletion) and serve as a fuel depot after dietary fat intake (repletion); this has been shown in skeletal muscle [2]. In overweight individuals ectopic lipids are increased indicating that excess fat accumulates in non-adipose tissue [1]. The consequences of increased ectopic lipids are dual: (1) they may alter organ function directly (lipotoxicity); and (2) metabolites of ectopic lipids may be involved in mediating insulin sensitivity [2].

If the physiological role of ectopic lipids is to provide fuel in working tissues (skeletal and heart muscle), the capacity to deplete and replete these stores may be critical for the occurrence of pathological effects such as insulin resistance and lipotoxicity. While the exercise-induced usage of IMCL has been shown [2], it is not established whether a single bout of exercise induces changes in IHCL or ICCL. Several factors may be involved in regulating ectopic lipids: (1) fat availability (both visceral [VAT] and subcutaneous [SCAT] adipose tissue) and the systemic availability of NEFA; (2) insulin sensitivity; and (3) exercise capacity [3]. The contribution of these factors may vary in different physiological conditions (i.e. exercise vs rest; fasted vs postprandial) [3].

We hypothesise that a single bout of physical exercise will reduce ectopic lipids in working tissues (skeletal muscle and myocardium) whereas excess fat from lipolysis will be stored in the liver thereby increasing IHCL. Exercise capacity, fat availability and insulin sensitivity influence ectopic lipids.

The aims of the current study were to investigate IMCL, ICCL and IHCL before and after a single bout of physical exercise. In parallel, fat availability, insulin resistance and exercise capacity were assessed.

Methods

This prospective study was performed at the University Hospital of Bern, Switzerland. All investigations were carried out at the Department of Clinical Research (Division of Endocrinology, Diabetes and Clinical Nutrition, and Division of MR Spectroscopy and Methodology). It was approved by the local review board (Kantonale Ethikkommission, Bern) and was performed according to the declaration of Helsinki, good clinical practice (GCP) guidelines, and Swiss health laws on clinical research. All participants provided written, informed consent. This study will be continued under clinical trial number NCT01467193.

Participants and study protocol Ten adult healthy male participants volunteered for the study, which included two visits. At visit 1, participants attended the investigation unit after an overnight fast. After blood sampling (insulin, glucose, NEFA), $\dot{V}O_{2max}$ was determined during an incremental workload on an electrically braked exercise bike. ECG was continuously monitored, blood pressure was measured every 2 min, and subjective level of exhaustion was assessed using the Borg scale [4].

During a period of 3 days before visit 2, subjects followed a standardised fat rich diet consisting of weight maintaining food intake with a supplementary fat intake of 0.75 g fat/kg body weight, administered as three additional snacks per day, distributed in pre-packed bags. This protocol has previously been shown to efficiently replete IMCL stores [5]. In addition, the subjects were asked to refrain from physical activity. This was monitored by a pedometer throughout the whole period (aim <5,000 steps/24 h). In addition, a food diary was kept during the last 5 days before visit 2.

At visit 2, the volunteers attended the hospital in the morning 1 h after the intake of a standardised breakfast. Body fat mass (both VAT and SCAT) was assessed using MRI and ectopic fat stores (IMCL, IHCL, ICCL) were measured by ^{1}H-MR spectroscopy before and after a 2 h exercise session on an exercise bike at 50–60% of $\dot{V}O_{2max}$. Indirect calorimetry and blood sampling (including NEFA and glucose) were performed during exercise.

Further details of measurements of ectopic lipids, VAT and SCAT, and metabolites and assessment of insulin sensitivity are provided in the electronic supplementary material (ESM) Methods.

Statistical analysis

Statistical analysis was performed using STATA 12 (StataCorp, College Station, TX, USA). Results are expressed in means ±1 SD. After checking for normal distribution, paired *t* tests were applied to analyse pre- and post-exercise IMCL, IHCL and ICCL and the impact of exercise on hormones and metabolites.

Univariate correlation coefficients were calculated between exercise-induced changes in ectopic lipids (Δ) and fat availability (VAT and SCAT, NEFA concentrations), insulin sensitivity (using the HOMA index [6]), and exercise capacity and hormone/metabolite levels during exercise (expressed as peak value minus baseline value; Δ). A p value of <0.05 was considered significant.

Results

Subjects: clinical and metabolic characteristics The clinical and metabolic characteristics, the results of VAT and SCAT assessment, the exercise variables and the evaluation of insulin sensitivity are summarised in Table 1.

Effect of physical exercise on ectopic lipids The 2 h exercise sessions resulted in a decrease in IMCL (4.64±1.76 to 3.86±1.83 mmol/l; p=0.008; ΔIMCL −16.8±21.9% from baseline), a decrease in ICCL (3.82±1.95 to 3.2±1.83 mmol/l; p=0.002; ΔICCL −16.8±13.7%) and an increase in IHCL (13.73±10.41 to 18.86±13.81 mmol/l; p=0.004; ΔIHCL +41.9±28.8%); (Fig. 1).

Correlations There was a tendency for a positive correlation between ΔIHCL and ΔNEFA (r=0.57; p=0.08). ΔIHCL did not significantly correlate with insulin sensitivity or exercise capacity.

Neither ΔIMCL nor ΔICCL significantly correlated with insulin sensitivity, fat availability or exercise capacity. For details, see ESM Table 1.

Discussion

This is the first study that has concomitantly analysed IMCL, IHCL and ICCL before and after physical activity. The results indicate that a single bout of exercise leads to a reduction in IMCL and ICCL (in working tissues) and an increase in IHCL (fuel store).

The exercise-induced significant decrease of IMCL is consistent with previous studies on the effects of physical exercise on IMCL [2]. Exercise capacity and fat availability (VAT, SCAT and NEFA levels) were not related to the changes in IMCL, indicating that these factors alone did not regulate IMCL in a significant manner, but that it is rather the physical exercise with its panoply of local and systemic mechanisms that may explain these findings [7]. The fact that insulin sensitivity was not related to ΔIMCL may be due to the limited range of insulin sensitivity in this group and the small number of participants investigated.

ICCL significantly decreased following a 2 h aerobic exercise session, suggesting that ICCL behave similarly to IMCL. This also indicates that the fat oxidation within the contracting myocardial cell outweighs the supply of lipids from the circulation during exercise. Recently, Bilet et al [8] reported an increase in ICCL following exercise in the fasting condition (mean peak NEFA concentration ≈1,800 μmol/l) and a non-significant decrease in ICCL in the presence of low NEFA concentrations (mean nadir concentration ≈200 μmol/l) in young men. The latter condition was established by glucose administration with concomitant hyperinsulinaemia. Peak NEFA concentrations in the present study were considerably lower than in Bilet's study and glucose was not administered to the volunteers in the present study, probably explaining the discrepant findings.

ΔICCL was not related to insulin levels, insulin sensitivity or fat availability. Recently, Winhofer et al [9] have shown that short-term hyperinsulinaemia leads to an increase in ICCL (and a decrease in systemic NEFA concentrations) with a significant correlation between ΔICCL and insulin exposure. However, in a physiological condition, as presented in the current study, euglycaemia and low insulin levels were recorded, which may explain the lack of significant correlation between ΔICCL and insulin exposure. Similarly, it is likely that the clinical conditions (exercise vs non-exercise, fasting vs non-fasting) with the concomitant changes in insulin and metabolites are more important in regulating ICCL than fat availability alone.

The finding that ΔICCL did not correlate with exercise capacity has to be interpreted with caution since only a small

Table 1 Clinical and metabolic characteristics

Characteristic	Mean value	SD	Range
Age (years)	28.90	± 6.37	23–46
BMI (kg/m^2)	22.75	± 1.40	20.57–24.49
Waist (cm)	83.10	± 5.57	72–92
HOMA index	0.63	± 0.17	0.4–0.8
Total cholesterol (mmol/l)	4.98	± 0.89	3.73–6.28
LDL-cholesterol (mmol/l)	3.21	± 0.70	2.14–4.46
Triacylglycerol (mmol/l)	0.71	± 0.11	0.55–0.85
Total fat mass (% of body weight)	17.90	± 1.37	15.44–20.31
VAT (% of body weight)	2.02	± 0.29	1.64–2.54
SCAT (% of body weight)	15.89	± 1.19	13.70–17.77
$\dot{V}O_{2max}$ (ml kg^{-1} min^{-1})	56.30	± 6.39	43–64
Workload during exercise at 50–60% $\dot{V}O_{2max}$ (W)	134.60	± 20.27	106–158
ΔNEFA (during exercise) mmol/l	0.92	± 0.40	0.45–1.95
ΔGlucose (during exercise) mmol/l	−0.61	± 0.55	−0.1–−1.43

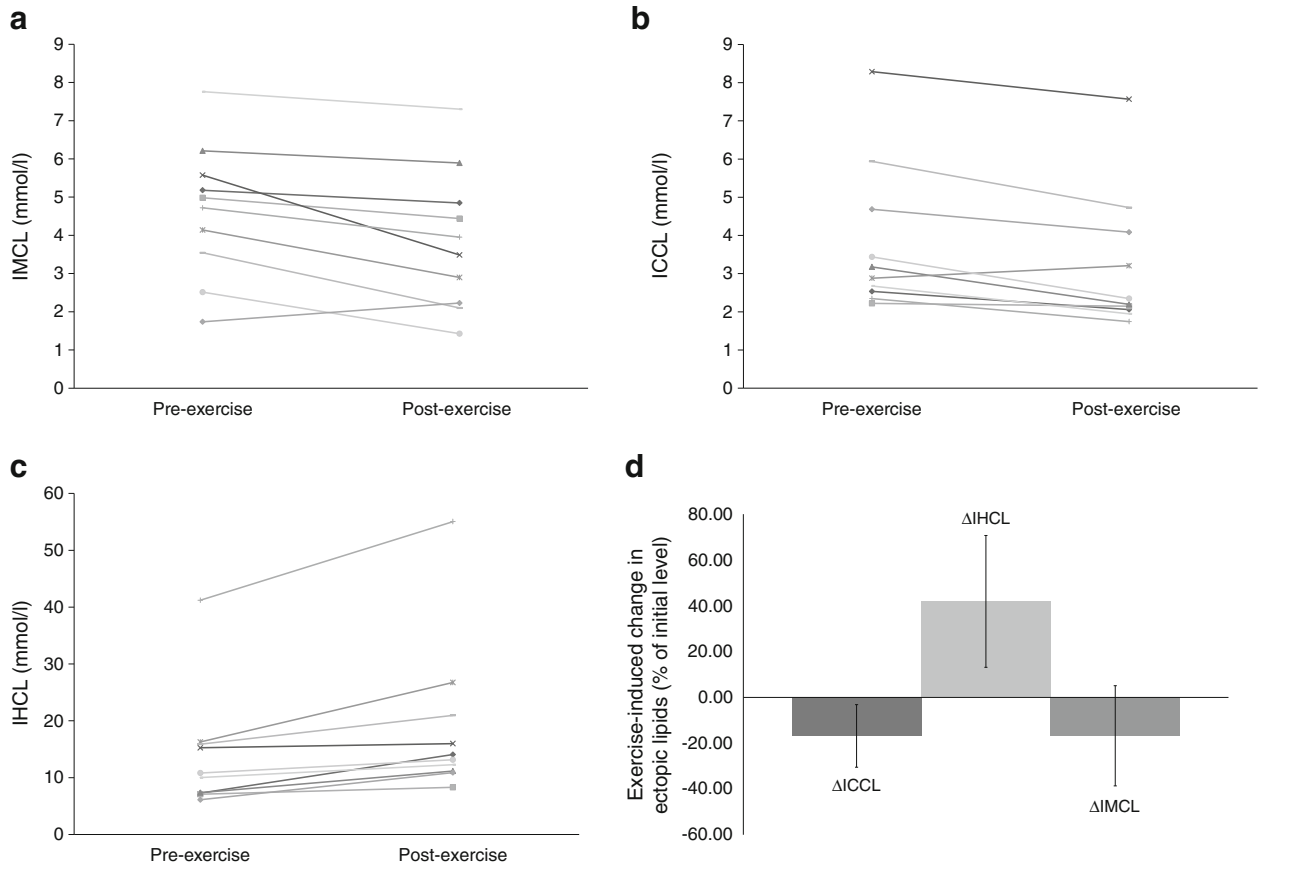

Fig. 1 Individual and mean data of exercise-induced changes in IMCL (**a**), ICCL (**b**) and IHCL (**c**). Pre- and post-exercise refer to the investigations before and after the 2 h physical exercise session at an intensity of 50–60% of $\dot{V}O_{2max}$. (**d**) Mean exercise-induced changes in ectopic lipids (in % of initial levels), with error bars showing ±1 SD

and, with regard to exercise capacity, homogenous group was investigated.

In our study, a 2 h aerobic exercise session led to a significant increase in IHCL, validating earlier preliminary results [10]. A short-term starvation period (48 h), which results in a similar increase in systemic NEFA concentrations to that shown in our study, leads to an increase in IHCL, in keeping with our findings [11].

Several studies have shown that insulin resistance is related to IHCL [12]. However, exercise-induced ΔIHCL has not been shown to be related to insulin sensitivity [10], consistent with the data presented. It may be that the relationship between IHCL and insulin action is not always straightforward, as suggested by recent data generated in genetically modified mouse models and in clinical studies [13].

Previous studies not involving exercise have shown that IHCL levels are related to fat mass, in particular VAT [12, 13], as also evidenced in this study (data not shown). Interestingly, there was a strong trend towards a positive correlation between NEFA exposure and ΔIHCL during exercise, indicating that the exercise-induced increase in lipolysis outweighs the transport capacity in peripheral tissues to take up NEFA, resulting in a rise in systemic NEFA. We can speculate that the superfluous, systemically available NEFA are taken up by the liver and re-esterified into triacylglycerol, leading to the observed increase in IHCL. This hypothesis is supported by a study in humans using stable isotope techniques and liver biopsies, which showed that nearly 60% of hepatic triacylglycerol arises from NEFA [14].

ΔIHCL was not significantly related to exercise capacity, consistent with previous data [10]. This is not a surprise if we assume that the liver only acts as a sink to store superfluous NEFA.

A major strength of the present study is the concomitant assessment of all ectopic lipids in a homogenous group of volunteers. Furthermore, potential influencing factors on ectopic lipids (fat availability, exercise capacity, insulin sensitivity) were measured in parallel. However, this study also has limitations: the advantage of having a homogenous group was offset by the fact that the range of the different variables of exercise capacity, insulin sensitivity and fat availability was small. It is, therefore, still conceivable that these factors play a role in a more heterogeneous group. We also acknowledge that the findings only apply to the specific workload intensity chosen.

In conclusion, in healthy male volunteers, a single session of aerobic exercise impacts on ectopic lipids, with a decrease in IMCL and ICCL (in working tissues) and an increase in

IHCL (fuel store), indicating that all ectopic lipids are flexible lipid depots. Fat availability may play a specific role in regulating ΔIHCL; in contrast, insulin sensitivity, fat availability and exercise capacity do not seem to influence changes in ICCL and IMCL significantly in this group of volunteers.

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