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THE INFLUENCE OF SKELETAL MUSCLE PROPERTIES, PHYSICAL ACTIVITY AND PHYSICAL FITNESS ON SERUM LIPIDS AND THE RISK OF CORONARY HEART DISEASE

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Academic Dissertation

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No aphorism is more frequently repeated than that we must ask Nature a few questions, or ideally, one question, at a time. I am convinced that this view is wholly mistaken. Nature will best respond to a logically thought out questionnaire. Indeed, if we ask Nature a single question, she will often refuse to answer until some other topic has been discussed.

Fisher RA. (1926) J Min Agr Gt Brit : 33, 503.

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications:

I Tikkanen HO, Härkönen M, Näveri H, Hämäläinen E, Elovainio R, Sarna S, Frick H.
Relationship of skeletal muscle fiber type to serum high density lipoprotein cholesterol and apolipoprotein A-I levels.
Atherosclerosis 1991; 90: 49-57.

II Tikkanen HO, Näveri H, Härkönen M
Skeletal muscle distribution influences serum high-density lipoprotein cholesterol level
Atherosclerosis 1996; 120: 1-5.

III Tikkanen HO, Näveri HK, Härkönen M
Alteration of regulatory enzyme activities in fast twitch and slow twitch muscles and muscle fibers in low-intensity endurance-trained rat.
Eur J Appl Physiol 1995; 71: 1-7.

IV Tikkanen HO, Hämäläinen E, Sarna S, Adlercreutz H and Härkönen MH
Associations between skeletal muscle properties, physical fitness, physical activity and coronary heart disease risk factors in men.
Atherosclerosis 1998;137: 377-389.

V Tikkanen HO, Hämäläinen E and Härkönen MH
Significance of skeletal muscle properties on fitness, long-term physical training and serum lipids.
Atherosclerosis 1999;142: 367-378.

VI Kujala UM, Sarna S, Kaprio J, Tikkanen HO, Koskenvuo M
Natural selection to sports, later physical activity habits and coronary heart disease.
Br J Sports Med 2000; 34: 445 - 449.

The articles are referred to in the text by their Roman numerals, I to VI. Some hitherto unpublished data which is presented and discussed is referred to as VII.

ABBREVIATIONS

ACOVA	analysis of covariance
ADP	adenosine diphosphate
AMP	adenosine monophosphate
ANOVA	analysis of variance
Apo A-I	apolipoprotein A-I
ATP	adenosine triphosphate
ATPase	myofibrillar actomyosin adenosine triphosphatase
BA	bile acids
BMI	body mass index
BP	blood pressure
BSA	bovine serum albumin
C	cholesterol
CE	cholesterol ester
CETP	cholesterol ester transfer protein
CHD	coronary heart disease
CI	confidence interval
CM	chylomicrons
CMR	chylomicron remnant
CPT	carnitinepalmitoyl transferase
DHEAS	dehydroepiandrosterone sulphate
DM2	type 2 diabetes mellitus
DTT	dithiothreitol
E2	estradiol
EDTA	ethylenediaminotetra-acetic acid
FA	fatty acid
F-Ac-CoA	fatty acyl-CoA
FC	free cholesterol
FT	fast twitch
HDL	high-density lipoprotein
HL	hepatic lipase
HSL	hormone sensitive lipase
IDL	intermediate density lipoprotein
KGDH	ketoglutarate dehydrogenase
LCAT	lecitin:cholesterol acyltransferase
LDL	low-density lipoprotein
LH	luteinizing hormone
LPL	lipoprotein lipase
LTPA	leisure-time physical activity
MET	metabolic equivalent of oxygen consumption
MHC	myosin heavy chain
mRNA	messenger ribonucleic acid
N-HDL	nascent HDL
NAD	nicotinamide adenine dinucleotide
OBLA	onset of blood lactate concentration
PDH	pyruvate dehydrogenase
PFK	phosphofructokinase
SEM	standard error of mean
SHBG	sex hormone binding globulin
SD	standard deviation
ST	slow twitch
ST-%	percentage of slow-twitch fibers
TET	trunk extension torque
TG	triacylglycerides
VLDL	very low density lipoprotein
VO_{2max}	maximal oxygen consumption

INTRODUCTION

The human body is designed for mobility and the instruments of movement are the skeletal muscles that are composed of muscle fibers with different metabolic and functional profiles. Type I or slow-twitch (ST) fibers have a high capacity for oxidative energy metabolism and especially fatty acid oxidation, whereas type II or fast-twitch (FT) fibers have a high capacity for glycolytic energy production. In the population, the mean value of the percentage of ST fibers (ST-%) in the vastus lateralis muscle of the thigh is close to 50 %. However, the power and speed athletes' ST-% may be as low as 10 %, whereas endurance athletes may have 90 % ST-fibers. In man, skeletal muscles constitute approximately 40% of the total body weight and they make a considerable contribution to the glucose and lipid metabolism of the resting body. Moreover, skeletal muscles may increase their oxidative activity to several times that of their resting level. Thus, the ST-% has an influence on the physical performance of an individual, but it may have significant health-related implications as well. The low incidence of coronary heart disease (CHD) found in physically active, fit people has been attributed to their high serum high-density lipoprotein cholesterol (HDL-C) that is shown to protect against the development of CHD. These individuals may also have some other metabolic factors that are related to the risk of CHD, like endogenous sex hormones, indicators of body fat, and fasting serum insulin concentration. Although observational studies regularly report that the physical activity, especially vigorous exercise in leisure time, protects against CHD, it is possible that inherited characteristics of the skeletal muscle like ST-% may influence these results. These findings have aroused some questions. Is there a selective process in operation that might render individuals both capable of achieving high levels of physical activity and fitness and endowing them with a metabolism with a favorable serum lipid profile? Recent findings that physical activity gives protection against CHD by modifying risk factors, weaken, but do not entirely negate the argument for a selection process. The present study was undertaken to explore the role that skeletal muscle properties might have in the modification of the risk of CHD. This was accomplished by **(i)** studying men with different levels of physical fitness and activity as well as men with defined CHD, **(ii)** by investigating what effects long-term physical activity has on skeletal muscle metabolism and health-related fitness, **(iii)** by studying what impact skeletal muscle properties have on physical fitness and activity, **(iv)** by studying former athletes and their participation in specific types of sports, their continuity of physical activity and the occurrence of CHD among them.

REVIEW OF THE LITERATURE

1. SKELETAL MUSCLE

A unique characteristic of the skeletal muscle is its diversity due to its design, i.e. its fiber composition and the heterogeneity of the individual muscle fibers as well as its design of metabolic adaptations due to physical activity. The skeletal muscles representing 35-45 % of body mass, play a central role in the whole-body energy metabolism, contributing approximately 20 % to the total energy expenditure in man (Zurlo et al. 1990). Vigorously working skeletal muscles may increase their oxidative activity to more than 50 times that of the resting level (Åstrand & Rodahl 1986). The skeletal muscles also possess a remarkable capacity to adapt to the changes in metabolic demand (Williams & Neufer 1996). Interest in the studies of human skeletal muscle adaptation has mainly focused on the effects of exercise training on human athletic performance (Saltin & Gollnick 1983), but recently, the studies on skeletal muscle metabolism in activity and inactivity has raised health-related attention as well (Rogers & Evans 1993, Cartee 1994, Passett Jr 1994).

1.1. Fast- and Slow-Twitch muscle fibers

The existence of two main fiber types was recognised in 1874, when the German physiologist Ranvier reported that (in animals) muscles which were slow in contracting appeared red, whereas fast-contracting muscles appeared white. A breakthrough in the delineation of the fiber types resulted from the combination of histological and physiological methods (Henneman & Olson 1965). The development of the percutaneous needle biopsy technique (Bergström 1962) permitted a rather untraumatic (Gerard et al. 1984) and suitable method to investigate human muscles.

The muscle fibers can be classified on the basis of myofibrillar actomyosin adenosine triphosphatase (ATPase) activity (Brooke & Kaiser 1970). Myosin, the major myofibrillar protein of the thick filament, influences the rate of tension and fatigue development during muscle contraction. The activity of ATPase in myosin correlates with the speed of muscle fiber shortening (Barany 1967). The observation that fast and slow myosins have different alkaline and acid stability formed the basis for ATPase-based fiber type delineation (Padykula & Herman 1955). The histochemically fast (fast-twitch, FT) fibers display high ATPase activity under alkaline staining conditions and low activity under acid staining conditions (alkaline-stable, acid-labile), whereas the slow (slow-twitch, ST) fibers exhibit the inverse (alkaline-labile, acid-stable) (Guth & Samaha 1970). The histochemical differences of the two types of muscle fibers correspond to the differences in contractile properties (Barnard et al. 1971, Burke et al. 1971). The fiber types are distributed in an apparently random or

mosaic pattern, and one motor unit (i.e. muscle fibers supplied by axonal branches of a single neuron) is histochemically homogenous (Burke et al. 1971, Edström & Kugelberg 1968). (Table 1)

Table 1. The main characteristics of the two major human skeletal muscle fibers.

Characteristics	ST fibers	FT fibers
Speed of contraction	Slow	Fast
Relaxation time	Long	Short
Myosin ATPase	Low	High
Lipid content	High	Low
Glycogen content	Low	High
ATP content	Same	Same
Creatine phosphate content	Same	Same
Mitochondrial content	High	Low
Capillary density	High	Low
Creatine kinase activity	Low	High
Glycogenolysis	Low	High
Krebs cycle enzymes	High	Low
Anaerobic capacity	Low	High
Efficiency	High	Low
Number of fibers in motor unit	Low	High

Different types of muscle fibers are distinguished on the bases of histochemical reactions for enzymes of their energy metabolism (Barnard et al. 1971, Peter et al. 1972). The ST fibers are generally characterised by high mitochondrial oxidative enzyme activity, whereas the FT fibers show less oxidative potential but a high glycolytic potential. The FT fibers may be divided in to FTa and FTb fibers as the former show both oxidative and glycolytic potential while the latter have primarily glycolytic potential (Brooke and Kaiser 1970). The development of a technique for single fiber dissection made it possible to determine enzyme activity profiles microbiochemically in the histochemically typed FT and ST muscle fibers (Essen et al. 1975, Saltin et al. 1977). In this method, a muscle sample is freeze-dried and individual muscle fibers are separated under a stereomicroscope, stained for ATPase to identify ST and FT fibers and analysed for enzyme activity. On the basis of this type of analysis, myosin-based functional and contractile properties seemed to correlate with metabolic profiles (Saltin et al. 1977). The activities of the glycolytic enzymes in the muscle fibers seem to differ markedly from each other (Essen et al. 1975, Henriksson & Reitman 1976, Spamer & Pette 1977, Lowry et al. 1978, Spamer & Pette 1979, Hintz et al. 1980, Essen-Gustavsson & Henriksson 1984, Hintz et al. 1984), but overlapping in the oxidative enzyme activities between the ST and the FT fibers has been observed in some

mammalian (e.g. rat, guinea pig) muscles (Lowry et al. 1978, Hintz et al. 1980, Lowry et al. 1980, Nemeth & Pette 1981, Hintz et al. 1984. In contrast to other mammals, the ST in human muscles fibers have substantially higher oxidative capacities and exhibit higher activity levels of enzymes representing aerobic oxidative pathways than the FT muscle fibers (Essen et al. 1975, Lowry et al. 1978, Saltin & Gollnick 1983, Pette & Staron 1990). The ST and the FT fibers with widely different metabolic profiles are dispersed throughout the muscle, and the distribution of the muscle fiber types is heterogeneous. The percentage of histochemically typed ST fibers (ST-%) in the vastus lateralis muscle of the thigh (the most commonly studied muscle in man) varies among individuals from 10 to 90 % with a mean value in the population usually close to 50 % (Saltin & Gollnick 1983).

1.2. Skeletal muscle metabolism

The skeletal muscles are able to use fatty acids, carbohydrates, ketone bodies and some amino acids as substrates to fulfil their energy requirements during rest and exercise (Newholme & Leech 1990). Under normal conditions, fatty acids and glucose are quantitatively the most important oxidizable substrates for muscle cells. In humans, both fat and carbohydrate is stored. The carbohydrate stores (muscle and liver glycogen, plasma glucose) are small, totally ca. 8000 kj (2000 kcal), when compared the amount of energy, ca 450000 kJ (110 000 kcal), stored as fat (intramuscular and adipose tissue) in an average body composition of an 80 kg man (Newholme & Leech 1990).

Glucose from glycogen in the liver has to be transported by the blood and taken up by the muscle before it can undergo glycolysis and be released as lactate, alanine or pyruvate, or be oxidized in the Krebs cycle. Glycogen stored in the skeletal muscle can undergo glycogenolysis, and hence directly glycolysis, and used for fuel in contractile processes. The most important physiological stimulator of the muscle glucose uptake is insulin, which influences on glucose transport into the muscle cells and enhances glycogen synthesis of the muscle in the fed state. During hyperinsulinemia and euglycemia, most glucose disposal occurs in the skeletal muscle (Yki-Järvinen et al. 1987b), skeletal muscle lipid oxidation is nearly entirely suppressed and glucose becomes the primary oxidative substrate (Yki-Järvinen et al. 1987a, Kelly et al. 1990). A decreased muscular activity causes various degrees of impairment of insulin action (insulin resistance) (Stuart et al. 1988, Richter et al. 1989, Mikines et al. 1991), whereas an increased activity level augments insulin action (Rodnick et al. 1987, Mikines et al. 1989). A greater response of insulin-stimulated glucose uptake has been observed in the ST versus the FT fibers (Kriketos et al. 1996). In addition, the glucose transporter protein GLUT-4 is more abundantly expressed in the ST fibers than in FT fibers (Gaster et al. 2000). Thus, skeletal muscle fiber type

(Lillioja et al. 1987) and oxidative capacity (Simoneau & Kelley 1997) may play a genetically determined and/or an environmentally modified role in the development of insulin resistance.

Lipids are the predominant fuel at rest in the postabsorptive and fasted state when the muscle glucose utilisation is low. The capacity of the muscle fibers to synthesise fatty acid (FA) de novo is limited. However, over 50 % of the energy requirement of resting muscle is derived from their oxidation and thus, FAs have to be supplied first from extracellular sources (van der Vusse & Reneman 1996). Fat is made available to the skeletal muscle cells via the blood as plasma nonesterified FAs liberated from the adipose tissue triacylglycerol (TG) storage after lipolysis or as TGs that form the lipid core of circulating TG-rich lipoproteins, very-low-density lipoprotein (VLDL) and chylomicrons (CM). From TG-rich lipoproteins, FAs are made available to the skeletal muscle after hydrolysis by enzyme lipoprotein lipase (LPL) attached to the luminal surface of the endothelial cells in the capillary bed of the skeletal muscle (Braun & Severson 1992). The gene expressing the LPL enzyme appears to be located in the muscle cells (Camps et al. 1990), and, when needed, LPL is sent to the capillary beds in search of a substrate by the same signal, which presumably activates intramuscular TG hydrolysing hormone-sensitive lipase (HSL) (Oscai et al. 1990). Intramuscular TGs accumulated in lipid droplets in the cytoplasm of skeletal muscle, especially in the ST fibers (Essen 1977), represent the major intracellular source of FAs, and contain more energy than the intramuscular carbohydrate pool (van der Vusse & Reneman 1996). The LPL activity in the muscles consisting predominantly of ST fibers is considerably higher (Linder et al. 1976, Hamilton et al. 1998), and they exhibit a greater capacity for both transporting long-chain fatty acids like palmitate into skeletal muscle cells and oxidizing them than muscles consisting predominantly of FT fibers (Dyck et al. 1997, Bonen et al. 1998).

Approximately 50 % of the FAs liberated from serum TGs are immediately extracted by the skeletal muscle cells (van der Vusse & Reneman 1996). In the resting muscle, most of the lipid oxidation is provided by exogenous FAs entering the cells (Dyck et al. 1997). In the fasted state, the LPL activity in skeletal muscle exceeds that in adipose tissue lipase. Fasting lowers the activity of lipase in adipose tissue but does not affect or even increase the LPL activity in the skeletal muscle (Nikkilä et al. 1963, Linder et al. 1976, Sugden & Holness 1993). These observations suggest that in the fasting state skeletal muscles are more apt to use FAs from the circulating lipoproteins for energy conversion and for intramuscular TG storage than adipose tissue to extract the FAs for storage (van der Vusse & Reneman 1996). Taking into account that the total skeletal muscle mass is on the order of 40 % of body mass, it can be inferred that the skeletal muscles may play an important role in the removal of lipoprotein TGs from blood, and, thus, the skeletal muscle morphology and metabolism

may significantly alter the plasma lipoprotein metabolism.

2. PHYSICAL ACTIVITY AND SKELETAL MUSCLE

2.1. Muscle fiber distribution

The question of whether FT fibers can be switched to ST fibers (or vice versa) is still somewhat controversial. Current data suggest, however, that significant transformations between the two histochemically typed main fiber types do not occur. Electric stimulation (Pette & Vrbova 1992) and cross reinnervation (Edgerton et al. 1996) as artificial tools in animal models have been used to induce changes in contractile properties that are commonly attributed to (partial or complete) transformation of the fiber types. However, when change in some aspects of the muscle fiber phenotypes has been observed, significant changes in the percentage of histochemically defined ST vs. FT fibers have not been observed (Pette & Staron 1990, Demirel et al. 1999).

Two changes in the human life span have been suggested to cause fiber transformation, namely changes in physical activity patterns and ageing. Many of the early histochemical studies found that exercise-training had no effect on the percentage of ST fibers (Barnard et al. 1970, Gollnick et al. 1973, Andersen & Henriksson 1977, Ingjer 1979, Schantz et al. 1982). Whenever change in the ST-% has been reported due to change in physical activity (Howald et al. 1985, Simoneau et al. 1985), the magnitude of change has been within the limits of a sampling error 10 - 15 % (Simoneau & Bouchard 1995), and the number of studied subjects has been low (Jansson et al. 1978, Howald et al. 1985, Larsson & Ansved 1985, Simoneau et al. 1985). Moreover, when the ST-% of highly trained endurance athletes have been reported (Costill et al. 1976a, Jansson & Kaijser 1977, Howald 1982), the difference in the ST-% between athletes and controls in these cross-sectional studies has been far more than the change observed in training studies, usually from 20 to 40 %. When the histochemically classified fibers have had a small percentage shift from fast to slow in some properties of the muscle fibers, the fibers had remained as fast or slow by histochemical analysis (Booth & Thomason 1991, Demirel et al. 1999, O'Neill et al. 1999). Some studies using biopsy samples provided evidence for a decline in the percentage of FT fibers (and a reciprocal increase in the ST-%) with ageing, but studies using larger sample sizes have not supported these results (Lexell et al. 1988, Rogers & Evans 1993). Evidently, with the training, endurance or resistance type, and regardless of age, the ST-% is unaltered although transformations occur within the FT fiber subtypes (Pette & Staron 1990, Rogers & Evans 1993, Cartee 1994). Thus, the current data suggest that it is unlikely that different types of exercise training or ageing would induce significant FT to ST transitions as a result of normal ambulatory

physical activity or inactivity in the human skeletal muscles (Fitts & Widrick 1996, Harridge 1996).

2.2. Skeletal muscle metabolism during exercise

During exercise the increased energy requirements are met by an increase in both carbohydrate (glucose and glycogen) and fat (fatty acid) oxidation (Newsholme & Leech 1990, Wolfe 1998). The FAs as energy sources for working muscle provide more ATP per molecule than glucose (Åstrand & Rodahl 1986). However, to produce the same amount of ATP, the oxidation of FAs requires more oxygen than the oxidation of carbohydrates, and per unit of time more ATP can be derived from carbohydrates than from the oxidation of FAs (Åstrand & Rodahl 1986, Newholme & Leech 1990). It is obvious that skeletal muscle cells need metabolic regulation when they use available energy sources in an integrated manner at rest, during exercise and during recovery from exercise. The percentage contribution of glucose, FAs and glycogen to oxygen consumption of working muscle is dependent on the type of exercise, its intensity and duration (Newsholme & Leech 1990). The proportion of oxidation of FAs increases as the intensity of exercise increases from low to moderate but decreases as the intensity exceeds 65 % of VO_{2max}, and the muscle uses preferentially carbohydrates at high intensity levels of exercise (Romijin et al. 1993). However, during exercise lipids remain important substrates for the muscle cells when the intensity of exercise does not exceed 80 - 90 % of VO_{2max}, and human subjects exercising at a rate as high as 85 % of VO_2max are still oxidising FAs at a rate 5 - 6 fold above that seen in the resting condition (Romijin et al. 1993).

In the proposed glucose-fatty-acid cycle (Randle et al. 1963, Randle et al. 1964), skeletal muscles possess mechanisms that allow carbohydrate and fat as oxidative energy sources to shift depending primarily on the availability of FAs. An increase in availability and greater oxidation of FAs leads to decreased utilisation of glycogen or extracellular glucose or both because the enzyme activities of phosphofructokinase (PFK) and pyruvate dehydrogenase (PDH) are inhibited by an accumulation of citrate and acetyl-CoA, respectively (Randle et al. 1963, Newsholme & Leech 1990). Several other levels of control mechanisms may exist for determining the selection of either fat or carbohydrates as the prime fuel in the skeletal muscle fibers (Richter 1996, van der Vusse & Reneman 1996, McGarry 1998, Wolfe 1998). Recently, some have suggested that the fuel selection and regulation operate by the action of malonyl-CoA that modulates the use of FAs (Ruderman et al. 1999, Båvenholm et al. 2000). Malonyl-CoA exerts an inhibitory action on the mitochondrial carnitine palmitoyltransferase I (CPT I) enzyme responsible for the conversion of fatty acyl-CoA derived from FAs from the blood stream or intramuscular TGs (van der Vusse & Reneman 1996) (Figure 1).

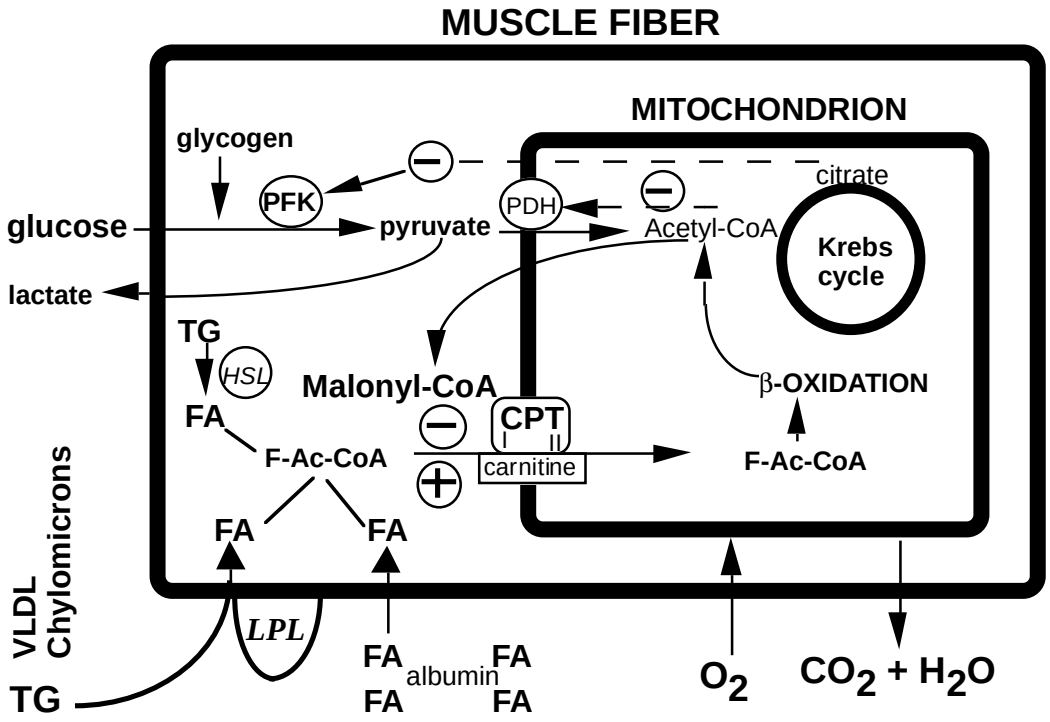

Figure 1. Features of the glucose fatty acid cycle and the "reverse fatty acid cycle". Fatty acids (FA) are taken up from the plasma either as free FAs bound to albumin or by the action of lipoprotein lipase (LPL) or from intramuscular TG by hormone sensitive lipase (HSL). The FAs are transported to the mitochondria by the CPT system, where they undergo B-oxidation to produce acetyl-CoA that enters the Krebs Cycle. Accumulation of acetyl-CoA and citrate inhibits pyruvate dehydrogenase (PDH) and phosphofructokinase (PFK), respectively. This results in a reduced glucose uptake when FA oxidation is increased. In the reverse glucose fatty acid cycle malonyl-CoA derived from acetyl-CoA inhibits the activity of CPT I when carbohydrate oxidation is increased.

The CPT enzyme system has a rate-limiting nature in governing the rate of FA oxidation in the mitochondrial level during acute exercise and after adaptation to exercise training (Mole et al. 1971, Newsholme 1980, Newsholme 1984). This malonyl-CoA/CPT I system also seems to be involved in the "reverse glucose fatty acid cycle" (Siddossis & Wolfe 1996, Jequier 1998), a corollary of the glucose-fatty acid cycle, in which increased glucose oxidation and decreased lipid oxidation results from hyperglycemia (Mandarino et al. 1993). Fatty acyl carnitine moves across the inner mitochondrial membrane, fatty acyl-CoA is regenerated, and carnitine is released by CPT II. Thereafter, fatty acyl-CoA can undergo β-oxidation and acetyl-CoA is formed for oxidation in the citric acid cycle. In the postabsorptive state or during exercise, an enhanced supply of FAs to skeletal muscle mitochondria is thought to mitigate the inhibitory effect of malonyl-CoA on FA oxidation because increased levels of fatty acyl CoA render CPT I less sensitive to malonyl-CoA (Ruderman et al. 1999). Thus, FA oxidation increases with a concomitant increment in both citrate and the acetyl-CoA/CoA ratio, resulting in the inactivation of PFK and PDH, and ultimately the depression of glucose utilisation according to the classic glucose-fatty acid cycle (van der Vusse & Reneman 1996). When carbohydrates become the primary oxidative

substrate as in high-intensity exercise (Gollnick 1985) or in hyperinsulinemia (Yki-Järvinen et al. 1987a), increased pyruvate availability from glycolysis results in the increased formation of malonyl-CoA (Ruderman et al. 1999), accelerated glucose metabolism inhibits FA oxidation resulting in a "reverse glucose fatty acid cycle". In addition, a decrease in pH associated with an increase in glycolytic flux decreases CPT activity which decreases FA oxidation (Starritt et al. 2000).

Fatty acids are the most important energy source for skeletal muscle, particularly during exercise of mild-moderate and prolonged duration (Martin III 1996) and especially in low intensity exercise (Romijin et al. 1993). Fatty acids have an inhibitory effect on glucose uptake and glycogen utilisation and during exercise this occurs especially in the ST-fiber rich skeletal muscles and in oxidative fibers (Rennie & Holloszy 1977, Kiens et al. 1993, van der Vusse & Reneman 1996). Fatty acids are also derived from the hydrolysis of intramuscular TG by HSL that is activated by a muscle contraction-induced mechanism similar to that which activates glycogen phosphorylase and glycogen depletion (Langfort et al. 2000). Contribution from FAs derived by hydrolysis of TG-rich lipoproteins by LPL in capillary beds of skeletal muscle has gained interest (Kiens et al. 1993, van der Vusse & Reneman 1996), and in the postprandial state high concentrations of TG-rich lipoproteins represent a potential source of FAs for contracting muscle (Hardman 1998). In addition, after exercise the concentration of malonyl-CoA in skeletal muscle remains depressed (Winder 1998) and thus, fat oxidation is elevated for relatively prolonged periods after a single bout of exercise (Rasmussen et al. 1998). This permits FAs from muscle triacylglycerols, and probably from TG-rich lipoproteins as well, to be used as an energy source during the recovery when glycogen resynthesis has high metabolic priority, and glycogen is being replenished (Kiens & Richter 1998).

Adaptive changes in skeletal muscles depend on the type of training and muscular activity (Holloszy & Coyle 1984). Endurance-type training, but not weight training (Tesch 1992, Kraemer et al. 1996), has been shown to increase enzymes involved with oxidative metabolism, and with endurance training, fat is used to a greater extent than carbohydrate at the same absolute exercise intensity (Saltin & Gollnick 1983, Holloszy & Coyle 1984, Brooks & Mercier 199). Glycolytic enzymes like PFK have been shown to be unaffected by heavy-resistance exercise training (Kraemer et al. 1996) and unaffected or decreased after endurance-type training (Henriksson & Reitman 1976, Saltin & Gollnick 1983, Blomstrand et al. 1986). Thus, endurance training results in a greater FA oxidation (Mole et al. 1971) and in a reduced rate of glycogen breakdown, glycolysis and lactate formation (Saltin & Gollnick 1983, Holloszy & Coyle 1984, Brooks & Mercier 1994). In addition, with training attenuated decrease in pH associated with a decrease in glycolytic flux allows CPT to remain active thus increasing FA oxidation (Starritt et al. 2000). This shift in metabo-

lism to greater oxidation of fat and concomitant sparing of the utilisation of glycogen stores is a contributing factor to the enhanced working capacity after endurance training. This shift to fat metabolism is even more pronounced than the change in VO_{2max} (Gollnick 1985). Although the LPL activity (Linder et al. 1976) and oxidative energy conversions of FAs (Okano & Shimojo 1982) in muscles with ST fiber predominance exceed that of muscles consisting predominantly of FT fibers, training can result in an increase in these activities in muscles with FT predominance to the level observed in muscles with high ST-% (Hamilton et al. 1998). In addition, the capillary supply of the vastus lateralis of the quadriceps femoris muscle in man increases with endurance training (Ingjer 1978) and the muscle blood flow also in resting state (fasting, nonexercised) is higher than in sedentary people (Ebeling et al. 1993).

2.3. The influence of skeletal muscle on physical fitness and activity

The plasticity of skeletal muscle energy metabolism is partly possible because of the mosaic pattern of different types of muscle fibers in skeletal muscle (Booth & Baldwin 1996). Glycogen depletion, an index of muscle fiber usage during exercise (Gollnick et al. 1974), shows selective recruitment of the muscle fibers during different types of exercises (Connet & Sahlin 1996). During light to moderate exercise, glycogen depletion and, thus fiber recruitment, is more pronounced in the ST fibers than in the FT fibers (Gollnick et al. 1974). Muscles with a high percentage of FT fibers generate a greater torque and higher power at a given velocity than those with predominantly ST fibers (Fitts & Widrick 1996). The ST fibers are designated for slow tonic activities such as the maintenance of posture (Saltin & Gollnick 1983), and they have a lower oxygen consumption while performing exercise at a given power output (Coyle et al. 1992). Therefore, when contractions are performed isometrically or at relatively slow velocities, the ST muscle fibers have been observed to be more efficient than the FT fibers (Rall 1985, Kushmerick 1987). Muscle efficiency also varies greatly in the highly trained athletes, and most of this variability is related to differences in their ST-% (Coyle 1995). In the studies of the physiological characteristics of elite runners during the 70's, Costill and co-workers observed both great aerobic power and a preponderance of ST fibers as well as high oxidative enzyme activities in the skeletal muscles of these athletes (Costill et al. 1976a). These studies also indicated that the muscle enzyme activities and the ST-% were quantitatively related to the distance performance (Costill et al. 1976b). When the results of these elite athletes were combined with the results of well-trained men with a lower quality of performance and maximal aerobic power, selection rather than that of training was concluded to be the reason for the invariably high values of maximal aerobic power noted in the elite runners (Foster et al. 1978). Performance was moderately correlated with ST-%, and there was a relationship between overall performance quality and

ST-% among different populations of runners (Foster et al. 1978). Thus, the characteristic values for ST-% reported in top-class sprinters, middle-distance runners and marathon runners (Gollnick et al. 1972, Costill et al. 1976a, Costill et al. 1976b) suggested that ST-% is related to one's aptitude for a particular sport event (Foster et al. 1978). Some other findings support the idea that the high ST-% is the result of the selection of individuals with suitable ST-% for the suitable sport rather than being an effect of training. Adult dancers have ST-% in their vastus lateralis muscle is similar to those of endurance trained athletes, and the ST-% of young dancers is higher than that of the average individual of the same age (Dahlström et al. 1996). The ST-% has been observed to correlate positively with the attitude to cross-country running, with the degree of physical activity and with the 9-min run performance in teenagers (Jansson & Hedberg 1991). Therefore, those persons who have a high ST-% have a natural endowment towards long-term endurance-type physical activity.

3. PHYSICAL ACTIVITY, FITNESS AND CARDIOVASCULAR HEALTH

Physical activity encompasses all forms of bodily movements, whether undertaken voluntarily (exercise), unavoidably (occupational and domestic chores), or deliberately, for example, the adoption of an active lifestyle. *Leisure-time physical activity* (LTPA) is a form of exercise that is usually performed on a repeated basis over an extended time (exercise training) with a specific external object such as improvement of fitness, physical performance, or health. *Physical fitness* is, in a very broad sense, determined by the individual's capacity for optimal work, and motor and sport performance (Åstrand & Rodahl 1986). *Health-related fitness* refers to those components of fitness that relate to the health status of the individual, and that may be influenced by regular physical activity (Bouchard & Shephard 1994). It is also defined as the states of physical and physiological characteristics that define risk levels for the premature development of several diseases or morbid conditions, where, on the other hand, these diseases or conditions are related to a sedentary lifestyle. Fitness is a physiologic attribute, which can be measured more accurately than physical activity, which is a behaviour. The level of fitness can be modified by physical activity over time but fitness, on the other hand, limits the quantity and quality of physical activity that may be performed. Inherited differences are likely to be involved in determining the health status of a person and influence on individual components of the physical activity-fitness-health paradigm.

3.1. Physical activity, fitness and CHD

Coronary heart disease (CHD) is still the leading cause of mortality in Western countries and the lifetime risk of developing CHD is one in two for men at age 40 (Lloyd-Jones et al. 1999). Participation in leisure physical activity has gained increasing

interest because physical inactivity as well as a low level of physical fitness has been identified as being independent predictors of cardiovascular disease and especially CHD (Powell et al. 1987, Berlin & Colditz 1990, Blair 1994, Lee & Paffenbarger Jr 1996). It has been estimated that there is an approximate doubling of CHD risk among inactive persons when they are compared with their active peers (Berlin & Colditz 1990). However, the review of national level surveys in many of the Western countries indicates that 5 to 15 % of the adult population might be called aerobically active, that is, engaging in vigorous activities during their leisure-time at least three occasions weekly for 20 to 30 min or more per occasion (Stephens & Caspersen 1994).

Cardiorespiratory fitness measured in exercise testing is a powerful predictor of cardiac events and CHD (Table 2). A low fitness level is associated with an increased risk for CHD (Peters et al. 1983, Ekelund et al. 1988, Farrell 1998), and a high fitness is associated with a decreased risk and mortality from CHD (Lie et al. 1985). In healthy men, the fitness level more than the physical activity pattern alone seems to be an independent protective factor against CHD (Sobolski et al. 1987). In some studies, no independent effect of physical activity has been observed on cardiovascular mortality after accounting for physical fitness (Slattery & Jacobs Jr 1988, Sandvik et al. 1993). A low level of fitness has been shown to be associated with an increased clustering of the metabolic abnormalities such as atherogenic dyslipidemia and glucose intolerance associated with the metabolic syndrome (Whaley et al. 1999, Carroll et al. 2000), and this finding connects a low fitness level with several adverse health outcomes including CHD (Blair et al. 1989, Blair et al. 1996, Roger et al. 1998).

Also the health benefits of leanness are limited to fit men (Lee et al. 1999). Because most of the studies indicate a dose-response effect of fitness (Lee & Paffenbarger Jr 1996), and a marked reduction in age-standardised cardiovascular mortality rates has been observed in initially sedentary or unfit men who become more physically active and improved their fitness (Blair et al. 1995, Erikssen et al. 1998), the conclusion has been drawn that the benefit of physical activity is mediated particularly by changes in physical fitness. The arguments against a causal relationship between level of physical fitness and CHD focus on selection bias that some subjects might self-select themselves into the lower spectrum of physical activity because of disability, occult disease or because of some constitutional characteristics reducing their fitness (Milvy et al. 1977).

Table 2. Some major prospective epidemiological studies of exercise capacity assessed by exercise testing as it relates to mortality in apparently healthy men.

First author and year	Follow-up (mean in years)	Assessment of fitness	Number of subjects	Conclusion
Peters 1983	4.8	BE	2779	Adjusted RR of 2.2 for a low exercise capacity if other risk factors also present
Lie 1985	7.9	BE	2014 (and 149 athletes)	Higher quintiles of fitness associated with decreased CHD and mortality
Sobolski 1987	5	BE	2363	Fitness level an independent protective factor against CHD
Slattery 1988	5	TM	3043	Middle-aged, unfit men are at greater risk of dying of CHD
Ekelund 1988	8.5	TM	4276	RR of 2.7 for cardiovascular death in men with a low exercise capacity
Blair 1989	8	TM	10 224	Physical fitness inversely related to all-cause mortality
Sandvik 1993	16	BE	1960	Physical fitness graded, independent, long-term predictor of mortality from cardiovascular causes
Blair 1995	5.1	TM Two exercise tests	9777	Improvement in fitness reduces mortality 44 %. Men who maintained or improved physical fitness were less likely to die from CVD.
Erikssen 1998	22	BE Two tests (7 to 10 y apart)	1756	Graded, inverse relation between change in fitness and mortality, irrespective of initial fitness level
Farrell 1998	8.4	TM	25 341	Moderate and high levels of fitness provide some protection from CVD mortality, even in the presence of CVD predictors.
Lee 1999	8	TM	21 925	Unfit men had a higher risk of CVD mortality than fit men in all fat and fat-free mass categories. The health benefits of leanness are limited to fit men.

BE = Bicycle ergrometer, TM = Treadmill, RR = relative risk, CVD = cardiovascular disease

Physical activity required by an occupation was first observed to protect against CHD (Morris et al. 1953, Morris & Crawford 1958, Paffenbarger et al. 1970) and protection against CHD can be achieved by LTPA as well (Morris et al. 1973). Many of the studies (Paffenbarger et al. 1978, Kannel et al. 1986, Leon et al. 1987, Slattery et al. 1989), but not all (Blackburn et al. 1970), have confirmed that low physical activity increases the risk of cardiovascular mortality and, on the other hand, high leisure physical activity decreases the risk (Epstein et al. 1976). More detailed analysis of many of these studies also suggest a dose-response effect of leisure physical activity: the higher the level of physical activity, the lower the risk of CHD (Lee & Paffenbarger Jr 1996). (Table 3)

Table 3. Some of the major epidemiological studies of physical activity in men as it relates to cardiovascular mortality.

Investigator, year, type	Activity assessment	Conclusions
Morris 1953, retrospective	Job description	PA at work is important in relation to CHD and active have less CHD
Morris 1958, retrospective	Determined by social class and occupation	Physical inactivity relates to class and occupation mortality from CHD
Blackburn 1970, prospective	Questionnaire	No difference between physically active and sedentary
Paffenbarger 1970, retrospective	Job description	Low PA level on the job doubles risk of fatal MI
Epstein 1976, prospective	Questionnaire	Vigorous PA at weekend protective
Paffenbarger 1978, prospective	Questionnaire	Low PA increases risk of MI and death
Kannel 1986, retrospective	Questionnaire	Low PA increases risk of cardiac mortality
Leon 1987, prospective	Questionnaire	Low PA increases risk of mortality
Slattery 1989, prospective	Questionnaire	Near 50 % increase of death from CHD in sedentary men
Lee 1995, prospective	Questionnaire	Total EE and EE from vigorous PA, but not non-vigorous PA inversely related to all-cause mortality
Lee 2000, prospective	Questionnaire	Light activities were not, moderate activities somewhat, vigorous activities clearly beneficial

PA = physical activity; MI = myocardial infarction, EE = energy expenditure

Physical activity can be divided by the intensity of the activity into light (e.g. strolling, ≤ 4 kcal/min of energy expenditure), moderate (e.g. walking, < 4-7 kcal/min of energy expenditure) and vigorous (brisk walking, jogging, running, cycling, > 7 kcal/min of energy expenditure) (Morris 1994). Some studies suggest a threshold effect for physical activity in protection against CHD (Leon et al. 1987). When data from five studies, the two studies of British civil servants (Morris et al. 1980, Morris et al. 1990), the study of Finnish men (Lakka et al. 1994), the Harvard Alumni Health Study (Lee et al. 1995, Lee & Paffenbarger 2000) and the US Railroad Study (Slattery et al. 1989), regarding the relative merits of vigorous and nonvigorous (light and moderate) physical activity were assessed, researcher observed that vigorous, but not nonvigorous physical activity, predicted a lower risk of cardiovascular disease. In the study of Finnish men (Lakka et al. 1994), in which fitness was measured by a symptom limited-exercise tolerance test on a bicycle ergometer, and physical activity was assessed using a validated questionnaire, both physical activity, especially conditioning physical activity (intensity of 6 kcal/min/kg) and fitness independently predicted a lower risk of CHD. However, increasing the time spent either in nonconditioning physical activity or walking or cycling to work was not associated with a significant lower risk (Lakka et al. 1994), suggesting that the benefit of physical activity is achieved only at higher physical fitness levels. It is conceivable that only vigorous (jogging, running, cycling, swimming, ball games) physical

activity is associated with lower incidences of CHD, because it is more efficient in improving physical fitness.

The question of a genetic contribution to fitness, as well as one's capacity to change fitness, e.g. to be engaged in physical activity, has been raised (Bouchard et al. 1997). It seems that a selective process might operate, rendering an individual capable of achieving high levels of physical fitness and life-time regular activity as well as averting adverse health outcomes (Noakes & Opie 1976, Lee & Paffenbarger Jr 1996). To evaluate the inheritance factor using data on participation in competitive sports has its limitations, but it is reasonable to conclude that persons who compete in various sports have above-average physical endowments and an enhanced ability to engage successfully in physical activity (Milvy et al. 1977). At first sight, this seems not to be the case because it has been reported that longevity is not affected by past athletic activity, and that previous vigorous exercise is not related to development of CHD (Schnohr 1971, Paffenbarger Jr et al. 1984, Morris et al. 1990). However, different athletic activities seemed to be related to differences in both life-expectancy and risk of CHD (Largey 1972, Polednack 1972, Prout 1972, Karvonen 1974, Sarna et al. 1993, Kujala et al. 1994, Lean & Han 1998). It has been suggested that the confusion surrounding studies of the longevity of athletes may best be explained by recognising the different somatotype (body build) of the athletes involved in various sports (Sheehan 1972, Sheehan 1973). Successful participation in sport activities that require power and speed capability and are not associated with a longer than normal life span (Yamaji & Shephard 1977) depends upon endomesomorphic (fat-muscular) body build (Carter 1970), a somatotype that appears to be associated with susceptibility to death from CHD (Spain et al. 1963, Damon et al. 1969). The first to observe this in athletes were Rook and co-workers, who reported that the prospect of longevity for heavily built athletes (hammer and weight men) was not as good as it was for those more lightly built (rowers, runners) (Rook 1954).

In 1956, two years after their first report (Morris et al. 1953). Morris and co-workers observed that not physical activity solely but self-selection may have influenced their earlier results on London busmen (Morris et al. 1956). In their study, the bus drivers had a higher death rate from CHD and tended to be heavier (larger waist and girth measurement) than bus conductors even at the beginning of their employment (Morris et al. 1956). The drivers also had higher serum cholesterol levels and blood pressure measurements than did conductors (Morris et al. 1966), and in addition, in a subsequent study it was documented that even the recruits for the jobs differed in lipid level and in weight (Oliver 1967).

A rational explanation for these findings is that some individuals are genetically more capable of staying or becoming physically fit or that physical activity is easily

acceptable to them because of some inherited or genetic predisposition. The protective effect of high physical activity carried out only at younger ages tends to disappear at the age of 50 (Schnohr 1971, Paffenbarger Jr et al. 1984, Paffenbarger Jr et al. 1997). Being fit, however, provides no protection against CHD in sedentary men (Hein et al. 1992), and only life-long, relatively intense, regular physical activity seems to reduce risk of CHD (Pomeroy & White 1958, Leon et al. 1997, Sherman et al. 1999).

3.2. Physical activity, fitness and serum lipids and lipoproteins

In observational studies physically active persons usually have more favorable serum lipid and lipoprotein values than age-matched sedentary counterparts (Dufaux et al. 1982, Durstine & Haskell 1994). Yet, many studies have not observed differences in serum total cholesterol or low-density lipoprotein cholesterol (LDL-C) concentrations between physically active and inactive persons (Tran et al. 1983, Durstine & Hasell 1994) but differences have been observed in cross-sectional studies in highly (endurance) trained individuals when compared with sedentary people (Wood et al. 1976, Williams et al. 1986). However, high serum HDL-C and low serum triglyceride levels are commonly observed in regular exercisers (Dufaux et al. 1982). An increase in serum HDL-C especially, observed after exercise training (see Table 4) has been suggested to indicate the protective effects of physical activity against CHD (Dufaux et al. 1982) because the serum HDL-C level is diminished in atherosclerosis (Barr et al. 1951, Nikkilä 1953, Miller & Miller 1975) and both epidemiological and observational studies have demonstrated that serum HDL-C is inversely related to the incidence of CHD (Gordon et al. 1977, Kannel 1983, Castelli 1984, Castelli et al. 1986, Gordon & Rifkind 1989). Serum HDL-C is thought to exert an antiatherogenic effect through its role in "reverse cholesterol transport" (see Figure 2), in which excess free cholesterol in peripheral tissues, including the arterial wall, is incorporated into HDL in plasma, esterified by lecithin:cholesterol acyltransferase (LCAT), transported to the liver and subsequently secreted into bile as cholesterol or bile acids (Hill & McQueen 1997). Because HDL-C has shown to be an independent and powerful predictor of the risk of CHD (Pocock et al. 1989) protection against CHD gained by regular exercise is biologically plausible. Indeed, physical activity reduces risk of CHD particularly in men (Haapanen et al. 1997). Thus, physiologically desirable and potentially effective means for increasing serum HDL-C concentration in men prone to CHD are being sought, and one method among those attracting wide attention today are programs for promotion of physical activity and fitness (Berg et al. 1994).

A high serum level of HDL-C has regularly been observed in endurance trained persons (Wood et al. 1976, Wood et al. 1977, Adner & Castelli 1980, Herbert et al. 1984,

Thompson et al. 1991) but not in individuals who engage primarily in anaerobic type power-speed activities or resistance exercises (Clarkson et al. 1981, Berg et al. 1982, Farrell et al. 1982, Kokkinos & Hurley 1990). Similarly, regular participation in endurance-type of physical activities is associated with lower serum TG concentration (Durstine & Haskell 1994) but not participation in anaerobic type of activities or resistance exercises (Kokkinos & Hurley 1990, Durstine & Haskell 1994). Thus, the type of training as well as the amount and/or intensity of exercise achieved by individuals relates to their serum lipid and lipoprotein levels (Durstine & Haskell 1994). Moreover, the results of many cross-sectional studies have shown a "dose-response" relationship (Kokkinos & Fernhall 1999). As the mileage of running increases (distance or frequency per week, as well as duration of exercise session), serum HDL-C concentration seems to increase (Higuchi et al. 1989, Kokkinos et al. 1995, Williams 1997, Williams 1998) and serum TG and LDL-C decrease (Kokkinos et al. 1995). Even at the extreme end of a continuum, such as represented by well-conditioned, middle-aged marathon runners, serum lipoproteins were related to the degree of fitness (measured by the marathon running time), as HDL-C was higher and LDL-C lower in the fastest when compared with the slowest runners (Ketelhut et al. 1996). Moreover, the fittest runners also showed greater increases in HDL-C after the marathon run (Ketelhut et al. 1996). In addition, when serum LDL-C concentration has been lower in trained persons, the concentration has been inversely related to the distance run each week (Tran et al. 1983, Wood et al. 1983). In the studies in highly active, regular runners both larger volume of training (Williams 1997) and faster running speed in 10-km running (Williams 1998) were associated with significantly higher serum HDL-C levels as well as lower serum LDL-C levels in a dose-response manner.

An issue often arising pertains to the volume of exercise necessary to induce the changes in lipid and lipoprotein profile. Usually prolonged exercise has been shown to elevate HDL-C immediately after exercise as well as in the days following exercise (Enger et al. 1980, Thompson et al. 1980, Durstin et al. 1983, Kantor et al. 1984, Dufaux et al. 1986). Serum lipids and lipoproteins were not changed after an 800-kcal treadmill running session but a 1600-kcal session caused a significant increase in HDL-C concentration (Visich et al. 1996). When two exercise intensities (50 and 75 % of VO_{2max}) were studied in trained runners, and energy expenditure was held constant at 950 kcal per session, no change was observed in HDL-C concentration immediately after or in the days following the exercise (Davis et al. 1992). However, in sedentary men a short duration (30 min) and low-to-moderate-intensity exercise has been reported to increase HDL-C immediately after and 24 hours after exercise (Angelopoulos et al. 1993). In healthy men, the 800-kcal session was able to decrease serum TG concentration, 1100 kcal of energy expenditure was needed to elicit a significant increase in serum HDL-C concentration, whereas a 1300-kcal energy expen-

diture was necessary for a decrease in LDL-C (Ferguson et al. 1998). Thus, considerable amount of exercise, at least in some individuals, is needed to induce significant changes in serum lipids. Moreover, the length of regular training in cross-sectional and observational studies has usually been for several years.

The magnitude of a favorable change in HDL-C level is often related to the amount of exercise performed per week. HDL-C concentration is frequently increased by an exercise regimen that requires 1000 - 1200 kcal of energy expenditure per week (Williams et al. 1982, Superko 1991, Wei et al. 1997). Usually favorable changes in HDL-C appear to reach statistical significance at an energy expenditure of 1200 to 1600 kcal per week (Kokkinos & Fernhall 1999). Epidemiological findings (Leclerc et al. 1985, Lakka & Salonen 1992) suggest a minimum level of exercise intensity for each session with an energy cost of 5 - 6 kcal/min/kg or more as the threshold for favorable changes in HDL-C. Thus, the findings on serum lipids and lipoproteins and especially on serum HDL-C support the epidemiological studies indicating that moderate-to-vigorous physical activities as a form of exercise that is usually performed on a repeated basis over an extended period of time (exercise training) are associated with a reduced risk of CHD (Morris et al. 1980, Slattery et al. 1989, Morris et al. 1990, Lee et al. 1995, Williams 1997, Williams 1998).

3.3. The influence of exercise training trials on serum lipids and lipoproteins

The results from exercise training trials have usually not revealed significant changes in total cholesterol concentration (Durstine & Haskell 1994). In some studies, but not in all (Thompson et al. 1988), LDL-C concentration has decreased in longitudinal endurance-exercise training studies (Wood et al. 1988, Stein et al. 1990), and the change has been inversely related to the distance run each week (Tran et al. 1983, Wood et al. 1983). In addition, an increase in physical activity, that has induced changes in cholesterol or LDL-C levels, has usually resulted concomitantly in weight loss and reductions of body fat (Tran et al. 1983, Wood et al. 1991, Durstine & Haskell 1994). Serum TG concentrations are usually reduced by exercise training when baseline concentrations are elevated (Huttunen et al. 1979, Thompson et al. 1988, Wood et al. 1991), but not always (Mann et al. 1969).

Aerobic training in randomised trials of exercise (Table 4) usually results in changes in HDL-C that are qualitatively similar to those observed in physically active persons or endurance athletes (Tran et al. 1983, Durstine & Haskell 1994) but not always (Juneau et al. 1987, Hellenius et al. 1993, Stensel et al. 1993, Suter et al. 1994). The mean changes induced by exercise training alone, however, are modest in magnitude (Huttunen et al. 1979, Kiens et al. 1980, Baker et al. 1986, Blumenthal et al. 1991) when compared with substantially (20-30 %) higher serum HDL-C values observed

in highly active individuals than in inactive controls in cross-sectional studies (Durstine & Haskell 1994).

Table 4. Randomised trials of training in healthy or hyperlipidemic middle-aged men.

First author and year	Number of subjects , age in years, lipid status, initial fitness level	Description of exercise program (type; intensity; time; sessions; duration,)	Change (%) in fitness and HDL-C ; Comments
Baker 1986	n=45, >50, HL, 31.5	Walking, running; 80 %; 48 min; 3 x wk; 20 wks	16.8 and 16,6 %
Blumenthal 1991	n=97, >60, HL, 19.5	Ergometer, jogging; 84 %; 55 min; 3 x wk; 17 wks	16 and 7 %
Hellenius 1993	n=78, 35 - 60, HL	Walking, jogging; 50 %; 30-40 min; 2-3 x wk; 26 wks	4 % and ns
Houmard 1994	n=20, 40-65, NL, 29.0	Treadmill and walking; 68 %; 30 - 45 min; 4 x wk; 14 wks	21 and 11 %
Huttunen 1979	n=90, 40-45 , HL, 43	Walking and jogging; 64 %; 30 min; 3 x wk; 17 wks	10 and 11 %
Juneau 1987	n=113, mean 48, HL, 31.9	Walking, jogging; 72 %*; 50 min; 5 x wk; 24 wks	15 % and ns
Kiens 1980	n=37, mean 40, NL, 37.8	Leisure-time conditioning; 80-85 %: 45 min; 3 x wk; 12 wks	12 and 8 %; Weight unchanged
King 1991	n=300, 50 - 65 , NL 28.7, 30.1 and 30.8	Walking, jogging,; 45 % and 76 %; 40 min; 3 x wk; 52 wks	6 , 4 , 5 % and ns; Training improved fitness but not risk factors
Stein 1990	n=49, mean 44 , HL 25.1-35.9, 28.4-35.9, 30.3-31.5	Ergometer; 42%, 57 and 72 %; 30 min; 3 x wk; 12 wks	mean 23, 13 - 19 %
Stensel 1993	n=65, 42-59, HL, 35.9	Brisk walking; 56 %; from 20 to 45 min; 7 x wk; 52 wks	6.5 % and ns
Suter 1994	n=75, mean 41, HL, 38.1 and 35.3	Jogging and walking; 50 % and 75 %; 30 min; 6 x wk	7% and ns; Association between amount of training and increase in HDL-C
Williams 1994	n=88, 30 - 59, NL	Walking and jogging; 56 %; 40/50 min; 5 x wk; 52 wks	na and 7-13 %; Greatest in normal-to high initial HDL- C
Wood 1983	n=81, 30 - 55, HL, 35.2	Jogging and running; 56 %; 3 x wk; 52 wks	21 and 8.7 %; Threshold of 12.9 km per wk
Wood 1988	n=81, 30 - 59, HL	Walking and jogging; 65 %; 40/50 min; 4 x wk; 52 wks	Fat loss by dieting or exercising produced comparable changes in plasma lipoproteins

NL=normolipemic, HL=high lipemic; Initial fitness = VO_{2max} (ml/min/kg);
Intensity as % of VO_2max or maximal heart rate*; ns= not significant, na= not available

Exercise training with (Wood et al. 1988) or without (Thompson et al. 1997b) changes in body weight induces increases in HDL-C but the mean increase (see Table 4) is usually less than that expected by most clinicians and the expected value based on

results in cross-sectional studies. Although exercise training benefits are small at the individual level, they may be significant for public health. It has been estimated that a 0.026 mmol/l increase in HDL-C would reduce the risk of CHD by 2 % in men (Gordon & Rifkind 1989). Thus, in a recent meta-analysis of randomised controlled trials, 0.05 mmol/l increase in HDL-C, represents a 3.8 % decrease in CHD risk (Halbert et al. 1999).

For individuals with a risk of CHD, exercise is often recommended for increasing HDL-C levels. Persons who have normal-to-high serum HDL-C levels have better ability to increase HDL-C levels through endurance exercise training (Williams et al. 1994, Thompson et al. 1997b, Zmuda et al. 1998), but this ability is limited in subjects with low initial HDL-C levels (Zmuda et al. 1998). On the other hand, individual responses in increasing HDL-C levels through endurance exercise training are highly variable (King et al. 1995a, Zmuda et al. 1998, Hagberg et al. 1999a, Hagberg et al. 1999b), and changes in lipids and lipoproteins may be genotype-dependent (Despres et al. 1988, Hagberg et al. 1999a, Hagberg et al. 1999b). Differences in the lipid levels between the trials (Tran et al. 1983, Halbert et al. 1999) are probably accountable to the varying responses of blood lipids within individuals. Possible contributors to the heterogeneity also include the variability in the age of the subjects, differences in pre-training fitness level and in pre-training lipid concentrations of the subjects, varying exercise programs and interaction amongst exercise intensity, frequency, duration of each exercise session and length of the exercise training period (Durstine & Haskell 1994).

Cross-sectional studies in exercisers vs. controls may be biased by a selection effect (Durstine & Haskell 1994), but there is also reason to suspect the same in randomised trials of exercise training. In one of their early studies, Wood et al. (1983) observed that although lipoprotein concentration change uniformly in the runners vs. controls and favored reduced risk of CHD, changes were not significant when all 46 participants with complete data were included (Wood et al. 1983). However, the 25 men who averaged at least eight miles (12.9 km) per week of running increased their HDL-C levels significantly when compared with controls (Wood et al. 1983). The finding that men with higher initial levels of serum HDL-C will tend to run more when undertaking an exercise program and that their concentrations of HDL-C and LDL-C did not begin to change until a threshold exercise level of 16 km run per week was maintained for at least nine months (Williams et al. 1982) also supports the possibility of the influence of selection. These results, among others, underline the importance of the intensity (Stein et al. 1990) and the duration (King et al. 1995b) of the exercise program, the determinants of exercise training influenced by inherited fitness characteristics of an individual (Bouchard et al. 1997).

3.4. The effects of skeletal muscle and exercise on serum lipids and lipoproteins

Plasma TG-derived FAs are taken up by skeletal muscle through the action of LPL found anchored to the luminal side of the capillary endothelium where its main function is to mediate the initial hydrolysis of triglycerides in circulating lipoproteins (Eckel 1989). The hydrolysis of lipoprotein triglycerides decreases the triglyceride content of the lipoprotein particles, a process that modifies both the blood lipid profile and the properties of the lipoprotein particles (Figure 2). The HDL level is dependent on the rate by which free cholesterol, phospholipids and apolipoproteins are released from TG-rich lipoproteins during lipolysis and taken mainly by HDL. Indeed, variations in the fractional catabolic rate of TG-rich lipoproteins have been shown to correlate positively with the HDL-C concentration (Hamsten 1990). In addition, the HDL particle distribution in plasma is determined by the transfer of cholesteryl esters and triglycerides between HDL and the TG-rich lipoproteins resulting in triglyceride-rich HDL (HDL2) that is a preferred substrate for hepatic lipase. By depleting triglycerides from HDL (HDL2) hepatic lipase remodels HDL towards HDL3. (Figure 2)

Lipid oxidation contributes about 50 % to the overall energy conversion in human subjects exercising at 65 % of VO_{2max} for 60 -100 min (van der Vusse & Reneman 1996). Exercise training increases LPL activity in skeletal muscle (Nikkilä 1987) and FA oxidating capacity of skeletal muscle (Mole et al. 1971). Thus, it is tempting to suggest that skeletal muscle may be responsible for increased hydrolysis of plasma TG during exercise and thus, may alter plasma lipoprotein metabolism after exercise training, inducing a favorable lipid profile, low serum TG and high serum HDL-C values, most often observed in regular exercisers (Figure 2). This presumption is also supported by the finding that the utilisation of FAs has been observed to be related to changes in serum lipoprotein concentrations (Kiens & Lithell 1989). Indeed, the metabolisms of serum TG and HDL-C are coupled (Eisenberg 1984, Sady et al. 1986, Sady et al. 1988) and skeletal muscle LPL plays a central role in this regulation (Nikkilä 1987, Berg et al. 1994). Although extraction rates of circulating triacylglycerides under resting conditions have been observed to be 8 % in untrained compared with 15 % in trained thigh muscles, differences in TG utilisation during exercise are relatively small (Kiens et al. 1993). The contribution of lipoprotein-derived FAs to muscle total lipid energy utilisation during exercise has been estimated at no more than 3-10 % (Nikkilä 1987, Oscai et al. 1990, van der Vusse & Reneman 1996). Moreover, endurance training enhances lipid oxidation at low relative (< 40 % of VO_{2max}) exercise intensities (Bergman & Brooks 1999). Most athletes and regularly physically active persons exercise at higher intensities, and therefore, the utilisation of FAs from plasma TG during exercise probably have a minor effect on plasma lipoproteins.

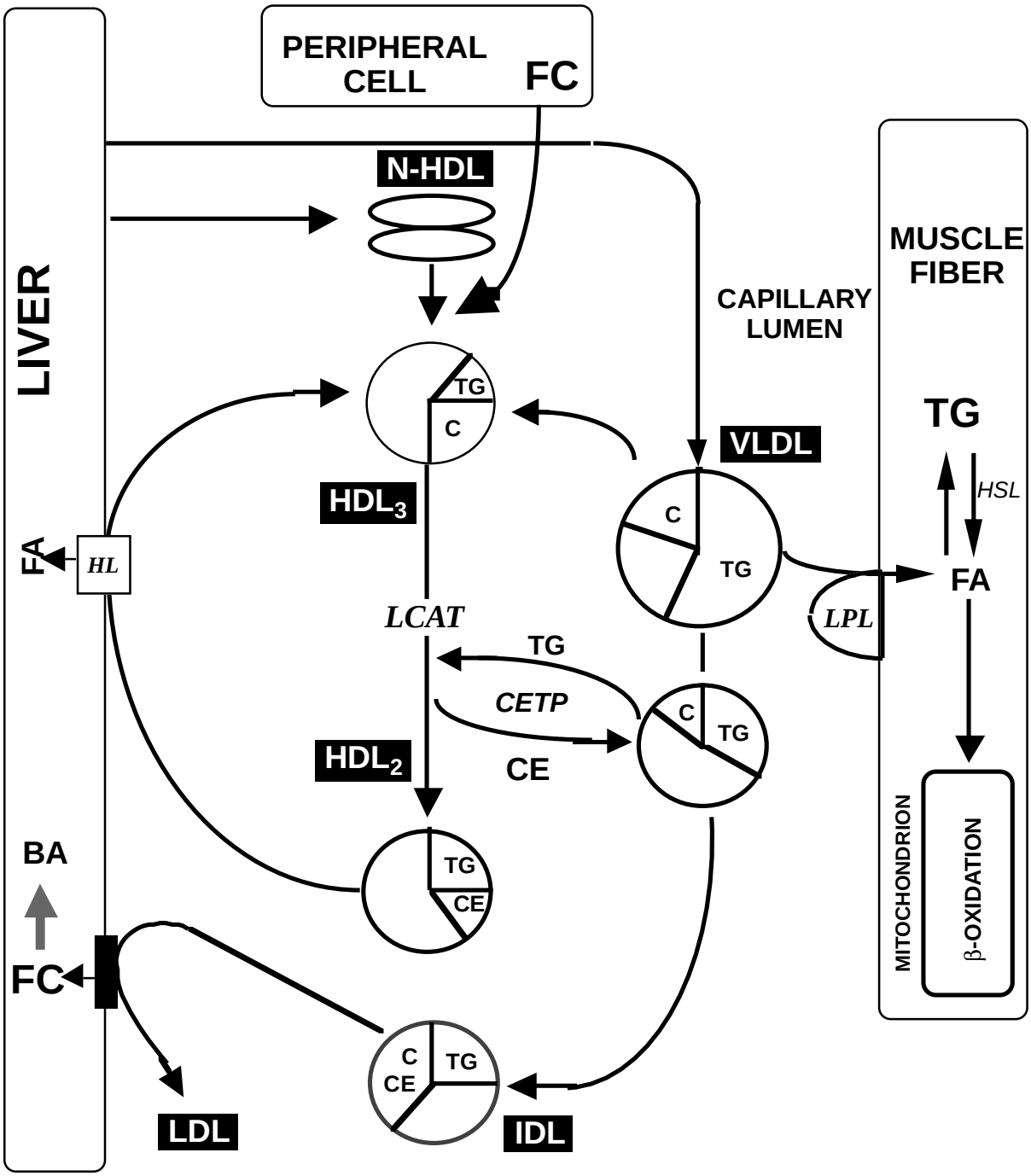

Figure 2. Overview of lipid and lipoprotein metabolism. Skeletal muscle contributes to lipid metabolism by using fatty acids from serum triglycerides as an energy source or by storing fatty acids as intramuscular triglycerides. Abbreviations see page 6.

During exercise intracellular stores of intramuscular TG can provide 20 - 25 % of energy requirements (Romijin et al. 1993, Guo et al. 2000). In untrained subjects, an exercise-induced increase in total lipid oxidation at low-intensity exercise is associated with an increase in the oxidation of both plasma TG- and intramuscular TG-derived FAs but as the intensity increases, but still remains below 65 % VO_{2max},

the contribution of intramuscular TG to total lipid oxidation increases (Turcotte 2000). With training, an increase in total lipid oxidation appears to be associated with the increased oxidation of FAs from both plasma and intramuscular TG (Turcotte 1992, Bergman 1999). However, regularly trained persons may use more fat from intramuscular stores during high-intensity exercise than untrained persons at the same relative exercise intensity (Coggan et al. 2000). Thus, the replenishment of intramuscular TG from FAs of plasma TG-rich lipoproteins after exercise by muscle LPL (Oscai et al. 1990), as well as FA utilisation from plasma TG-rich lipoproteins and from intramuscular TG after exercise when muscle glycogen stores are replenished (Kiens & Richter 1998), have been suggested to be crucial in influencing serum lipids and lipoproteins (Kiens & Lithell 1989).

The importance of skeletal muscle LPL to clear triacylglycerides from body circulation after exercise in replenishing muscle triacylglyceride stores (Oscai et al. 1990, van der Vusse & Reneman 1996) is supported by the results indicating that exercise before fat-rich meal can significantly attenuate a postprandial hypertriglyceridemia response (Zhang et al. 1998). Indeed, local muscle contractile activity is required for increasing muscle LPL expression (Hamilton et al. 1998), and the acute post exercise increase in muscle LPL mRNA and mass (Seip et al. 1995) as well as activity (Ferguson et al. 1998) coincidences with the post exercise fall in circulating TG (Cullinane et al. 1982, Seip & Semenkovich 1998) and increase in serum HDL-C later (Enger et al. 1980, Thompson et al. 1980, Durstin et al. 1983, Kantor et al. 1987). In sedentary men the elevation of HDL-C concentration has been observed 24-78 hours following the exercise session (Kantor et al. 1987) but in trained men the elevation of HDL-C concentration has been observed both immediately after (Kantor et al. 1987) and in hours following an exercise session (Kantor et al. 1984, Dufaux et al. 1986, Sady et al. 1986). The LPL activity and oxidative energy conversions of FAs in muscles consisting predominantly of ST fibers are considerably higher than in muscles with FT fiber predominancy (Linder et al. 1976, Okano & Shimojo 1982, Hamilton et al. 1998, but training causes a significant rise in LPL activity in FT fibers (Hamilton 1998). In addition, the FA transport rate into muscle cells is higher in ST- than in FT-rich muscles because FA transporters are abundantly available in the ST fibers (Bonen et al. 1998). These findings may explain the high LPL activity in endurance trained runners (Nikkilä et al. 1978) and enhanced TG clearance in endurance trained athletes (Sady et al. 1988, Cohen et al. 1989).

4. OTHER CHD RISK FACTORS

Endogenous sex hormones are candidate modulators of plasma lipoprotein metabolism and determinants of CHD. In general, steroids with estrogen activity increase plasma levels of HDL-C and steroids with androgenic activity have the opposite effects

(Crook & Seed 1990). Increased LDL-C and decreased HDL-C levels in post-menopausal women are believed to be the result of decreased estrogens associated with menopause (Crook & Seed 1990). This is consistent with the sex differences in HDL-C levels, in the CHD incidence and mortality in women and men (Jousilahti et al. 1999). An association between testosterone or estradiol and cardiovascular risk factors has not been consistently observed however (Haffner & Valdez 1995).

Serum testosterone may have an influence on the gender differences in CHD incidence, but studies of sex hormones and lipoprotein levels in men have yielded conflicting results. In adolescent males, puberty and increasing testosterone levels are associated with decreasing serum HDL-C levels (Morrison et al. 1979, Laskarzewski et al. 1983, Kirkland et al. 1987), and the suppression of endogenous testosterone in men has lead to an increase in serum HDL-C (Goldberg et al. 1985, Bagatell et al. 1992). Some studies have reported a positive relationship between testosterone levels and serum HDL-C (Stefanick et al. 1987, Khaw & Barrett 1991), which is inconsistent with the associations of CHD with low HDL-C levels and the male gender. Significantly lower testosterone levels in persons with prevalent CHD than persons without heart disease has been observed (Lichtenstein et al. 1987), it is not possible to conclude whether the difference in serum hormones between the CHD-patients and the healthy men is a consequence of the disease rather than a cause of CHD. Indeed, much of heterogeneity in HDL-C levels can be accounted for by environmental factors like strenuous physical activity (Duell & Bierman 1990). Although in healthy middle-aged men, single point plasma androgen measurements fairly reliably reflect the annual mean androgen level (Vermeulen & Verdonck 1992), exercise training may cause variations in hormone levels of physically active men (Hackney et al. 1988) and this may have an influence on serum lipoproteins and thus on the risk of CHD. Markedly reduced serum testosterone levels have been observed after prolonged exercise (Aakvaag et al. 1978, Kuoppasalmi et al. 1980, Kuusi et al. 1984) and after extreme physical training in athletes (Wheeler et al. 1984) but also after a person completes an exercise training program (Frey et al. 1983). In non-competitive joggers, a significant decrease in serum testosterone levels and increase in serum HDL-C levels has been observed after marathon running (Kuusi et al. 1984). After 10 weeks of training, serum testosterone has been observed to correlate positively with serum HDL-C, and, moreover, training-induced changes in these two variables correlated significantly and positively (Frey et al. 1983). Thus, physical activity may be one confounding factors in the androgen-lipoprotein relationships, and the exercise-induced changes in testosterone in men may mediate changes in the lipoprotein response to physical training.

Serum estradiol, in contrast to serum testosterone, is associated with a reduced CHD risk. Hepatic lipase (HL) (Figure 2), an enzyme inversely related to serum HDL-C

(Kuusi et al. 1980) because it catalyses the degradation of HDL lipids (Nikkilä et al. 1982), is jointly regulated by endogenous androgens and estrogens (Sorva et al. 1988). When there is an increase in physical fitness in men, there is a decrease in HL activity (Kuusi et al. 1982). It is possible that estradiol effects lipid and lipoprotein levels because of its estrogen-mediated or estrogen-androgen balance -mediated influence on HL activity. In addition to influencing the HL activity, estradiol may increase skeletal muscle LPL activity, which has been observed in exercised male rats (Ellis et al. 1994). An increase in estrogens in men in response to exercise training has been observed to correlate positively with changes in HDL-C (Frey et al. 1983). Serum LDL-C concentration, which is regulated by endogenous estradiol in women (Tikkanen et al. 1986), is suggested to be more sensitive to changes in endogenous estrogens than in androgens (Sorva et al. 1988). Serum estrogens in men were significantly increased by exercise training, and LDL-C concentrations decreased (Frey et al. 1983). Both more training and a faster running speed in male but not in female runners were associated with significantly lower serum LDL-C concentrations in a dose-response manner (Williams 1996, Williams 1997, Williams 1998). Thus, training seems to have different effects on serum LDL-C in men and women and various changes in endogenous estrogens may mediate this difference.

Sex hormone binding globulin (SHBG), a glycoprotein that transports sex steroids in human plasma, has been observed to associate positively with serum HDL-C concentrations (Semmens et al. 1983, Hämäläinen et al. 1986, Hämäläinen et al. 1987). This association may be important because serum SHBG may be a significant negative risk factor for CHD mortality (Lapidus et al. 1986). However, both serum SHBG (Adlercreutz et al. 1986, Hämäläinen et al. 1987) and serum HDL-C (Durstine & Haskell 1994) levels are increased by physical activity which may explain the positive association between them. When physical activity has been combined with a low-fat, high carbohydrate diet, an increase in serum SHBG level has been observed (Tymchuk et al. 1998). Whether this increase in SHBG is determined by the sex hormone balance of the subject (Semmens et al. 1983, Hämäläinen et al. 1986, Lapidus et al. 1986) or merely reflects differences of liver induction due to environmental factors such as diet (Adlercreutz et al. 1987) or due to physical activity (Hämäläinen et al. 1987) has not been established.

Dehydroepiandrosterone (DHEA), with its sulphate conjugate (DHEAS), is the major secretory steroidal product of the adrenal gland. The serum concentration of DHEAS is 300-500 times higher than that of DHEA and 20 times higher than that of any steroid hormones. Despite the high concentration of DHEAS in the blood, its physiological role has remained unknown. Low levels of DHEAS may predict cardiovascular disease in men (Barrett-Connor et al. 1986). In addition, the role of DHEAS as a possible cardiovascular risk factor has gained attention as cross-

sectional data relate low level of DHEAS to atherosclerosis as assessed by coronary angiography (Herrington et al. 1990). In female runners, regardless of menstrual cycle status, DHEAS has significantly correlated with HDL-C and apo A-I (Thompson et al. 1997a). In the Helsinki Aging Study however, low plasma DHEAS appeared to be a secondary phenomenon rather than a specific risk indicator (Tilvis et al. 1999) and, after checking for disease, DHEAS did not predict an increased risk of all-cause or cardiovascular mortality during the follow-up (Tilvis et al. 1999).

A high fasting insulin concentration is an important predictor of CHD in healthy middle-aged Finnish men (Pyörälä et al. 1998). Adjustment by multivariate analysis for several confounders do not significantly diminish this association (Despres et al. 1996). However, a negative association between fasting insulin concentration and physical activity has been observed among non-diabetic men and women (Regensteiner et al. 1991). Physical activity is perhaps the most important single determinant of insulin sensitivity (Rosenthal et al. 1983), and increasing the intensity or duration of exercise has a graded relation to improvements in insulin sensitivity (Mayer-Davis et al. 1998). Interestingly, skeletal muscle fiber type has been suggested to determine and modulate whole body insulin action synergistically with physical activity (Storlien et al. 1996), and skeletal muscle oxidative capacity and ST-% have been suggested to play a role in the development of insulin resistance (Lillioja et al. 1987, Kriketos et al. 1996). A variation in subcutaneous adipose tissue in the abdominal region is also an important determinant of individual differences in insulin sensitivity (Goodpaster et al. 1997).

An excess of body fat is shown to be associated with unfavorable risk profiles for cardiovascular disease (Anderson et al. 1988, Seidell et al. 1991). Body mass index (BMI, the weight in kilograms divided by the square of the height in meters), is an indirect but simple and commonly used indicator of general adiposity (Garrow & Webster 1985). The categories and cut-off points widely used in Europe are for normal weight 20-25, overweight 25-30 (Bray 1985). BMI over 30 is a commonly used criterion for defining obesity (Ravussin & Swinburn 1992), but a BMI already greater than 25 is known to be related to an increased risk for cardiovascular disease (Hubert et al. 1983, Calle et al. 1999). Subcutaneous adipose tissue, especially subscapular and abdominal fat, is closely associated with an unfavorable lipid profile in men (Despres et al. 1985).

Overweight men with low fitness have a high risk for developing CHD (Wei et al. 1999), but moderate-to-high fitness has been shown to reduce the risks of obesity (Lee et al. 1999). A low body fat content may partly explain favorable lipid levels associated with a high fitness and physical activity level (Marti et al. 1989). Thus, the changes in body weight and adiposity may be responsible for the exercise-induced

changes in serum lipids and lipoproteins (Williams et al. 1983) as well as influencing the risk of CHD (Wood et al. 1988, Williams et al. 1990, Williams et al. 1992). Subjects regularly involved in vigorous activities are leaner than those not participating in these activities (Tremblay et al. 1990). Indeed, vigorous LTPA, despite a lower total energy cost, induces a greater loss in subcutaneous adipose tissue, increases oxidative potential and is more effective for stimulating fatty acid oxidation than a moderate intensity program (Tremblay et al. 1994, Chilibeck et al. 1998).

A reduced capacity for fat oxidation related to a reduced capacity in skeletal muscle to take up and oxidize the circulating lipids may be an important factor for obesity (Ravussin & Swinburn 1992). Indeed, leanness is associated with an increased oxidative capacity in skeletal muscles (Kriketos et al. 1996), and a low activity of oxidative enzyme KGDH in the Krebs cycle in skeletal muscle has been observed to contribute to one's proneness to gain subcutaneous adipose tissue over time (Simoneau et al. 1996). Adiposity seems to be inversely related to ST-% (Lillioja et al. 1987, Wade 1990, Kriketos et al. 1996, Kriketos et al. 1997), but coincident correlations between fitness, physical activity and fatness may be confounders in these studies.

THE AIM OF THE STUDY

In the present study, investigations on skeletal muscle properties (muscle fiber distribution and regulatory enzyme activities in energy metabolism), physical fitness and physical activity and their influence on variations of serum lipids and lipoproteins, and eventually on the risk of CHD were carried out. In addition, the influence of fitness and physical activity on serum sex hormones, serum insulin and body adiposity were also examined in order to study the effects of skeletal muscle properties on variations of these risk factors.

The specific aim of this work was to study the following hypotheses:

1. The percentage of ST and FT muscle fibers in the vastus lateralis muscle of the thigh may affect serum concentrations of lipids and lipoproteins, especially HDL-C, which may influence an individual's risk of CHD.

2. Progressive exercise training has a significant influence on the regulatory-enzyme activities of energy metabolism in slow- and fast-twitch skeletal muscle fibers though the effects of training may differ depending on the muscle fiber type and the proportion of these fibers in the muscle.

3. Skeletal muscle properties, especially the percentage of ST fibers (ST-%), have an effect on physical fitness and physical activity. Moreover, the ST-%, fitness and physical activity may have significant interrelationships in their influence on serum lipids and lipoproteins.

4. Natural selection to different types of sports at a young age may bias the association between physical activity and the occurrence of CHD, but the continuity of physical activity may have an influence on the risk of CHD also later in life.

5. Physical fitness and physical activity affect serum hormones, body adiposity and serum lipoproteins and, thus, may significantly confound the associations observed between these variables.

MATERIALS AND METHODS

1. Study population

A total of 122 men (mean age 41.8 years, SD 6.9 years) volunteered for this study and they were recruited as follows. Of the total, 60 sedentary men offered either to begin an exercise training program arranged by their employer or to participate in one arranged by the city of Helsinki, 36 physically active men came from sports clubs from the Helsinki area, and 26 were CHD patients who had undergone coronary angiography at the First Department of Medicine, University Central Hospital of Helsinki. Data on cigarette smoking and alcohol consumption were obtained from questionnaires and by personal interviews. All had a Western-type diet and no heavy drinkers or those with vegetarian or other special diets were included in the study. The study protocol was approved by the Ethical Committee of the First Department of Medicine in Helsinki University Central Hospital.

1.1. Healthy men (Study I, IV ,V)

The healthy men (n= 96, mean age 40.0 years, SD 5.6 years) were not under long-term medication, and they had no history of endocrinological, liver, kidney or gastrointestinal diseases. They all had negative histories of CHD, which were confirmed by exercise electrocardiograms (ECG) in progressive cycle ergometer tests. Their maximal oxygen uptake (VO_{2max}) showed a mean value of 35.2 ml/min/kg with SD 7.4 .

Forty-one of the healthy men (mean age 39.5 years, SD 5.6 years), who had not participated in regular intense leisure-time physical activity before the study, but reported to have had irregularly participated in ball games, jogging, swimming or walking 0 - 3 times per week, gave muscle samples for ST-% analysis and blood samples for lipid and lipoprotein analysis (I). Detailed information about the leisure physical activity of 36 of these men was obtained from questionnaires and personal interviews (IV).

Nineteen of the healthy men (mean age 38.7 years, SD 3.4 years), sedentary white-collar office workers, volunteered to begin an exercise training program arranged by their employer (V). These men were selected to the training group, because they were employed by the same car-selling company and, thus, it was easy to keep close contact with them. They had not participated in any competitive sport or regular intensive exercise training before. Two of the men did not complete the training program (one moved to Lapland during the study year and was not able to attend meetings and come to the laboratory, and the other stopped training after two

months because of observed hypertension needing treatment with medication). Five of the men, after receiving information about the training, did not want to participate in the program because they felt that it was too demanding and time-consuming. These five men did volunteer for the exercise testing and gave blood and muscle biopsy samples at the beginning of the training year and attended all the monthly meetings. As they received the same information as the 12 men (aged 37.4 years, SD 3.4 years) who completed the 12-month training program, these five men (mean age 38.6 years, SD 3.3 years) formed an internal control group

Thirty-six physically active men (mean age 43.0 years, SD 5.6 years) had trained (running and cross-country skiing, swimming, bicycling, ball games) regularly 3 to 7 times per week for the last five years (I, IV). They displayed no evidence of coronary heart disease, as judged by their negative histories and normal exercise electrocardiograms during a graded, maximal bicycle-ergometer test. In this test their VO_{2max} was determined and showed a high level of fitness with a mean value of 54.2 ml/min/kg, and SD 7.9. Detailed information of their leisure physical activity was obtained from questionnaires and personal interviews (IV).

1.2. CHD patients (Study I, IV ,V)

The twenty-six CHD patients (mean age 47.9 years, SD 7.9 years) showed none of the following: unstable angina pectoris, hypertension (diastolic blood pressure ≥105 mm Hg), diabetes, thyroid, liver, kidney, gastrointestinal or endocrinological diseases. They did have significant coronary artery narrowing (more than 50 % of the vessel diameter) of at least one main artery (6 with a one-vessel disease, 10 with a two-vessel disease and 10 with a three-vessel disease). In 14 of the 26 patients, at least one previous acute myocardial infarction, based on the typical history of chest pain, enzymatic changes (aspartate aminotransferase, creatine kinase or creatine kinase MB-isoenzyme) and positive signs of myocardial damage in the ECG, had been diagnosed 8 to 13 years before they were included in the study. The patients had never taken any lipid-lowering drugs, and none had a recent cardiac event. However, at the time of the study the patients were taking ß-blockers (12 took metoprolol, 100 - 200 mg /day and 14 took either pindolol, 5 - 10 mg /day or atenolol, 50 - 200 mg /day), and, in addition, 23 were taking either prazosine, mexilethinehydrochloride, a thiazide diuretic, nifedipin or sublingual nitroglycerides. All CHD patients smoked, mean of 3.7 (SD 3.3) cigarettes per day. In twenty CHD patients (mean age 47.9 years, SD 5.2 years), serum hormones were also analysed, and the results were compared with the 72 healthy men with different level of fitness and physical activity (IV).

1.3. Former athletes and controls (Study VI)

The associations between natural selection to sports at a young age, continuity of physical activity, occurrence of CHD, and physical activity among former athletes and controls were studied using questionnaires, three nationwide registers and death certificates. The extent to which selection to specific types of sports at a young age predicted later physical activity (in 1985) and prevalence of coronary heart disease (in 1985 and 1995) was analysed. Male athletes who had represented Finland between 1920 and 1965 at least once at the Olympic games, world or European championships, or other international competitions in selected sports were identified (Sarna et al. 1993). The full name, place, and date of birth were traced for 98% of the athletes from selected sports. Controls were selected from among Finnish men who at about 20 years of age had been classified as completely healthy (military class AI, fully fit for ordinary military service) at the medical examination preceding their conscription (Sarna et al. 1993). They were drawn from the public archives of the register of men liable for military service and matched for birth cohort and area of residence with the athletes. The original cohort of athletes included 2401 men and the reference group 1712 men (Sarna et al. 1993).

In 1985, 1282 (response rate 80-90% by sport of those alive in 1985) athletes and 777 (response rate 77%) controls responded to the questionnaires. For the present study, those athletes and controls who provided complete data on the 1985 questionnaire were selected. From the athletes, two extreme groups according to the presumed type of muscle fiber composition (Saltin & Gollnick 1983) suspected as giving the best advantage for a specific type of sports event, and thus being a selective factor for natural selection to different types of sports. Endurance runners (n=101) and cross-country skiers (n=65) were assigned to the 'endurance' athlete group, and sprinters (n=73), weight-lifters (n=66) and track and field throwers (shot putting, n=22; discus, n=24; hammer, n=20; javelin, n=30) to the 'power-speed' athlete group. The third athletic group 'other athletes', comprised 154 soccer, 108 ice-hockey and 67 basketball players, as well as 131 boxers, 131 wrestlers and 243 men from other than the aforementioned track and field disciplines.

Data were collected from the 1985 and 1995 questionnaires, and morbidity data were compiled from three nationwide registers covering all citizens in Finland: the registers for hospital inpatient discharges, reimbursable medications and disability pensions (Kujala et al. 1994). In addition, causes of death were available from the Cause of Death Bureau files at the Central Statistical Office of Finland. The personal identification (social security) code assigned to all Finnish citizens permitted accurate computerised record-linkage. Based on registers and death certificates, the occurrence of coronary heart disease was analysed using the International

Classification of Diseases (ICD) codes (ICD-8 from 1970 to 1985 and ICD-9 from 1986 to 1995) (WHO 1969, WHO 1977), rubric code 410-414 indicating CHD.

From 1986 to 1995, 232 (18.8%) of the 1235 athletes and 158 (21.3%) of the 743 controls died. In 1995, 931 (91.9%) of the 1013 athletes and 535 (91.5%) of the 585 controls alive in 1995 responded to the second questionnaire. For those who died during the last 10-year follow-up or who did not respond to the 1995 questionnaire, the follow-up data were derived from death certificates and morbidity registers. The 1985 and 1995 questionnaires included items on physical attributes (height, current weight), occupation, smoking, physical activity, and any diseases verified by a doctor. The questionnaire included the question: "Has a doctor said that you have or have ever had angina pectoris (chest pain due to coronary heart disease) or myocardial infarction?"

Cause of death data were available from 1936 to December 31, 1995. Since 1967, all hospital discharges in Finland have been recorded annually in a nationwide register at the National Board of Health. The reports are obligatory for all public and private hospitals. Record linkage was not possible until 1969, because the data were too incomplete for accurate identification. Thus, the follow-up with hospital records in the present study started from the beginning of 1970 and continued to December 31, 1995. The validity of the register for epidemiological studies of coronary heart disease and myocardial infarction has been shown to be very good (Heliövaara et al. 1984, Pietilä et al. 1997). Data on disability pensions and reimbursable medication were obtained from the register of the Social Insurance Institution, the public agency responsible for basic social security covering all residents of Finland (Kujala et al. 1994). The follow-up of the disability pension records started from 1970, when pensions granted earlier in surviving subjects were coded and continued to 1985. Detailed data on reimbursements due to CHD were available from 1986 to 1995.

Subjects who had CHD, including physician-diagnosed angina pectoris or myocardial infarction, based on the 1985 questionnaire or at least on one of the registers before January 1, 1986, were determined to have coronary heart disease in 1985. Those with CHD in 1985 or coronary heart disease based on the 1995 questionnaire, or on the hospital discharge registry, the reimbursable medication registry or death certificates before January 1, 1996, were determined to have CHD in 1995. Those who had CHD in 1995 but not in 1985 were considered to be incident cases of CHD during the last ten-year follow-up. Occupational data were collected partly from the Central Population Registry and partly from the 1985 questionnaire study. The main occupational groups were as follows: executives, clerical staff, skilled workers, unskilled workers and farmers (FCSO, 1972). Each person was classified according to the occupation they had held longest during their lifetime. The calculation of body-mass

index [weight (kg) by height (m) squared] in 1985 was based on the self-reported height and weight in the questionnaire. The tobacco smoking status of the subjects was classified according to their responses to a detailed smoking history (Kaprio & Koskenvuo 1988). Non-smokers were men who had smoked no more than 5 to 10 packs of cigarettes (or the equivalent of other tobacco products) throughout their lifetime. Other subjects were classified as current smokers or ex-smokers according to whether they were smoking daily at the time of the questionnaire or had quit.

1.4. Animals (Study III)

Thirty-two 2-3 -month-old male rats of the Sprague-Dawley strain were used in a training study, after which selected hind limb muscles and muscle fibers from these muscles were analysed for regulatory enzymes. The rats were provided with food (62 % carbohydrate) and water ad libitum. Sixteen rats were randomly selected for training five days per week over an eight-week period on a tread-mill. The other sixteen rats served as sedentary controls.

2. Collection and storage of samples

2.1. Blood sampling (Study I, IV,V)

The blood samples of the healthy men were collected when they came to the laboratory for the personal interview. To avoid the acute effect of physical strain on serum parameters, the physically active subjects were asked to refrain from competitive sport for one week and from exercise for one day before the blood sampling. The blood samples of the CHD patients were collected when they came to the hospital for coronary angiograms. After an overnight fast, two blood samples (within 15 min intervals) were drawn between 7:30 and 9:30 a.m. by venopuncture from the antecubital vein with the subjects in a sitting position. A small portion of the pooled serum samples was stored at 4 °C for lipoprotein analysis, which was completed within two days. The other half of the pooled sample was frozen immediately and stored at -20 °C for hormone analysis.

The blood samples for lipid and hormone measurements from different groups were collected during the same time of the year (I, IV) and the training of the sedentary men (V) lasted for one year. Thus, the possible confounding influence of the annual variation of sex hormones and serum lipids were minimised. Pulsative secretion and cicardian variation were controlled by careful timing of blood sampling and by using a pooled serum of at least two consecutive blood samples for each subjects. In addition, blood sampling of the men was performed in the same body position after a half hour rest.

2.2. Muscle sampling in men (Study I, IV, V)

The skeletal muscle samples were taken from the lateral portion of the quadriceps femoris muscle with a biopsy needle (Tru-Cut®, Travenol Laboratories Inc., Illinois, U.S.A.). After local anesthesia of the skin, a small incision (1-2 mm) was made with a scalpel, and the biopsy needle was advanced 3-4 cm into the muscle at the mid-point between the greater trochanter and the articular cavity of the knee. Two samples were taken through the same incision: the first sample was taken by advancing the needle toward the more distal and the second by advancing the needle toward the more proximal part of the muscle to avoid the possibility of taking the second sample from a previously sampled muscle area. The muscle specimens (ca. 10 mg, each sample consisted of 100-300 muscle fibers) were frozen immediately in liquid nitrogen and stored at -75°C until analysed.

2.3. Muscle sampling in rats (Study III)

At the end of the training period, four trained and four sedentary rats were killed by decapitation without anesthesia, at rest. The gastrocnemius muscle (mosaic mixture of both FT and ST muscle fibers and therefore called in this study "mixed muscle"), tibialis anterior muscle (predominantly FT fibers, called "fast muscle") and soleus muscles (predominantly ST fibers, called "slow muscle") were excised (Rice et al. 1988). Muscle samples were frozen immediately in liquid N_2 and stored at –75°C until analysed for enzyme activities. Gastrocnemius muscles from eight trained rats and eight sedentary rats, killed at rest, were used for the single muscle fiber analysis. The effect of training on skeletal muscle was studied by investigating muscles containing different proportions of ST and FT fibers as well as investigating single ST and FT fibers of gastrocnemic muscle.

3. Analytical methods

3.1. Serum lipids and lipoproteins (Study I, IV, V)

Serum total cholesterol and triglyceride (TG) concentrations were analysed with a Hitachi 705 automatic analyser utilising enzymatic methods (Boehringer-Mannheim GmBH, West-Germany). Cholesterol in the high density lipoprotein fraction (HDL-C) was measured after precipitation of other lipoproteins (LDL and VLDL) by dextran sulphate-Mg^{++}. Serum apo A-I was determined immunoturbidometrically using a Kone-CD compact Clinical Analyser (Kone OY, Helsinki, Finland) and apo A-I reagents (Orion Diagnostica, Espoo, Finland). The between-assay imprecisions for serum cholesterol, serum HDL-C, serum TG and serum apo A-I were 2.1, 2.5, 3.5,

3.5 %, respectively. Serum LDL-C was calculated according to the formula of Friedewald et al. (1972).

3.2. Serum sex hormones, sex hormone binding globulin and insulin (IV, V)

Serum testosterone (Nordiclab, Oulunsalo, Finland) and serum estradiol (Sorin Biomedica S.p.A., 13040 Saluggion (VC), Italy) were measured by radioimmunoassay. The serum sex hormone binding globulin (SHBG) was measured by an immunoradiometric assay (IRMA) method of Orion Diagnostica (Oulu, Finland). The serum free, non-protein bound testosterone (free testosterone) was calculated using the results of total testosterone and SHBG (Anderson' 1976). The serum free, non-protein bound estradiol (free estradiol) was calculated using results of total estradiol and SHBG (Moore 1982). Serum insulin was measured with a radio-immunoassay kit from Pharmacia (Uppsala, Sweden). Serum dehydroepiandrosterone sulfate (DHEAS) was determined using a commercial RIA kit (Wien Laboratories, Inc., Succasunna, NJ, USA). The serum free, non-protein bound estradiol (free estradiol) was calculated using the results of total estradiol and SHBG (Moore et al. 1982). Serum luteinizing hormone (LH) was determined utilising a commercial RIA kit from CIS International (France).

3.3. Muscle fiber distribution analysis (I, IV, V)

The skeletal muscle fiber distribution (the percentage of ST fibers, ST-%) was analysed from samples taken from the lateral portion of the quadriceps femoris muscle with a biopsy needle. The muscle samples were sectioned in a cryostat and stained for ATPase, preincubated at pH 4.3 (Guth & Samaha 1970). In this staining, the two main fiber types, ST and FT fibers, can be separated clearly: ST fibers are stained dark but FT fibers remain unstained (Guth & Samaha 1970, Saltin et al. 1977, Saltin & Gollnick 1983). The number of ST and FT muscle fibers was calculated from both samples, and the proportion of the fiber types was used as a muscle fiber distribution percentage. The muscle samples were coded and the (same) technical worker did not know which samples she was analysing. Repeated sampling from the same muscle gave a coefficient of variation for the fiber composition of less than 15 % which has been similarly observed in earlier studies due to sampling and technical errors (Simoneau & Bouchard 1995).

3.4. Measurement of enzyme activities in muscle samples

The activities of phosphofructokinase (PFK), ketoglutarate dehydrogenase (KGDH) and carnitine palmitoyl transferase (CPT) were measured because these enzymes are rate limiting (key-enzymes) and provide quantitative information on the maximal

capacities of glycolysis, oxidative metabolism in the citric acid cycle (Krebs cycle) and fatty acid metabolism, respectively (Newsholme 1980, Newsholme 1984). Skeletal muscle lipoprotein lipase (LPL) plays a central role in the trafficking of lipoprotein derived FAs (Nikkilä 1987), and it hydrolyses circulating lipoprotein triacylglycerols, liberates FAs for tissue uptake and changes lipoprotein composition in a way that may lower atherosclerotic risk (Nikkilä 1987, Seip & Semenkovich 1998).

3.4.1. Chemicals, enzymes and equipment

Carnitine acetyltransferase (CAT) from pigeon breast muscle (5 g/l), acetyl-CoA (trilithium salt), adenosine 5' monophosphate (AMP), adenosine 5' diphosphate (ADP), adenosine 5' triphosphate (ATP), nicotinamide adenine dinucleotide (NAD^+) (free acid) and NAD reduced (NADH) were from Boehringer Mannheim, Germany. Alpha-ketoglutarate dehydrogenase (KGDH), alpha-ketoglutarate (KG), L-carnitine-HCL, bovine serum albumin (BSA), imidazole base (grade III, low fluorescence blank) were from Sigma Chemical CO, St. Louis, MO, U.S.A.. Dithiothreitol (DTT) was obtained from Calbiochem, San Diego, CA, U.S.A.. All the other reagents were of analytical grade from Merck AG, Germany. The water used in analysis was prepared by the Millipore Super Q system. Transcon 102 FN Fluoro-nephelometer (Elomit Ltd, Helsinki, Finland) was used in skeletal muscle enzyme measurements. The excitation wavelength was 360 nm (Corning filter no. 5840) and emission wavelength 460 nm (Corning Nos. 4303 and 3387 with the latter facing the phototube).

3.4.2. The preparation of muscle samples for enzyme analysis (III, IV, V)

Human muscle samples. For the determination of enzyme activities, the muscle samples (ca. 10 mg) were homogenised in 10 volumes of 50 mmol/l Tris-HCl buffer, pH 7.4, containing 0.5 mmol/l DTT, 2 mmol/l $MgCl_2$ and 1 mmol/l ethylene-diaminetetra-acetic acid (EDTA). Later, the protein content of the water-diluted homogenates was measured (Lowry et al. 1951), and for better comparison of results, enzyme activities are expressed as µmol/min/mg protein.

Rat muscle samples. Twenty mg of muscle tissue was homogenised in ground-glass homogenizer with 10 vol of homogenising medium containing 50 mmol/l Tris-HCl buffer, pH 7.5, 0.5 mmol/l DTT, 2 mmol/l $MgCl_2$ and 1 mmol/l EDTA. The protein content of the water diluted homogenates was measured (Lowry et al. 1951), and for a better comparison of the results, enzyme activities are expressed as µmol/min/mg protein.

3.4.3. The preparation of muscle fibers for enzyme analysis (III)

Approximately 20 mg wet weight of gastrocnemius muscle was freeze-dried in aluminium holders in glass vacuum tubes in the freeze-dryer (Heat, Brokered, Denmark) at –45° C for 48 h. The dried samples were stored in vacum tubes at -25° C, and the tubes were always warmed up to room temperature before they were opened. Because freeze-dried material may absorb moisture and labile compounds may therefore be destroyed, dissection was done only on days when the relative humidity of the air was less than 50 %. The preparation of single muscle fibers (dissecting and weighting per sample) took 10 - 15 min, and according to knowledge and experience, this has no effect on the enzyme activities of frozen dried muscle samples at room temperature in relative humidity of below 50 %.

For the individual muscle fiber analysis, freeze-dried muscle fibers were separated under a stereomicroscope at room temperature at x 15 magnification, stained for ATPase to identify ST and FT fibers and analysed for enzyme activity. In this process, individual fibers (2–5 mm) were teased apart at room temperature and stored under vacuum at -25° C. For muscle fiber analysis, a piece of freeze-dried muscle fiber was cut from individual fibers for ATPase staining to identify the fiber as ST or FT before enzyme analysis. The fiber pieces to be identified were placed into water droplets on the microscope slide and the droplets were dried at room temperature for at least 30 min. To minimise the problem of identifying the fiber pieces as either ST or FT fiber, 20 µm sections of rat muscle were cut in a cryostat at - 28° C with a microtome and placed on the same microscope slide as the fiber pieces. Stained muscle sections were then used as reference guides for stained fiber pieces. In staining for actomyosin ATPase, the method adapted from Guth and Samaha (1970) was used.

When enzyme activities are measured in very small samples as in the present study, the weighing and the analysis must be performed with exceeding accuracy and precision. Two self-made, quartz-fiber fishpole balances, one ranging from 0.3 - 3.0 µg and another ranging from 1.0 - 10.0 µg, were used for weighing individual muscle fiber pieces (Lowry et al. 1972). The coefficient of variation of these balances were 1-2 %. The balances were calibrated and the linearity checked with p-nitrophenol crystals (Sigma Chemical Company, St. Louis, MO, U.S.A.). After measuring the deflection on the fishpole balance, the crystals were dissolved in 1 ml 0.1 mol/l NaOH and the absorbance measured in a spectrophotometer. A Sartorius analytical balance was used in reference weighings. For better comparison of results, enzyme activities are expressed as µmol/min/mg dry weight.

3.4.4. The measurement of enzyme activities in muscle samples (Study III, IV, V)

Phosphofructokinase. Assay medium was principally as described by Lowry et al. (1978) and contained 50 mmol/l Tris-HCl, pH 8.1, 1 mmol/l ATP, 2 mmol/l $MgCl_2$, 0.02 % BSA, 10 mmol/l K_2HPO_4, 1 mmol/l AMP, 1 mmol/l DTT, 10 µmol/l NADH, 50 U/ml triose-P-isomerase, 90 mU/ml aldolase and 1 U/l glyserin-3P-DH. The reaction was started by adding 2 mmol/l of fructose-6-P and followed kinetically at +25°C for 1-2 min. Two duplicated calibrators, containing 2 µmol/l and 4 µmol/l of fructose-1,6-P in final concentrations in the assay medium, were used and treated in the same way as the samples.

Ketoglutarate dehydrogenase. Assay medium in rats was principally as described by Read et al. (1977) with modifications for fluorometric determination. Assay medium contained 100 mmol/l Tris-HCl, pH 7.4, 2 mmol/l DTT, 1 mmol/l KCN, 0.4 mmol/l ADP, 1 mmol/l NAD^+ and 0.5 mmol/l CoA. The reaction was initiated by adding 1 mmol/l of KG. Formation of NADH was followed kinetically at +25° C for 2-3 min. In calculations, 5 µmol/l of NADH in the final concentration in assay medium was used as calibrator. Assay medium for KGDH in human skeletal muscle was principally as described by Cooney et al. (1981) with the following modifications for fluorometric determination: Assay medium contained 100 mmol/l Tris-HCl, pH 7.4, 250 mmol/l mannitol, 10 mmol/l KH_2PO_4, 10 mmol/l KCl, 5 mmol/l $MgCl_2$, 1 mmol/l DTT, 0.05 % Triton X-100, 1 mmol/l NAD^+ and 0.5 mmol/l CoA. The reaction was started by adding 1 mmol/l of KG. Formation of NADH was followed kinetically at +25 °C for 2-3 minutes.

Carnitine palmitoyl transferase I. The release of CoA in reaction with palmitoyl-CoA and carnitine was used as an index of enzyme activity. Assay medium was principally as described by Yates and Garland (1970) and contained 100 mmol/l Tris-HCl, pH 7.4, 80 mmol/l KCl, 1 mmol/l KCN, 1 mmol/l EDTA, 0.1% BSA, 1 mmol/l KG, 0.5 mmol/l NAD^+, 1 mmol/l carnitine and 50 mU/ml of KGDH. The reaction was started by adding 50 µmol/l of palmitoyl-CoA and followed kinetically at +25°C for 2-3 min. In calculations, 5 µmol/l of NADH was used as calibrator.

Carnitine palmitoyl transferase II. Formation of carnitine in the reaction between palmitoyl-carnitine and CoA was used as an index of enzyme activity. The method of Deufel and Wieland (1983) was used with the following modification: Carnitine formed in the reaction was measured using CAT. CoA formed in this reaction from acetyl-CoA was measured in the medium containing 50 mmol/l Imidazole-HCl buffer, pH 6.7, 1 mmol/l $MgCl_2$, 0.5 mmol/l EDTA, 0.5 mmol/l NAD^+, 0.5 mmol/l KG, 1 mmol/l DTT, 0.02 % BSA and 0.1 mmol/l acetyl-CoA and 40 mU of KGDH. Both 400 µl of this solution and 20 µl of a CoA-containing medium were pipetted to Transcon microcuvettes, initial fluorescence was read and the reaction started by adding 20 mU of CAT. The reaction was completed in 5 min at +25°C and the formation of carnitine was calculated. Carnitine in a concentration of 5 µmol/l was

used as calibrator.
Skeletal muscle LPL Skeletal muscle lipoprotein lipase (LPL) activity was measured from 3-8 mg of skeletal muscle pieces assayed for heparin-releasable lipoprotein lipase activity (Taskinen et al. 1980). Enzyme activity was expressed as µmol of FAs released from the triacylglyceride substrate in 1 h by 1 g of tissue.

3.4.5. The measurement of enzyme activities in muscle fibers (Study III)

Phosphofructokinase. For measurement of PFK activity, 0.30 - 0.50 µg fiber pieces were placed close to the bottom of the tube. The reaction was started by adding 100 µl of assay reagent to the tubes in an ice bath. The assay reagent was essentially the same as in homogenate measurement except that 100 µmol/l of NADH was used. The tubes were transferred to a 37° C water bath and incubated for 30 min. The reaction was stopped by boiling the tubes for 2 min. One ml of 50 mmol/l Tris-HCl buffer, pH 8.1, was added, and the tubes were cooled to +25° C before the fluorescence was read. Triplicate calibrators of fructose-1,6-P (in 4 µmol/l and 8 µmol/l final concentrations) were treated in the same way as the samples.
Ketoglutarate dehydrogenase. In the analysis, 0.5 - 2.0 µg of individual muscle fiber pieces were used. Because the muscle fiber pieces did not disintegrate in aqueous droplets (see Lowry et al. 1978), the fibers were placed in the tubes and incubated at +25°C for 30 min in 5 µl of reagent containing 100 mmol/l Tris-HCl, pH 7.4, 2 mmol/l DTT, 0.02 % BSA, 1 mmol/l $MgCl_2$, 0.5 mmol/l EDTA and 0.6 mol/l KCl. Thereafter, 10 µl of assay reagent containing 100 mmol/l Tris-HCl, pH 7.4, 2 mmol/l DTT, 0.02 % BSA, 2 mmol/l KCN, 1 mmol/l ADP, 2 mmol/l NAD+, 1 mmol/l CoA, 1 mmo/l $MgCl_2$, 0.5 mmol/l EDTA and 2 mmol/l KG was added in the tubes, and samples were incubated at 37° C for 60 min. The reaction was stopped by adding 5 µl of 6 mol/l NaOH and heating at +80° C for 20 min. To increase the sensitivity of the measurement, enzymatic cycling (Lowry & Passonneau 1972, Lowry, 1980) was used. The method, adapted from Hintz et al. (1980), in which glutamate dehydrogenase and glucose-6-phosphate dehydrogenase are used, gave a 300-fold amplification in one hour.
Carnitine palmitoyl transferase II. The measurement was performed with samples pooled from 3 - 6 individual fibers of the same type (total weight 5 - 7 µg from the same rat). The volume of the assay reagent was reduced to half of that used in the homogenate measurement. The tubes were incubated at +37° C for 60 min, and the reaction was stopped by adding 10 µl of 0.7 mol/l perchloric acid. After neutralisation with 3 µl of 2.5 mol/l $KHCO_3$ and centrifugation for 10 min at 3000 g, 20 µl of supernatant was taken for carnitine measurement as described earlier.

4. The measurement of physical fitness (Study I, IV, V)

The fitness of the healthy men was assessed during a graded bicycle-ergometer test to volitional exhaustion. In this test, expiratory gas flow, oxygen and carbon dioxide concentrations were measured with an automated open circuit gas analysis system (Oxycon-4, Mijnhardt, the Netherlands), which was calibrated against gas mixtures of known concentrations before each test. The highest oxygen uptake per minute reached was defined as the maximal oxygen uptake (VO_{2max}) and expressed as ml/min/kg of body weight. In these tests, no evidence of coronary heart disease (as judged by a normal exercise ECG and a symptomless performance at heart-rate level above 90 % of the age-related maximum) was observed. The fitness of CHD patients was assessed with a progressive, symptom-limited exercise-tolerance test on an electrically braked cycle ergometer, and VO_2max was estimated using the formula: 12.3 x peak power in Watts (Mertens et al. 1994). When fitness was expressed (in the study IV) as metabolic equivalents (METs, see below) VO_{2max} as ml/min/kg was divided by 3.5 which is the oxygen consumption at rest.

5. The assessment of physical activity (Study IV, V, VI)

A questionnaire as well as a personal interview provided the data for the assessment of the healthy men's leisure-time physical activity (LTPA). The intensity of physical activity was expressed in metabolic units (metabolic equivalents of oxygen consumption, MET). MET is a ratio of the metabolic rate during exercise to the metabolic rate at rest, which corresponds to oxygen consumption of 3.5 ml/min/kg body weight. The list of activities included the most common leisure-time activities of Finnish men. The healthy men (Study VI, V) were asked to record the frequency and average duration in hours and minutes per session. Physical activity was categorised according to type: walking 4.2 MET, jogging 10.1 MET, cross-country skiing 9.6 MET, cycling 5.8 MET, swimming 5.4 MET, rowing 5.4 MET, ball games 6.7 MET, gymnastics or weight lifting 5.0 MET. The former athletes and their controls (VI) were divided into two classes according to their participation in vigorous activity in 1985. The volume of physical activity in 1985 was computed from responses to three structured questions. Those whose exercise intensity usually corresponded to jogging or running were vigorous exercisers. By assigning a MET score to activity and calculating the product of intensity times duration times frequency of activity, the cumulative leisure-time MET-hours per week (LTPA index) was calculated (Study IV,VI) (Kujala et al. 1994). Since one MET corresponds to an energy expenditure of approximately one kilocalorie per kilogram of body weight per hour, the LTPA index (in kcal/wk) was calculated by multiplying body weight by activity (in METs) and duration (in hours) of activities per week (Study V).

6. Exercise training programs

6.1. Healthy men (Study V)

The training of the 12 men consisted of self-conducted, mostly home-based exercise. In order to have long-term compliance, the participants were encouraged to increase the kind of physical activity (mostly ball games) they were already engaged in. The men either had no leisure-time physical activity or it was at the lower range of the recommendations (Pate et al. 1995) but according to the activity level reported they can be considered as sedentary before training (Stephens & Caspersen 1994). In order to have long-term compliance, the men were encouraged to increase the kind of physical activity they were familiar with. According to the training diaries their physical activities were jogging, cross-country skiing, cycling and ball games, and such activites usually classified as vigorous. An exercise training prescription and general information on the health benefits of exercise training were given to the whole study group of 17 men (the 12 Sedentary men and 5 men in the internal control group) at the monthly meetings. The subjects submitted their LTPA diaries from the previous month at these meetings. They were also interviewed to confirm their continuous training, and those needing individual guidance for training received this. The weekly goal of the exercise program for LTPA was set at 1000 - 2000 kcal divided into 3 - 5 sessions per week. Thus, the men were instructed to increase their LTPA ca. 100 kcal/wk every month. All 17 men attending the meetings were discouraged from altering their diet composition during the year.

6.2. Animals (Study III)

Sixteen rats trained five days per week over an eight-week period on a tread-mill. The running speed on the treadmill was 10 m/min and the running time per day was progressively increased from 15 min during the first week to 2 h during the last week of training. The training of the rats represented intensity approximately 50 % of maximum (Gillespie et al. 1982, Sonne 1989, Patch & Brooks 1980) and thus, it can be consider as low- to moderate intensity exercise. Sixteen sedentary rats served as controls. For the acute exercise experiment eight randomly selected sedentary rats were made to run on a treadmill for 15 min twice a week. To study the acute effects of exercise, four trained and four sedentary rats ran on a treadmill at a speed of 20 m/min until exhaustion. Running time for trained and sedentary rats was 2-3 and 0.5–1.5 h, respectively.

7. Other methods

Body mass index (BMI) was used as a measure of relative body weight and was calculated as weight (in kg) divided by height (in m) squared (kg/m^2).
Subcutaneous adipose tissue of the healthy men (in Study IV and V) was estimated by measuring skinfold thicknesses from four standard sites - subscapular, triceps, biceps and suprailiac skinfold regions - from the right side of the body were measured with a Harpenden calliper by the same experienced technician. The sum of the four skinfolds was used as indicator of subcutaneous adipose tissue. The intra-assay imprecision of this method is 3 %.
Onset of blood lactate accumulation (OBLA), For the measurement OBLA (in Study V), venous blood was collected by a catheter from an antecubital vein during the last 15 s of every 3-min stage in their exercise testing. The OBLA was determined from these venous blood samples by using both the increase of lactate 1 mmol/l above the baseline criterion (OBLA-1) as described by Coyle et al. (1995), and the fixed level of 4 mmol/l (OBLA-4) criterion as described by Karlsson et al. (1984). The OBLA points are the work loads in Watts in corresponding levels of blood lactate. Perchloric acid extracts of venous blood were made for measurement of blood lactate, which was determined using fluorometry (Lowry & Passonneau 1972).
The total blood volumes of the men before and after training (Study V) were measured using an isotope dilution method in which autologous red cells were labeled with radioactive ^{99m}Tc (Thomsen et al. 1991).
Isometric trunk extension torque (TET) in Newtons per kilogram of body weight (N/kg) was measured (Study V) on a dynamometer. For the measurement of TET, the subjects were pulling a handle in a sitting position. Three maximal isometric trials lasting 3 - 5 s were performed, and the best TET was used for further analysis.

8. Statistical methods

For the statistical analyses, computer programs StatView 4.5 and SuperAnova (Abacus Concepts, Inc., 1984 Bonita Ave., Berkeley, California, USA) and JMP statistical software (SAS Institute Inc., Cary, NC, USA) were run in an Apple Macintosh (model Quadra 650 and 700) computer. The Confidence Interval Analysis (CIA) (Gardner & Altman 1990) program and BMDP (Dixon 1992) were run in a PC. Results are presented as mean values and standard deviations (SD) (IV,V), mean values and standard error means (SEM) (III) or mean values with 95 % confidence intervals (95 % CI) (I,V). All P-values are based on a two-sided alternative hypothesis, and P less than 5% was considered statistically significant.

Univariate associations between the variables were estimated as Spearman's rank correlation coefficients (r_s). The Wilcoxon Signed-Rank test was used in the paired

comparison of the results. The Mann-Whitney U test was used in the unpaired comparison of the results. When the overall differences among the study groups were tested with the Kruskal-Wallis test, the pairwise comparisons of the group means were performed using the Mann-Whitney U-test and the significance levels in these comparisons were adjusted using the Bonferroni method. Shapiro-Wilk W-test in JMP statistical software (SAS Institute Inc., Cary, NC, USA) (Lehman 1989) was used to test the normality of the distributions, and the variables were transformed, when necessary, to correct for the marked skewness of the distributions.

Analysis of variance (ANOVA) and Fisher's Protected Least Significant Difference (PLSD) or Dunnett's Test for multiple comparisons were used in post-hoc multiple comparison procedures when comparing the groups. When the two qualitative factors, time of the measurements (rest or exercise), and the training state (sedentary or trained) and their influence on the enzyme activities in each muscle were compared in study III, statistical analyses were performed using the two-way analysis of variance. Analysis of covariance (ACOVA) and multiple regression analysis were used as the multivariate techniques in order to control the possible confounding effects of other variables (age, BMI, smoking, alcohol consumption, group).

The K-means clustering technique (Dixon 1992) was used in order to see whether the individuals studied could be formed into a natural system of groups by using variables having strong associations with HDL-C. In this method, the number of groups is not specified in advance. The subjects fall into groups determined by the values of the variables. The individuals within a group resemble each other in the values taken by the variables more than the individuals in different groups do.

The extent to which selection to specific types of sports at a young age predicts later physical activity in 1985 and prevalence of CHD in 1985 and 1995 were analysed. Differences between groups in 1985 were analysed using ANCOVA, and the Newman-Keuls test was used for post hoc comparisons. The odds ratios (OR) and their 95 percent confidence intervals (95% CI) for CHD in 1985 and 1995 for different athletic groups were compared to controls using logistic regression models (Dixon, 1992). In addition, to test the hypothesis endurance athletes were tested with power and speed athletes. Age, BMI, smoking and occupational group were included as confounding factors in the analysis and P values here are from Wald's test. In analysing those without CHD in 1985 to determine whether selection to sports at young age or activity in 1985 was the stronger predictor for CHD in later life, the OR and their 95% CIs for the occurrence of CHD from 1986 to 1995 were calculated using logistic regression models, adding the physical activity variables in 1985 to the aforementioned confounding factors.

RESULTS

1. Muscle fiber properties and CHD risk (Study I, IV, V, VII)

The mean value of the muscle fiber distribution in all men studied (VII) was 51 %, ranging from 12 to 88 %, and the mean value in all the CHD patients studied was significantly lower than that in all the healthy men studied (Figure 3). The percentage of ST-fibers in nine out of ten men who participated in jogging (Study I) was more than 50 whilst that in more than half of sedentary men and in nearly two thirds of the CHD patients was less than 50.

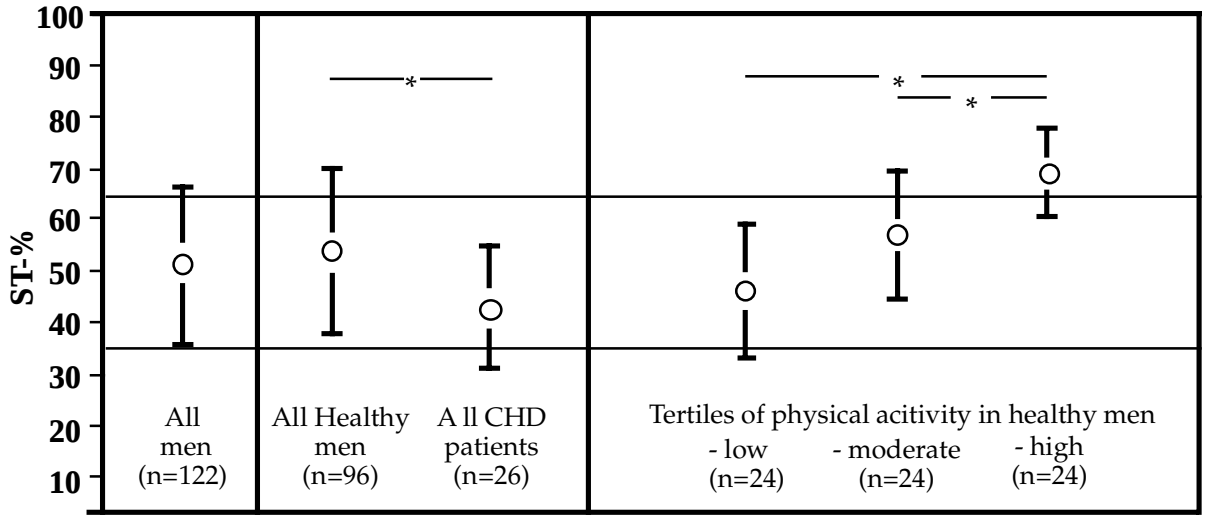

Figure 3. Muscle fiber distribution (ST-%) in all men studied, in all healthy men and in all CHD patients. Tertiles of physical activity according to LTPA index in 72 healthy men are from the Study IV. Values are mean ± SD. Horizontal lines are in 35 and 65 % of the distribution. * denotes significant difference $p<0.01$.

When the 72 healthy men (Study IV) were divided into tertiles according to their physical activity, the men in the highest tertile of activity had higher ST-%s than the men in the other two tertiles (Figure 3). Similarly, the men in the highest tertile of VO_{2max} had higher ST-%s than the men in the other two tertiles (Table 4). However, the distribution of muscle fibers in the men in the lowest tertiles of VO_{2max} and physical activity did not differ significantly from that in the CHD patients (Table 4 and see Study IV, Tables 5 and 7). In addition, the CPT enzyme activities in the skeletal muscle of the men in the lowest tertiles were similar to those observed in the CHD patients. The activities of CPT in the men in the highest tertiles of VO_{2max} and physical activity were higher than the activities of the men in the lowest tertiles.

Table 4. Body adiposity (BMI and sum of skinfolds), maximal oxygen uptake (VO_{2max}), leisure-time physical activity index (LTPAI), skeletal muscle properties, serum lipids and lipoproteins and serum levels of insulin, sex hormone binding glopbulin (SHBG) and testosterone in the CHD patients, in the healthy men and in healthy men divided in tertiles of fitness according to their VO_{2max}. Mean (SD).

Variable		Healthy men (n=72)	Low fitness (n=24)	Moderate fitness (n=24)	High fitness (n=24)
BMI (kg/m^2)	28.0 (3.6)$^{(\#,1,2,3}$	23.6 (2.5)	24.7 (2.1)	23.9 (2.6)	22.3 (2.3)
Sum of skinfolds (mm)	-	40.1 (13.0)	47.7 (11.8)	41.3 (10.4)	31.2 (11.5)
VO_{2max} (ml/min/kg)	16 (7)$^{(\#,1,2,3}$	46 (11)	34 (5)	46 (3)	59 (5)
LTPAI (METs x hour /wk)	-	32.6 (29.1)	8.4 (13.4)	27.3 (16.8)	62.0 (24.5)
Skeletal muscle					
ST-%	44 (13)$^{(\#,2,3}$	57 (15)	46 (13)	56 (13)	69 (9)
KGDH (µmol/min/mg prot)	4.9 (2.8)$^{(\#,1,2,3}$	8.8 (3.1)	7.8 (2.4)	9.4 (2.3)	9.3 (4.1)
CPT (µmol/min/mg prot)	0.28 (0.18)$^{(\#,2,3}$	0.48 (0.22)	0.39 (0.22)	0.52 (0.18)	0.54 (0.22)
PFK (µmol/min/mg prot)	45.0 (31.0)	38.1 (33.3)	39.4 (26.3)	40.7(35.4)	34.3 (38.1)
CPT/KGDH x 10^{-3}	53 (28)	57 (33)	47 (18)	57 (22)	67 (50)
PFK/CPT	210 (154)$^{(\#,1,2,3}$	99 (99)	139 (115)	92 (102)	69 (61)
Serum lipids					
Triglycerides (mmol/l)	2.39 (1.00)$^{(\#,1,2,3}$	0.99 (0.32)	1.01 (0.29)	1.04 (0.32)	0.92 (0.33)
Cholesterol (mmol/l)	6.9 (1.5)$^{(\#,1,2,3}$	5.6 (0.9)	5.9 (1.0)	5.8 (0.9)	5.3 (0.8)
HDL-C (mmol/l)	0.91 (0.18)$^{(\#,1,2,3}$	1.55 (0.32)	1.34 (0.18)	1.54 (0.32)	1.76 (0.30)
LDL-C (mmol/l)	4.90 (1.30)$^{(\#,1,2,3}$	3.64 (0.95)	4.05 (0.95)	3.76 (0.91)	3.11 (0.76)
Apo A-I (g/l)	1.36 (0.22)$^{(\#,1,2,3}$	1.98 (0.36)	1.76 (0.32)	1.99 (0.33)	2.20 (0.29)
Hormonal variables					
Insulin (mU/l)	13.8 (7.5)$^{(3}$	11.0 (11.0)	17.1 (13.5)	11.5 (10.5)	5.0 (2.0)
SHBG (nmol/l)	29 (13)$^{(3}$	32 (12)	23 (9)	33 (10)	38 (10)
Testosterone (nmol/l)	19.6 (6.6.)$^{(\#,1}$	23.1 (6.3)	24.6 (8.8)	21.9 (4.7)	22.7 (4.3)
Free-Testosterone (pmol/l)	324 (87)$^{(1}$	372 (100)	435 (132)	346 (58)	337 (63)

$^{\#)}$ significant ($p<0.05$) difference from all healthy men
$^{1,2,3)}$ significant ($p<0.05$) difference from low, moderate or high fit men, respectively .

A high positive correlation was found between the ST-% and both the VO_{2max} and the LTPA index in all the healthy men (Table 5). A high correlation between VO_{2max} and LTPA index was observed as well. Both ST-% and VO_{2max} correlated positively with KGDH activity and with CPT activity. The LTPA index correlated positively with CPT activity. The PFK activity did not show significant correlations with ST-%, LTPA index or VO_{2max}, but its ratio to CPT correlated negatively with ST-%, VO_{2max} and LTPA index.

Table 5. Significant correlations (r_S) between skeletal muscle properties, VO_{2max} and physical activity (LTPA index) in healthy men (n=72) in Study V.

Variable	VO_{2max}	LTPAI	KGDH activity	CPT activity	PFK/CPT
ST-%	0.62***	0.62***	0.25*	0.29**	- 0.33**
VO_{2max}	-	0.81***	0.29*	0.36**	- 0.32**
LTPA index	-	-	-	0.33**	- 0.35**

* = p<0.05, ** = p<0.01, ** *= p<0.001

2. Muscle fiber properties and serum lipids and lipoproteins (Studies I, IV)

High positive correlations between the ST-% and serum HDL-C concentration and between the ST-% and serum apo A-I concentration were found (Table 6) in all men investigated (see Study I, Table 3 and Figures 2 and 3).

Table 6. Correlations (r_S) between ST-% and serum levels of HDL-C, apo A-I and triglycerides in all men (n=102) in Study I.

Variable	HDL-C	Apo A-I	Triglycerides
ST-%	0.57*	0.60*	- 0.43*

* = p<0.001

On the contrary, a negative correlation was observed between the ST-% and concentration of serum triglycerides (Table 6). This negative association was especially observed in sedentary men and in CHD patients separately (see Study I, Table 3). The ST-% significantly associated with serum HDL-C and serum apo A-I values after taking into account age, body mass index, smoking, alcohol consumption and physical activity in the regression model.

The ST-%, VO_{2max} and LTPA index correlated positively with serum HDL-C and with apo A-I but negatively with serum LDL-C in the healthy men of Study IV (Table 7). The most physically active and fit men had the lowest serum LDL-C concentrations (Table 4), (see also Study IV, Tables 4 and 5). Serum cholesterol did not show significant correlations with ST-%, VO_{2max} or LTPA index. Significant differences in serum cholesterol or serum triglyceride concentrations between the tertiles of physical activity or VO_{2max} were not observed (Table 4).

Table 7. Correlations (r_S) of ST-%, VO_{2max} and physical activity (LTPA index) with serum HDL-C, LDL-C and apo A-I in the healthy men in Study IV.

Varibles	HDL-C	LDL-C	Apo A-I
ST-%	0.54 ***	-0.26 *	0.54 ***
VO_{2max}	0.54***	- 0.37**	0.56***
LTPA index	0.51***	- 0.32**	0.53***

* = p<0.05, ** = p<0.01, *** = p<0.001

3. Other risk factors for CHD (Study IV)

In the healthy men, BMI correlated negatively with the ST-%, and a negative correlation was found between subcutaneous adipose tissue measured as skinfolds and the ST-% as well (Table 8). The strongest association was observed between ST-% and the skinfold of spina iliaca in the abdominal region (r_s = -0.42, p<0.001). Serum triglycerides correlated positively with BMI and with the sum of skinfolds. On the contrary, negative associations were observed between BMI and serum HDL-C concentration as well as between the sum of skinfolds and serum HDL-C concentration (Table 8).

Table 8. Correlations (r_S) of body adiposity (BMI and sum of skinfolds) with ST-% and serum levels of triglycerides and HDL-C in healthy men in Study IV.

Variable	ST-%	Triglycerides	HDL-C
BMI	- 0.38	0.44***	- 0.30 *
Sum of skinfolds	- 0.34	0.35**	- 0.41***

* = p<0.05, ** = p<0.01, *** = p<0.001

When the healthy men were divided into tertiles according to their VO_{2max} and LTPA index (see Study IV, Tables 4 and 5), the men in the highest level of VO_{2max} and physical activity were characterised with a lower BMI and subcutaneous adipose tissue when compared with most unfit and sedentary men. When the healthy men were divided in two groups according to their BMI, the 25 overweight men had higher serum TG concentrations than the 47 lean men. No other significant differences in risk factors for CHD were observed between the overweight and the lean men, however.

Serum total testosterone and estradiol or free estradiol were not significantly different between the fitness and physical activity tertiles. Serum total and free testosterone or estradiol, or serum DHEAS did not significantly differ between the

CHD patients and the healthy men after adjusting age (see Study IV, Tables 1 and 7). Serum free testosterone was the highest in the men with the lowest VO_{2max} and physical activity. On the other hand, serum SHBG, was the highest in the men in the highest tertile of VO_{2max} (Table 4) and physical activity (see Study IV Tables 5 and 7).

In healthy men, significant correlations were not observed between serum total testosterone and serum lipids and lipoproteins. However, serum free testosterone correlated negatively with serum HDL-C and with apo A-I levels and, moreover, with VO_{2max} and with LTPA index (see Study IV, Tables 2 and 3). Serum SHBG showed positive correlations with serum HDL-C and apo A-I levels as well as with VO_{2max} and LTPA index (Table 9). Serum SHBG associated negatively with sum of skinfolds, serum LDL-C and with serum insulin (Table 9). Serum insulin correlated negatively with serum levels of HDL-C and apo A-I but positively with serum LDL-C level and sum of skinfolds. The correlation between VO_{2max} and LTPA index with serum insulin level was highly negative. (Table 9)

Table 9. Significant correlations (r_S) of serum SHBG and insulin with other clinical variables

Variables	HDL-C	Apo A-I	LDL-C	VO_{2max}	LTPA index	ST-%	Sum of skinfolds	Insulin
SHBG	0.47	0.48	-0.28	0.54	0.54	0.47	-0.37	-0.53
Insulin	- 0.45	-0.45	0.39	-0.54	-0.63	-0.63	0.35	-

* = p<0.05, ** = p<0.01, *** = p<0.01

The ST-% showed a strong negative correlation with serum insulin level (rs = -0.63, p<0.001) but correlated positively with serum SHBG level (rs=0.47, p<0.001). A high serum insulin concentration (> 20 mU/l) was found only in 15 sedentary or moderately active men but not in any of the highly active men.

4. The effects of progressive training on skeletal muscle fibers of rats (Study III)

In the sedentary rats, the activity of the glycolytic enzyme PFK in fast muscle was 1.4 times as high as that in the the mixed muscle and 3.4 times as high as that in the slow muscle. The activities of the mitochondrial enzymes CPT and KGDH in the slow muscle were 1.3 and 1.7 times as high as these activities in the fast muscle. Correspondingly, these activities were 1.3 and 2.8 times as high as those in the slow muscle than in the mixed muscle. (Study III, Table 1)

In the acute exercise experiment, the trained rats ran twice as long as the sedentary rats. After acute exercise, the activities of mitochondrial enzymes KGDH and CPT in

the skeletal muscles of both trained and untrained rats tended to increase, but the activity of PFK did not increase (see Study III, Table 1). Similarly, higher activities of the mitochondrial enzymes KGDH and CPT were observed in the gastrocnemic muscle of the trained rats when compared with untrained rats (Figure 4). However, the activity of PFK in the gastrocnemic muscle did not differ between the untrained and trained rats. After training, a higher activity of the oxidative enzyme KGDH was observed both in the ST and in the FT fibers, but the activity of the enzyme in fatty acid metabolism, CPT, was higher only in the ST fibers (Figure 4).

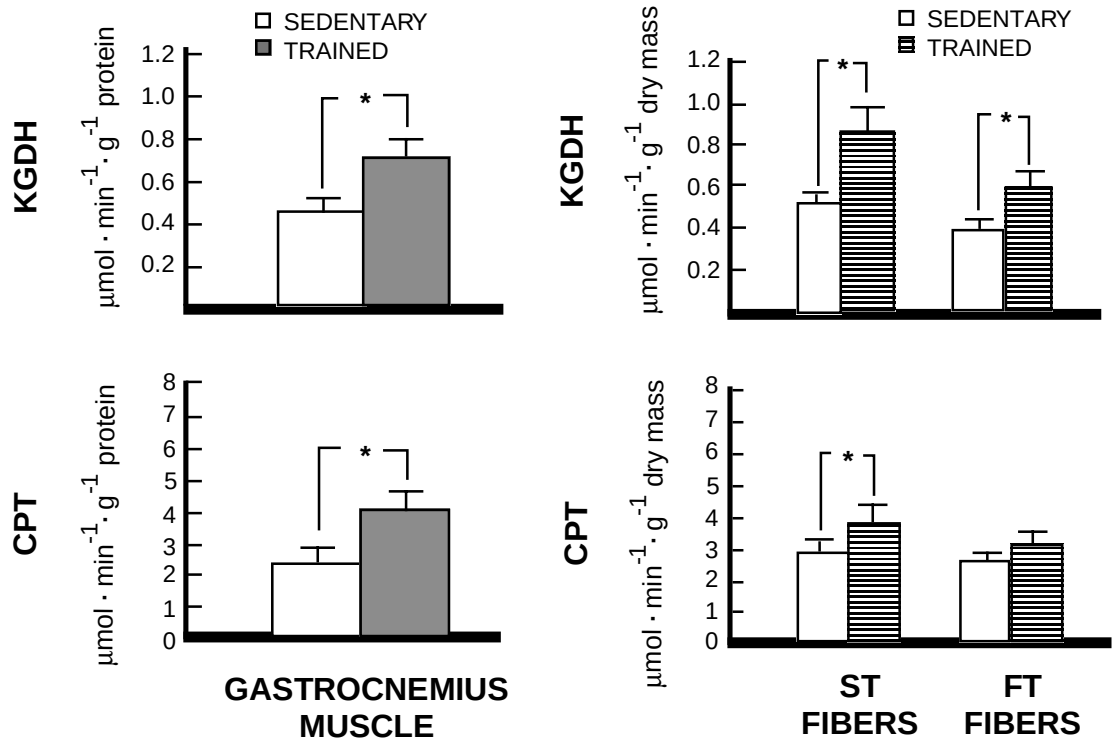

Figure 4. The activities of KGDH and CPT in the gastrocnemic muscle and in ST and FT fibers of sedentary and trained rats. * denotes $p < 0.05$

5. The effects of training in sedentary men (Study V)

Before training, the VO_{2max} mean value of of the sedentary men was 32 ml/min/kg. In 12 months, the men increased their LTPA from a mean of 728 to 1526 kcal/wk. Thus, the recommended level of physical activity (Pate, 1995) was reached during the study year. Their training was effective because the men increased their VO_{2max} and isometric trunk extension torque (Figure 5). Although VO_{2max} increased, the total blood volume before and after training was the same. The VO_{2max} level of the five men in the internal control group was similar to that of the men in the training group. VO_{2max} did not change significantly during the follow-up in the control group. The weight of the trained men did not change but their skinfold thickness

measured from the abdominal (spina iliaca) area decreased by 12 %.

The skeletal muscle LPL activity increased by 65 %, and the higher the activity was before training, the larger the increase tended to be (see Figure 2 in Study V). Skeletal muscle KGDH and CPT enzyme activities were markedly increased after training and attained the level observed in previously physically active men (see Tables 10 and 11). Training also markedly increased the work loads in Watts in the two OBLA points determined (Figure 5). However, neither PFK activity nor the PFK to KGDH ratio changed significantly, but a marked decrease in the PFK to CPT ratio was found (see Tables 10 and 11).

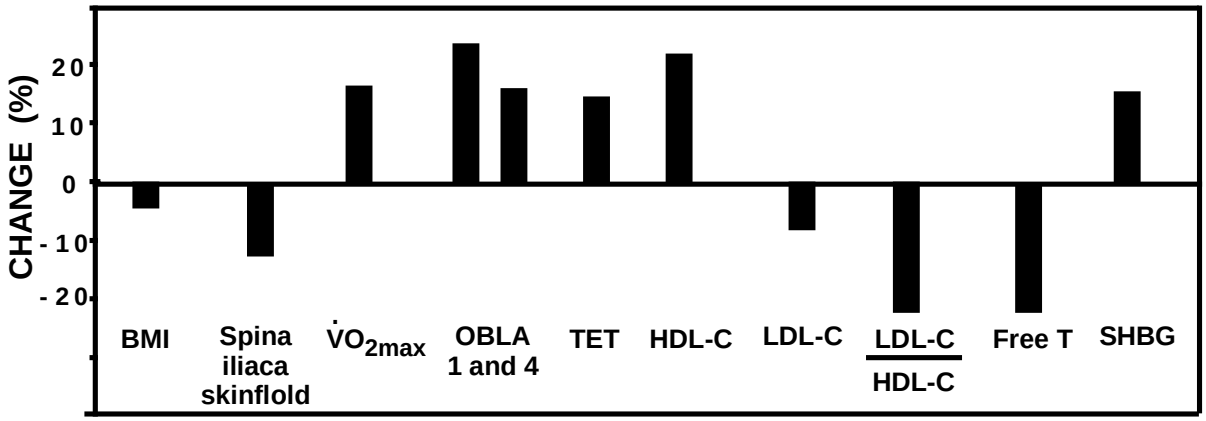

Figure 5. Effects of 12 months of training in 12 previously sedentary men. Mean of change (%) from pre-training values presented. TET is trunk extension torque and Free T is free testosterone. For other abbreviations, see text.

After training, a large increase in serum HDL-C and a large decrease in serum LDL-C and in the ratio of LDL-C to HDL-C were observed (Figure 5). The higher the serum HDL-C concentration was before training, the larger the increase in it tended to be (see Figure 2 in Study V). The increase in skeletal muscle CPT activity correlated (r_S = 0.81, $p < 0.01$) with the increase in HDL-C concentration. Training did not significantly alter serum triglyceride or cholesterol levels but the ratio of cholesterol to HDL-C decreased by 17 %. Total and free testosterone concentrations decreased considerably and serum SHBG concentration increased after training. Serum LH concentration also decreased by 32 %. The concentrations of serum estradiol, free estradiol or DHEAS were not changed after training, but the ratios of free estradiol to free testosterone and serum DHEAS to free testosterone were higher after training (95 and 25 %, respectively).

6. The comparison of healthy men before and after training with CHD patients and with physically active men (Study V)

Test results of sedentary men before and after training were compared with results of twelve CHD patients and 30 physically active men of the same age (Tables 10 and 11). The ST-% and the skeletal muscle enzyme activites of the sedentary men and CHD patients did not differ significantly. However, the CHD patients had higher serum SHBG and lower serum free testosterone levels than the sedentary men before training (Table 10).

Table 10. ST-%, activities of skeletal muscle enzymes and their ratios and serum levels of testosterone and sex hormone binding globulin (SHBG) of the previously sedentary men before training compared with the CHD patients. Mean (SD).

Variable	Sedentary men before training (n=12)	CHD patients (n=12)
ST-%	42 (12)	44 (16) #
KGDH (µmol/min/mg prot)	6.3 (2.3)	4.9 (2.4) #
CPT (µmol/min/mg prot)	0.29 (0.12)	0.23 (0.16) #
PFK (µmol/min/mg prot)	42 (36)	47 (39) #
PFK/KGDH	7.2 (5.9)	9.7 (5.9) #
PFK/CPT	204 (176)	241 (150) #
Testosterone (nmol/l)	24.5 (9.1)	20.7 (8.1)
SHBG (nmol/l)	21 (5)*	31 (15)
Free Testosterone (pmol/l)	448 (144)*	331 (106)

* = $p<0.05$ between sedentary men before training and CHD patients
# $p < 0.05$ between CHD and physically active High-ST-Men (see Table 11).

After training, the KGDH and CPT activities of the healthy men were higher than activities observed in CHD patients. After training total and free testosterone of the healthy men decreased to the same level observed in both in the CHD patients and in the healthy, physically active men. (Tables 10 and 11).

The 30 physically active (activity more than 3 times per week) men were divided equally into two groups according to their muscle fiber distribution (high-ST-men and low-ST-men). The mean difference in the ST-% between the high-ST-men and low-ST-men was 24 %. The sedentary men and the physically active low-ST-men did not differ from each other with respect to ST-%, but the difference between the high-ST-men and the sedentary men was 31 %.

The high-ST-men and low-ST-men had higher VO_{2max} than the previously sedentary men both before and after the training. The LTPA index for the high-ST-men was 9 times as high as that of the sedentary men before training and 2.5 times as high as that after the training. Moreover, the high-ST-men engaged in almost twice as much leisure physical activity as the low-ST-men. (Table 11)

After training both KGDH and CPT activities in skeletal muscle of the previously sedentary men increased to the levels observed in physically active men, but PFK activity and the ratios of PFK to KGDH and PFK to CPT remained higher after training in the previously sedentary men when compared with physically active high-ST-men.

Table 11. Maximal oxygen uptake (VO_{2max}), leisure-time physical activity (LTPA), activities of skeletal muscle enzymes and their ratios and serum levels of testosterone and sex hormone binding globulin (SHBG) of the previously sedentary men after training compared with the physically active men with low ST-%s and with physically active men with high ST-%s. Mean (SD).

Variable	Men after training (n=12)	Low-ST-Men (n=15)	High-ST-Men (n=15)
VO_{2max} (ml/min/kg)	37 (5)*	46 (5)	56 (7)
LTPA (kcal /wk)	1526 (459)*	2137 (1367)	3845 (2043)
KGDH (µmol/min/mg prot)	8.9 (3.0)§	9.1 (2.4)	9.7 (3.)
CPT (µmol/min/mg prot)	0.44 (0.18)§	0.47 (0.16)	0.56 (0.23)
PFK (µmol/min/mg prot)	48 (43)#	32 (23)	19 (16)
PFK/KGDH	4.9 (3.5)#§	3.7 (2.7)	2.1 (1.4)
PFK/CPT	110 (83)#§	79 (64)	38 (28)
Testosterone (nmol/l)	18.8 (3.5)	22.5 (5.5)	23.0 (3.3)
SHBG (nmol/l)	24 (9)#	31 (15)	36 (10)
Free Testosterone (pmol/l)	335 (62)	358 (67)	350 (48)

* = $p<0.05$ between men after training and both groups of physically active men.
# $p < 0.05$ between men after training and physically active High-ST-Men.
§ $p<0.05$ between men after training and CHD patients (see Table 10)

The high-ST-men had the highest serum HDL-C levels but also the lowest serum LDL-C levels when compared with the low ST-men. These groups did not differ from each other with respect to serum testosterone, estradiol, free estradiol and DHEAS concentrations. The sedentary men had the lowest serum SHBG concentrations and the highest free testosterone concentrations and the highest ratios of free testosterone to free estradiol before they started training (Tables 10 and 11). After training, the corresponding values were similar to those observed in CHD patients and in active men.

7. Cluster analysis in healthy men (Study IV)

Multiple regression analysis showed that ST-%, fitness and LTPA index explained 32 % of the variation in HDL-C in the healthy men. Cluster analysis was used to find out which parameters create natural groups in the study population, thus exhibiting strong associations with serum HDL-C levels. The number of groups was not specified in advance. The best result was obtained in three clusters, when leisure-time physical activity (categorised as no leisure-time physical activity per week, physical activity 1-3 times per week, physical activity more than 3 times per week), fitness, ST-% and serum SHBG were taken into the clustering model. In this model, the first cluster showed the physically active men (n=20) of whom 60 % of the cluster members had leisure-time exercise sessions more than 3 times per week. In the inactive men (n=27) in the second cluster, 67 % of the members reported no leisure-time physical activity per week. In the third cluster (n=25) the men were fit but only 44 % of these men reported exercising 1- 3 times per week, and 20 % of them reported no leisure-time activity per week. (Table 12)

Table 12. Mean values (SD) of the characteristics of 72 healthy men divided into three clusters in Study IV.

Variable	Active (n=20)	Inactive (n=27)	Fit (n=25)	P-value*
ST-%	66 (10)	42 (9)	66 (9)	0.0001
VO_{2max} (ml/min/kg)	52 (10)	37 (8)	51 (9)	0.001
CPT-activity (µmol/min/ mg prot)	0.57 (0.21)	0.38 (0.21)	0.52 (0.22)	0.006
PFK/CPT	55 (45)	149 (129)	81 (68)	0.002
HDL-C (mmol/l)	1.83 (0.35)	1.36 (0.20)	1.54 (0.24)	< 0.0001
LDL-C (mmol/l)	3.30 (0.92)	4.00 (0.81)	3.52 (1.02)	0.03
Insulin (mU/L)	5.8 (4.5)	19.3 (13.7)	3.3 (3.9)	<0.0001
Free testosterone (nmol/l)	340 (54)	417 (132)	351 (61)	0.01
SHBG (nmol(l)	46 (7)	23 (8)	29 (5)	0.0001

* from ANOVA

The physically active men had high ST-%s, high levels of fitness, and high serum HDL-C and serum SHBG concentrations but low serum insulin concentrations and low serum LDL concentrations. On the contrary, the inactive men had low ST-%s, low levels of fitness and their mean values for skeletal muscle enzyme activities, serum HDL-C, LDL, SHBG and insulin were different when compared with physically active men. In addition, they had higher serum free testosterone concentrations than the men in the other two clusters. The fit men had high levels of

VO_{2max} high ST-%s, and their mean values for HDL-C, LDL-C, SHBG and serum insulin were between the values of the two other clusters.

8. Physical activity and CHD in former athletes and controls (Study VI)

More members in all groups of the former athletes (endurance, power-speed and others) participated more often in vigorous physical activity in 1985 than the controls (Table 13). The mean total volume of leisure-time physical activity in 1985 in terms of the LTPA index was significantly higher in all former groups of athletes than in the controls. The former endurance athletes participated more often in vigorous physical activity than the former power-speed athletes (age-adjusted P = 0.006) and had a higher LTPA index (age-adjusted P = 0.015). In addition, BMIs in the former endurance athletes were lower than in the former power-speed athletes, in other athletes and in controls. BMIs of the former power-speed athletes were higher than those of controls.

Table 13. Percentage of participation in vigorous physical activity in the groups of former athletes and controls in 1985. Mean total volume of leisure-time physical activity in terms of the LTPA index and body mass index (BMI) of groups of athletes and controls.

Variable	Controls (n=743)	Power-speed athletes (n=235)	Other athletes (n=834)	Endurance athletes (n=166)	P-value *
Participation for vigorous activity	12 %	28 %	38 %	37 %	< 0.001*
LTPA index (METs x hour /wk)	15	32	29	38	< 0.001*
BMI (kg/m^2)	26.4	27.1	26.1	24.4#	

* age-adjusted P-value for all comparisons between former groups of athlete and controls.
# significantly lower than in other groups.
significantly higher than in controls.

In 1985 and in 1995, after adjustment for age, occupational group and smoking, both endurance and other athletes had less CHD compared with controls, while the difference between controls and power-speed athletes was non-significant. After the same adjustments, former endurance athletes had significantly less CHD than power-speed athletes both in 1985 with the odd ratio for CHD, 0.34 (95%CI from 0.17 to 0.73, P = 0.004) and in 1995 with the odd ratio for CHD, 0.51 (95%CI from 0.30 to 0.87, P = 0.015).

Even though the 1985 group differences in CHD prevalence persisted in 1995, there were no group differences in CHD incidence from 1986 to 1995 among those without manifest CHD in 1985 (n=1688). In contrast, among those free of CHD in 1985 but the

non-participants in vigorous physical activity, 15 % had CHD before 1996, compared with 6 % who did participate in vigorous activity (age-adjusted $P < 0.001$). The mean LTPA index in 1985 was 27 for those who did not acquire CHD during the next ten years, compared with 20 for those who did (age-adjusted $P = 0.042$). In order to determine the most important predictors of new CHD cases from 1986 to 1995, all variables (age, occupational group, smoking, BMI, study group, participation in vigorous activity, LTPA index) were included in a fixed logistic regression model. In this model, the OR for CHD increased by 4% per year as age increased, and 7% per unit of BMI increase; the odds ratio was increased for smokers compared with non-smokers, and decreased (OR 0.57, 95% CI from 0.34 to 0.94, $P = 0.025$) for those who participated in vigorous activity compared with those who did not.

DISCUSSION

1. Muscle fiber distribution and CHD risk

The men with defined CHD had the mean ST-% less than that in the healthy men. On the contrary those healthy men that were physically highly active and had a high level of cardiorespiratory fitness had the mean ST-% higher than that of sedentary men. Thus, the present study shows that the ST-% is quite heterogeneous among individuals and among different populations. This has previously been observed in athletic populations: a low ST-% in power and speed athletes and a high ST-% in endurance athletes. The findings of the present study suggest that the inherited ST-% is not solely attributed to athletic endowments but that the low ST-% of an individual increases the risk for CHD. Indirect support to this observation gives the finding that CHD was more common in former power and speed athletes than in former endurance athletes.

The distribution and the mean of ST-% in all the men in the present study agrees wholly with the results of the previous studies (Saltin & Gollnick 1983, Simoneau & 1989). It is estimated (Simoneau & Bouchard 1995) that about 75 % of North American men and women of European ancestry have ST-% between 35 % and 65 % and this figure corresponds to the mean ± SD of the present study (see Figure 3.). Normal ambulatory activity does not result in a significant conversion of the histochemical appearance of the two main fiber types, although changes can occur in some other particular (metabolism, contractile proteins, etc.) property of fiber phenotype (Baumann et al. 1987, Pette & Staron 1990, Fry et al. 1994, Demirel et al. 1999, O'Neill et al. 1999). Familial concentration and heritability are noteworthy in the ST-% but the extent to which human skeletal muscle fiber types are under the control of genetic factors is not currently known. Nevertheless, the variability in ST-% within a pair of twins (Komi et al. 1977) suggests that there are genetic factors that predispose some individuals to higher or lower ST-% (Komi et al. 1977, Bouchard et al. 1997). Thus, the level of individual or group differences in ST-% cannot be explained by differences in the life-style, age or sampling error.

Based on the findings of the present study it can be hypothesised that the inherited distribution of ST and FT fibers in the skeletal muscle of an individual, low or high ST-%, may increase or decrease the risk of developing CHD. The possible mechanism by which ST-% may influence the risk of CHD is that ST-% may have effects on the known risk factors of CHD such as cardiovascular fitness, physical activity and serum lipoproteins. In addition, because ST-% contribute to athletic ability, the influence of ST-% may account in part for the variations in physical activity. Alteration of physical activity of an individual is known to influence the serum

levels of lipoproteins, sex hormones, fasting insulin and the body distribution of fat. Thus, ST-% may have direct influence on the risk factors for CHD or ST-% may affect the mechanisms by which these risk factors influence the risk of CHD.

2. Physical inactivity and activity: The influence of ST-% on the risk of CHD

Those men in the present study, who were considered to be sedentary according to their history and who had a low measured cardiorespiratory fitness (Studies I, IV, V) also had a mean ST-% lower than average and similar to the mean ST-% of the CHD patients. Accordingly, it suggests that a low ST-% is associated with a low level of physical activity and cardiorespiratory fitness, and both are associated with an increased risk of CHD (see Tables 2 and 3). Thus, a low ST-% of an individual may influence the level of physical activity and cardiorespiratory fitness and ultimately the risk of developing CHD (Figure 6).

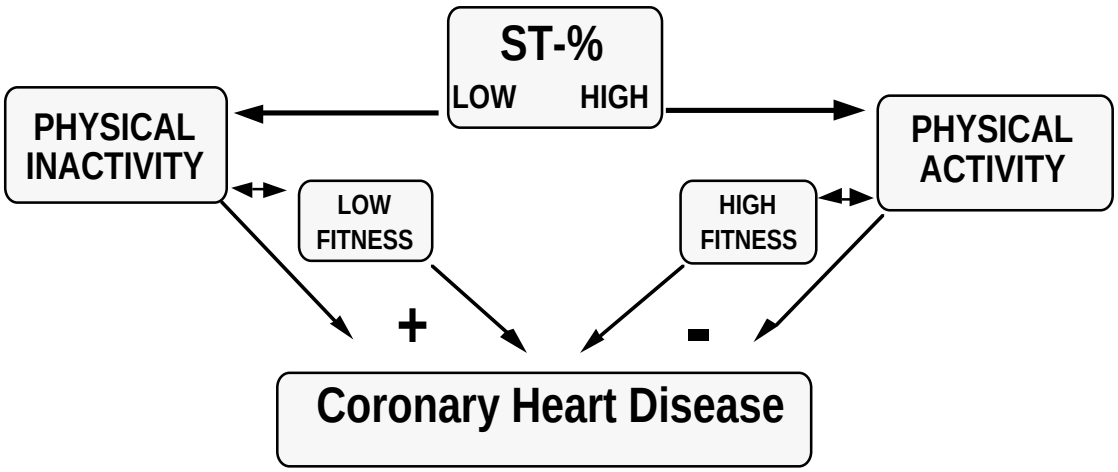

Figure 6. Outlook of the possible influences of muscle fiber distribution (ST-%) on physical activity and fitness, and their relationships to the risk of coronary heart disease. (+ and - denotes increased and decreased risk, respectively).

The ST-% correlates strongly and positively with both cardiorespiratory fitness and physical activity (Study IV) and those men who had a high ST-% have a high level of both physical activity and cardiorespiratory fitness (Study V). Another important issue that arises from these findings is "positive" selection: the individuals capable of achieving a high level of physical activity will constitute a group with an inherently low risk of CHD. The participation in competitive sports from as early as at the age of 10 - 19 years has been observed to be a powerful predictor in maintaining a high level of physical activity independent of the presence of chronic disease (Hirvensalo et al. 2000). Moreover, it has been argued that individuals with a natural ability in

selected types of physical activity are at a decreased or an increased risk of developing CHD (Noakes & Opie 1976, Milvy et al. 1977, Lean & Han 1998). Indeed, in this study the former athletes were more physically active than the control group. Furthermore, the former endurance athletes who participated in vigorous physical activity more often, had significantly less CHD than the former power-speed athletes. Thus, aptitude for endurance sport is associated with protection against CHD. The findings of this study are the first that give direct evidence that the lower rates of CHD in active men may be due to inherited factors that lead to a more active lifestyle and to a decrease in the risk of CHD. Because ST-% was low in both CHD patients and in sedentary men but high in physically active fit persons, inherited skeletal muscle properties may give an explanation for these findings. Therefore, ST-% may be one factor that is associated with "positive" selection, ie that some individuals like former endurance athletes are capable of achieving a high level of physical activity also in later life (Figure 6).

It has also been argued that it is current activity that is protective (Paffenbarger Jr 1984, Morris 1990). The protective effects of physical activity on CHD in former athletes (Study VI) as well as findings of others (Paffenbarger Jr et al. 1993, Blair et al. 1995) showing that an increase in physical activity gives protection against CHD, weaken, but not entirely negate the argument for a positive selection process. These findings merely suggest that physical activity or inactivity amplyfies or diminishes the effects of variations in inheritance, like ST-%, on the development of CHD (Figure 7).

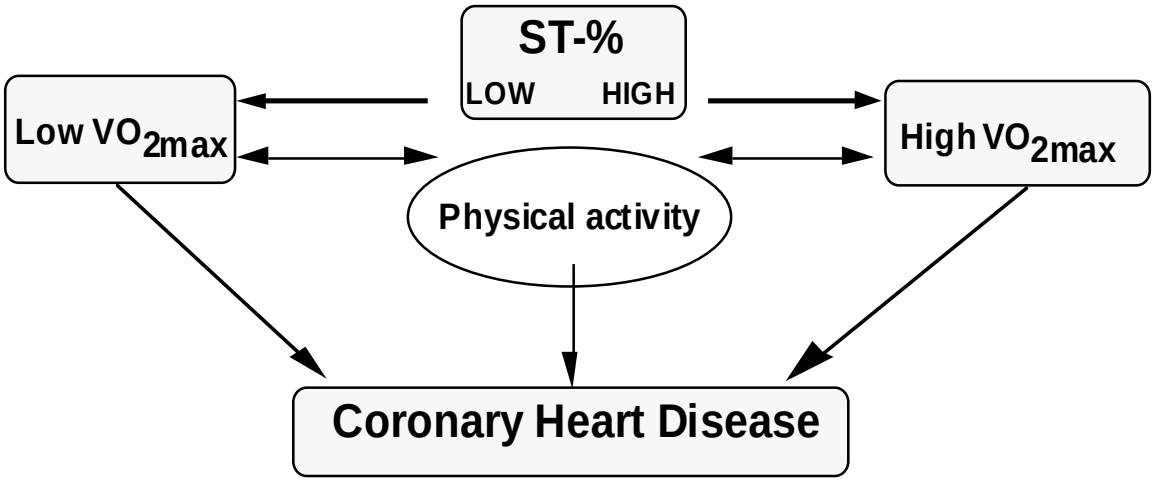

Figure 7. The possible influence of muscle fiber distribution (ST-%) on physical fitness (measured as VO_{2max}) and physical activity, and their relationships to coronary heart disease. Inactivity and physical training modifies the influence of inherited ST-% on CHD.

The endowment of athletic activities increases participation in leisure activity in older age. As shown in this study, those with a high ST-% (Study IV) and who have an aptitude for endurance sports (Study VI) readily engage in regular physical activities which provide health benefits. The cluster analysis indicates that those who have a high ST-% have a high level of cardiorespiratory fitness. Inherited factors, like ST-%, explain in part the individual variation in participation in physical activities (Maes et al. 1996, Bouchard et al. 1997, Beunen & Thomis 1999). The aerobically fit middle-aged individuals are observed to be more physically active than persons with low VO_{2max} (Brochu et al. 1999). During light to moderate physical activity, the ST fibers are recruited easily (Connet & Sahlin 1996), and the activity of normal ambulatory individuals has shown to correlate closely with the ST-% (Monster et al. 1978). Thus, the reason for the association between the ST-% and physical activity may be that skeletal muscles in those with a high ST-% are more aerobic and therefore less susceptible to fatigue during physical activities (Figure 8). This suggestion is supported by the findings that VO_{2max} correlated with KGDH and CPT activites, which provide an assessment of the capacity for aerobic ATP generation; these enzyme activites were the highest in the muscles of most active and fit men, which suggests that the men will depend less on anaerobic metabolism in ATP formation and therefore less lactic acid is produced resulting in less fatigue and lower level of perceived exertion. Thus, a high ST-% is advantageous for regular physical activites because it encourages those with a high ST-% to exercise regularly compared to those with a low ST-% (Figure 8).

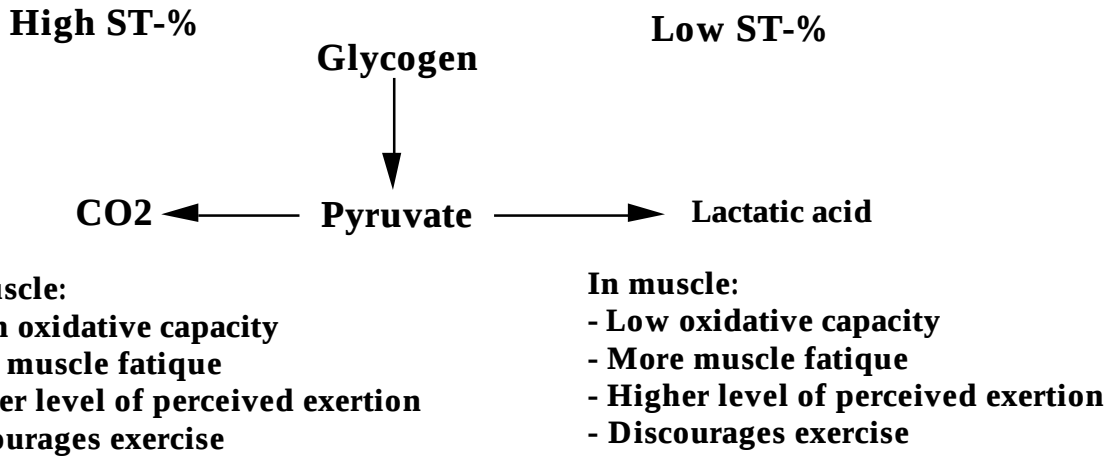

Figure 8. Those who have a high ST-% have a higher oxidative capacity than those who have a low ST-% and consequently in latter muscles have to rely more on lactic acid formation. Those with high ST-% suffer less muscle fatigue which encourages them to exercise regularly.

3. The influence of skeletal muscle on serum lipids and lipoproteins

A positive correlation between ST-% and serum HDL-C but a negative correlation between ST-% and both serum LDL-C and serum TG levels could explain the association of low ST-% with high prevalence of CHD. Studies by Frick (1987) and Manninen (1988) show an inverse relationship between serum HDL-C and the incidence of CHD, and a slow rate of progression of coronary atherosclerotic lesions correlates with serum HDL-C (Levy et al. 1984, Nikkilä et al. 1984, Brown et al. 1990). Two factors concerning skeletal muscle, namely the distribution of the two muscle fiber types and the effects of physical exercise on the metabolism of the fibers, result in variations in the metabolic characteristics of the skeletal muscle that may give an explanation for the associations between ST-% and serum lipoprotein levels.

The variations in the fractional catabolic rate of TG-rich lipoproteins and serum HDL-C concentrations have been shown to correlate positively (Hamsten 1990). The initial hydrolysis of triglycerides in circulating TG-rich lipoproteins is mediated by the LPL enzyme anchored to the luminal side of the capillary endothelium (Figures 2 and 10). It is suggested that the percentage of ST fibers in the skeletal muscle may explain in part the hydrolysis of serum triglycerides and the increased level of HDL-C. Skeletal muscle uses fatty acids from circulating triglycerides for oxidation (Cryer 1987). In the present study the activity of CPT, the key enzyme in the pathway of the oxidation of fatty acids, in skeletal muscle correlated negatively with serum triglyceride levels but positively with HDL-C and ST-% (Study V). These results suggest that increased capacity for oxidation of fatty acids is associated with the ST-%. Thus, variations in ST-% may influnce serum triglyceride hydrolysis in skeletal muscle and may have influence on the formation of HDL-C.

In the present study the ST-% correlated positively with the activity of KGDH which is in accordance with previous reports (Blomstrand et al. 1986) and suggests that ST-% and oxidative capacity of the muscle are coupled. In earlier studies muscle LPL activity has been shown to be directly related to ST-% (Jacobs et al. 1982) and to the capillary supply (Lithell et al. 1981). In the ST fiber-rich muscles the hydrolysis of triglycerides is supported by the surrounding capillaries that also provide oxygen for the oxidation of the fatty acids in the muscle fibers. The number of capillaries surrounding an individual fiber is between 2-3 for FT fibers and 3-4 for ST fibers but in locally homogenous areas, where an individual muscle fiber is surrounded only by those of a similar type, as many as 4-11 capillaries per ST fiber can be found (Saltin et al. 1977, Saltin & Gollnick 1983). In addition, a high ST-% and a high CPT acitivity were clustered together. Consequently, the muscles with a high ST-% are highly capable of participating in the interaction of the TG-rich lipoproteins with LPL in the abundant capillary bed, and of liberating and using FAs from TG-rich

lipoproteins. Taking into account that total skeletal muscle mass is about 40 % of body mass, the lipolysis of TG-rich lipoproteins in the capillaries in peripheral skeletal muscles may be quantitatively important in the whole body turnover of lipoproteins and therefore in increasing the serum HDL-C level (Figure 9).

Cross-sectional studies in athletes show a high ST-% in endurance athletes who also have a high HDL-C level (Durstine & Haskell 1994). However, athletes participating primarily in speed or power events have a low ST-% (less than 50 %) (Bergh et al. 1978, Saltin & Gollnick 1983), and they also have serum HDL-C levels similar to those of sedentary subjects (Clarkson et al. 1981, Farrell et al. 1982, Durstine & Haskell 1994) or even lower (Berg et al. 1982). Therefore, inherited muscle ST-% alone may be an important factor altering the metabolism of lipoproteins. Indeed, factors other than physical exercise have been suggested to account for differences in HDL-C (Sady et al. 1988) and current results suggest that the ST-% of a person may be one factor influencing HDL-C levels without physical exercise by the mechanisms outlined above. Indeed, in cluster analysis those fit men who did not have much leisure-time activity but had a high ST-% also had high HDL-C and high CPT activity in their skeletal muscles. The importance of ST-% is supported by the finding that the ST-% of an individual significantly contributed to the serum HDL-C level after the regression model takes into account several confounding factors including physical activity.

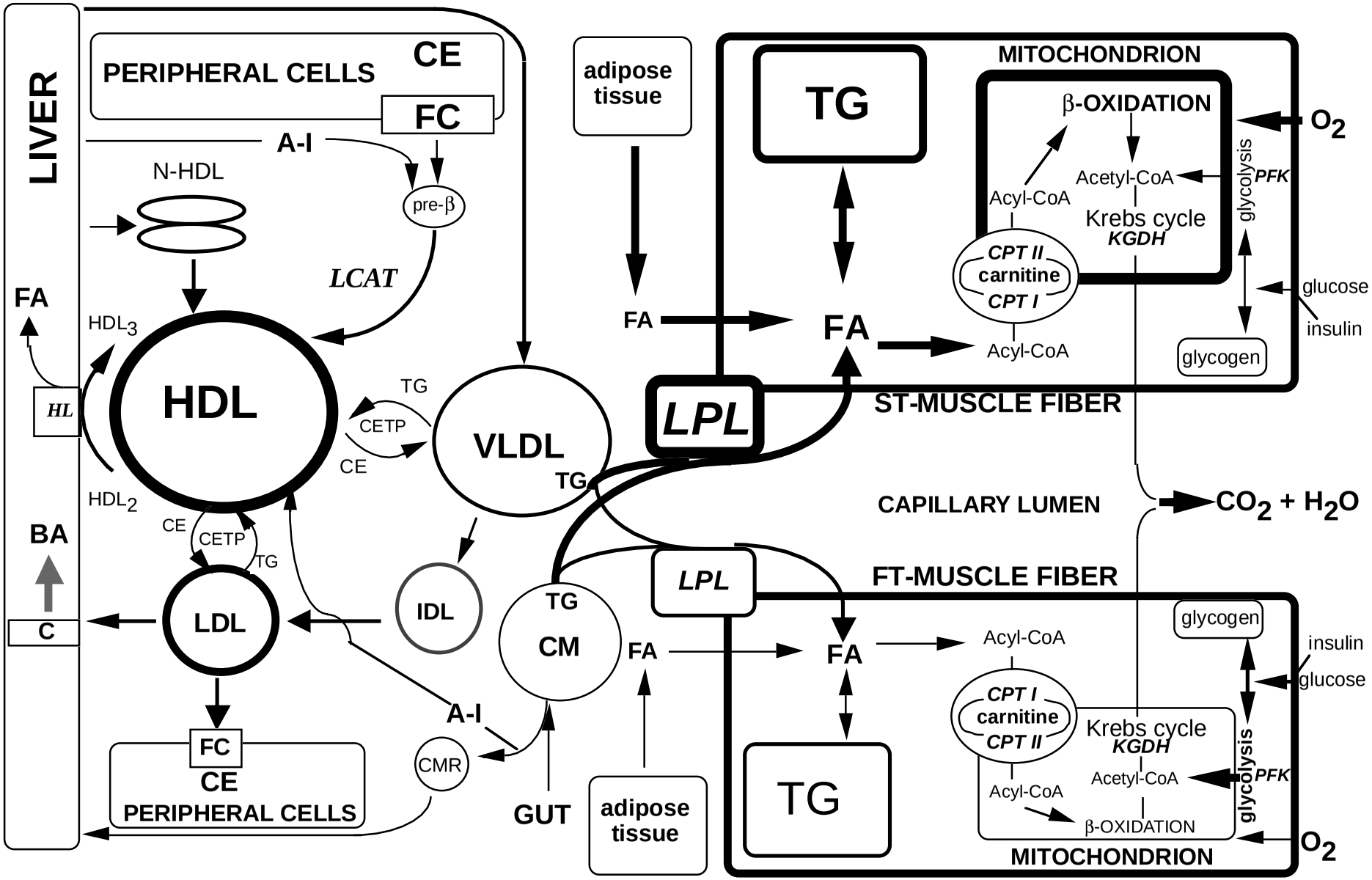

Figure 9. Overview of lipoprotein metabolism and energy metabolism in ST and FT fibers. Muscle fibers participate in lipid metabolism by using fatty acids from serum triglycerides as energy sources or by storing fatty acids as intramuscular triglycerides. Thickness of the line denotes relative flux through the pathway. Abbreviations see page 6.

4. The effects of exercise and muscle metabolism on serum lipoproteins

The training in rats increased KGDH activity in ST and FT fibers suggesting that oxidative capacity in the whole muscle increases in both fiber types. In FT fibers this adaptation offers better oxidative metabolism and thus, reduces the formation of lactic acid and spares the muscle glycogen stores during exercise (Figure 10). The increase in CPT activity in ST fibers (see Figure 4) but not in FT fibers suggests increased capacity to oxidise fatty acids in the trained ST fibers. Increase in fatty acid oxidation during low- to moderate-intensity exercise is important because it also spares glycogen (Golnick 1985). This could be another factor reducing fatigue during physical activity. Thus, the muscles composed of predominantly FT or ST fibers have the metabolic profile already needed for their function: high oxidative and lipid oxidating capacity in ST fibers and high glycolytic capacity in FT fibers. Moreover, physical exercise evokes a set of metabolic adaptations that streghtens the characteristics of these fibers for their functions during exercise.

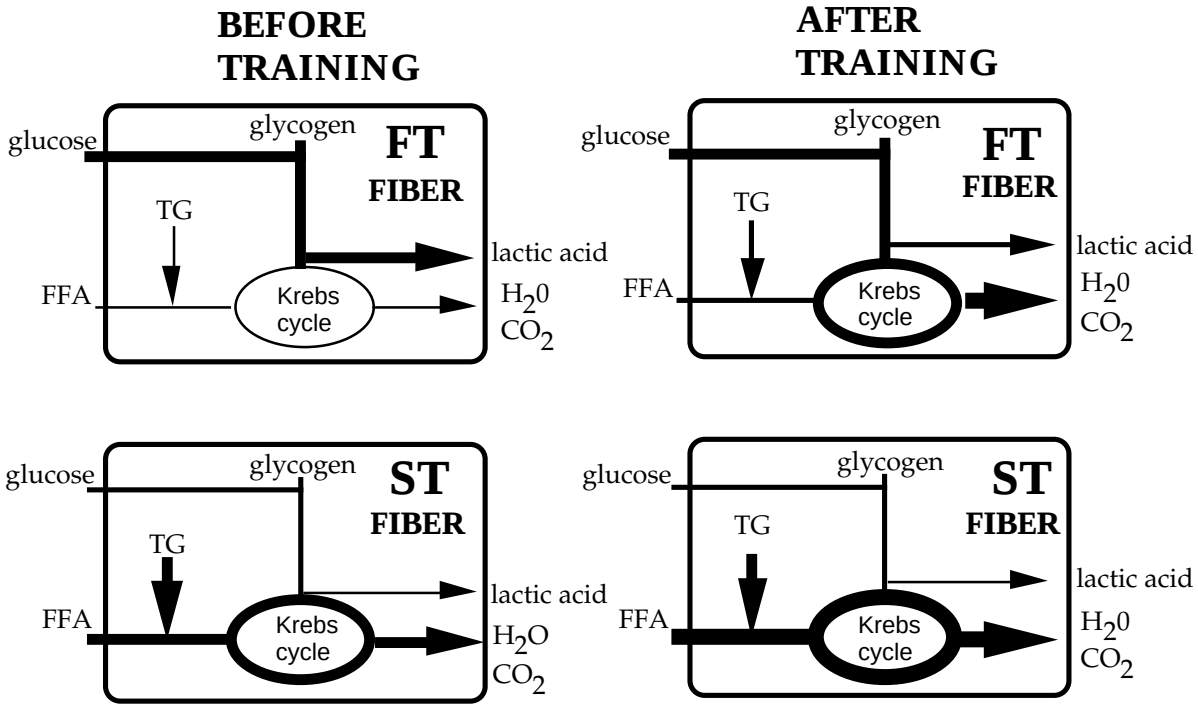

Figure 10. Overview of the effects of endurance training on the pathways of energy metabolism in FT and ST muscle fibers. Thickness of the line denotes relative flux through the pathway. FA= fatty acids, FT=fast twitch, ST=slow twitch, TG=triacylglyceride

To summarise, these observations in rats strengthen the idea that due to the different metabolic profiles of the two fiber types, the ST-% of the muscle influences the metabolic response in the muscle during acute exercise and during exercise training. There is an increase in the oxidative capacity for oxidative metabolism in ST fibers and, especially, an increase in lipid oxidation within these fibers; this decreases the formation of lactate, decreases the use of glycogen and prevents fatigue in the muscles for the same amount of work (Figure 10). Thus, those with a high ST-% have a metabolic "advantage", they can more easily cope with reglular physical exercise since less lactic acid is produced and training further increases the metabolic capacity in their skeletal muscle (see Figure 8).

Physical exercise increased the activites of LPL and KGDH suggesting that exercise increases skeletal muscle oxidative and lipid oxidating capacities. After exercise training the increase in CPT activity in skeletal muscle and the increase in serum HDL-C concentration correlated positively suggesting that skeletal muscle fatty acid oxidation may influence serum lipoprotein metabolism. This is also supported by the finding that the onset of lactic acid accumulation in the blood decreased after training suggesting a lower lactic acid level in skeletal muscle, attenuated decrease in pH, less inhibition of CPT activity and, thus favouring the oxidation of fatty acids during exercise (Bergman & Brooks 1999, Starritt et al. 2000). An increase in the uptake and oxidation of fatty acids may facilitate the hydrolysis of TG-rich lipoproteins by reducing the end-product inhibition of muscle LPL (Kiens & Lithell 1989). The degradation of circulating TG-rich lipoproteins by muscle LPL and the formation of HDL has been shown to be significantly higher in the trained than in the untrained leg both when individuals are at rest and exercising (Kiens & Lithell 1989). Although the contribution of lipoprotein derived fatty acids to total lipid energy utilisation of the muscle may be small in magnitude, it may be significant if exercise is performed on a regular base for longer periods like in the training program in this study (van der Vusse & Reneman 1996). Indeed, the activity of CPT seems to be sensitive to exercise. Physically active men with low ST-% had 50 % higher CPT activity than the sedentary men and the CHD patients suggesting that a daily physical activity does not markedly increase the activity of CPT in sedentary individuals who have a low ST-%. However, after the training the activity of CPT in former sedentary men was at the same level as observed in highly active men with similar ST-%. Nevertheless, the activity after training was nearly one third lower than the CPT activity observed in physically active men with a high ST-% suggesting that both high physical activity and high ST-% contributed to the high activity of CPT in their skeletal muscle.

Significant alterations in the level of fasting serum triglyceride level after physical exercise was not observed and fasting serum triglycerides in healthy men did not

differ between the tertiles of physical activity and fitness nor did they correlate with cardiorespiratory fitness or LTPA index. Physical exercise however reduces serum triglycerides only when the baseline concentration has been elevated (Huttunen et al. 1979, Thompson et al. 1988, Wood et al. 1991, Halbert et al. 1999) which was not the case in the healthy men of this study. Before training the healthy men had normal levels of HDL-C (see Study V) suggesting that they had a normal and effective clearence of TG-rich lipoproteins (Patsch et al. 1983, Patsch et al. 1992). On the contrary, physically inactive CHD patients had higher levels of serum triglycerides and lower levels of HDL-C and, in addition, lower levels of skeletal muscle oxidative and lipid oxidating enzyme activites. The earliest effect of physical activity is suggested to be the decrease of the levels of fasting and postprandial TG-rich lipoproteins and this probably precedes the effect exercise has on HDL levels (Weintraub et al. 1989). Moreover, physical activity should be taken frequently to maintain the improvement in serum lipids and lipoproteins (Hardman et al. 1998). According to the present study those persons who have a high ST-% partake in regular exercise and they are better able to exercise at the level of intensity that is beneficial.

Subcutanous adiposity in the abdominal region decreased after training. In addition, in the tertiles of physical activity subcutaneous adiposity decreased with the increase in leisure physical activity. Thus, physical exercise may prevent adipose tissue accumulation by partitioning more fatty acids from lipoprotein triglycerides into muscles for oxidation instead of their storage in adipose tissue. Fatty acids for oxidation in skeletal muscle are hydrolysed also from adipose tissue TG-storage. Thus, the increased oxidation of fatty acids in skeletal muscle may be one explanation why adiposity is usually inversely associated with HDL-C concentration and why a higher HDL-C is associated with leaness and an increase of serum HDL-C after exercise training is suggested to be due to a decrease in adiposity.

The influence of increased LPL activity of the skeletal muscle on the serum level of HDL-C in previously sedentary adults was confirmed in the present study. The activity of LPL is usually increased during the hours after the exercise (Seip & Semenkovich 1998). This explains why HDL-C may be higher several hours after the exercise (Kantor et al. 1987) and why exercising before fat meal has beneficial effects on the triglyceride response and HDL metabolism in several investigations (Kantor et al. 1984, Zhang et al. 1998, Malkova et al. 1999). This was also seen in this study since fasting serum HDL-C levels were high and serum triglyceride levels were low for at least 48 h after the last exercise session because the men did not exercise on the day before the blood sampling.

Intense exercisers like the physically active men in this study use more intramuscular energy sources, glycogen and intramusclular TG store, during exercise.

The trained persons may use more fat from intramuscular stores than untrained persons at the same level of relative exercise intensity (Coggan et al. 2000). Thus, an increase in LPL activity after exercise may facilitate the replenishment of muscle TG storage from fatty acids of serum TG-rich lipoproteins (Oscai et al. 1990). In addition, the increase in fatty acid utilisation for energy from plasma TG-rich lipoproteins after exercise has been suggested to occur when muscle glycogen stores are replenished (Kiens & Richter 1998). Therefore, the degradation of TG-rich-lipoproteins after intense exercise is suggested be more important for its potential long-term influence on blood lipid profiles than only the contribution of exercise to a higher rate of oxidation during exercise (Kiens & Lithell 1989). Again those with a high ST-% readily accept long term regular exercise gaining the most of these possible benifits of exercise on lipid metabolism.

5. Physical exercise and CHD risk factor modification

One of the key findings of this study is that a home-based exercise training programme can increase fitness and improve the serum lipid and lipoprotein profile in a sedentary population prone to CHD. During the training year the men reached the recommended level of 1500 kcal/week (Pate et al. 1995). The increase in their cardiorespiratory fitness was comparable to that observed in some similar studies (Stein et al. 1990, Shoemaker et al. 1996, Zmuda et al. 1998). Their VO_{2max} after training was still 20 % lower than that of the men with similar ST-% who had been physically active for longer period and reported a mean leisure-time energy expenditure a more than 2000 kcal/week. Moreover, the physically active men with a high ST-% had a mean leisure-time energy expenditure almost two times and VO_{2max} 20 % higher than that of physically active low ST-men. These results suggest that although persons who have a low ST-% can significantly increase their fitness by regular training, the persons with a higher ST-% may have a superior ability for high level physical activity and fitness and have long term adherence to vigorous physical activities. In addition, the findings in the former endurance athletes suggest that ST-% may influence the long term adherence to leisure activity and to a continuity of vigorous physical activity. Inherited characteristics that are regulating skeletal muscle properties, and especially ST-%, may be linked to both athletic performance and health-related physical activity. The large variation of ST-% in the population may explain why the responses to exercise interventions are often highly variable. In addition, when in cross-sectional studies athletes and sedentary persons are compared with each other, the large differences in some CHD risk factors like cardiorespiratory fitness and serum lipids and lipoproteins between them may be explained by the possible influene of ST-% on these variables.

The twelve men in training attained leisure-time energy expenditure levels in which

favorable changes in serum HDL-C appeared to occur (Kokkinos & Fernhall 1999). After the 12-month home-based moderate to vigorous physical exercise, an increase in the mean level of HDL-C in these twelve men was similar to that observed in healthy outpatients (Stein et al. 1990) and in men with premature myocardial infarction (Mendoza et al. 1991) but the increase was more than had been observed in some of the earlier studies (Tran et al. 1983, King et al. 1991, Halbert et al. 1999). Half of the men had a modest increase in HDL-C but in the other half the increase was substantial (see Study V, Figure 2). Indeed, after similar types of exercise, some subjects may have markedly higher HDL-C levels than others (King et al. 1995a, Thompson et al. 1997b). Because exercise training has little effect on HDL-C levels in healthy men with initially low HDL-C (Stefanick et al. 1998, Zmuda et al. 1998) the normal levels of HDL-C in these twelve men in training may have influenced the results. This study showed that the higher HDL-C was, the larger the increase tended to be after training, and similar finding has been observed in one earlier study (Williams et al. 1982). New observations in this study were that the higher the LPL activity in the skeletal muscle was before training, the larger the increase in LPL activity. Although in cross-sectional studies ST-% correlated with HDL-C and LTPA index, in the training study ST-% did not correlate with an increase in the level of physical activity or with an increase in HDL-C concentration. The lack of correlations there may be due to the small number of subjects or a too homogenous ST-% of these men. These findings may indicate that, in addition to the ST-%, some other inherited properties in skeletal muscle such as capillary density and metabolism capacity may have influenced the results of training.

The findings that serum LDL-C was the lowest in highly active men and that regular training significantly reduced serum LDL-C in previously sedentary men with high serum LDL-C may have clincial importance. Currently there is considerable disagreement in the exercise research community over the benefits of low and moderate amounts of physical activity recommended to the public (Barinaga 1997). In a recent meta-analysis the comparison of intesities showed that exercise programmes at intensities greater than 70 % of VO_{2max} produced larger chages in LDL-C while programmes at lower intesitites modified triglyceride and HDL-C levels (Halbert et al. 1999). It is suggested that a higher level of physical activity increases the oxidative capacity of the skeletal muscle and the hydrolysis of triglycerides in muscle and that the oxidation of fatty acids is increased. This could result in an increased turnover of serum triglycerides in the body which could result in a greater uptake of LDL and a greater production of HDL from the liver (Figure 9). This assumption is supported by the findings that skeletal muscle capillary density, a sign of oxidative capacity in the muscle, is related to serum levels of LDL-C (Shone et al. 1999). Because there is a negative association between LDL-C lowering and the reduced rate of progression of atherosclerosis, cardiac events and mortality (Barth

1995) the results of this study give support to many epidemilogical studies suggesting that only high levels of physical activity and cardiorespiratory fitness prevent premature progression of CHD in middle-aged.

Two studies have investigated the influence of physical exercise on the development of coronary lesions in CHD patients and they show that an advance of lesions may be stabilised if a person expends 1500 to 1700 kcal/week, however, about a 2200 kcal/week expenditure is needed to induce a possible coronary lesion regression (Hambrecht et al. 1993, Niebauer et al. 1997). Thus, at least healthy persons who are low level exercisers like most men with a low ST-% in this study, should try to engage in more vigorous regular physical activities (jogging, cycling, swimming, ball games) than has been recommended to the general public as initial step. It is however possible that a high level of physical activity can only be achieved by some persons, and the observation that vigorous physical activity was performed especially by former endurance athletes supports this assumption. The results from this study suggest that these persons are those who have a high ST-%.

It should be remembered however that high level regular endurance exercisers may develop CHD (Rennie & Hollenberg 1979, Eichner 1983, Noakes 1987). Nevertheless regular moderate to high intense physical activity does give protection against the risks of unaccustomed acute vigorous exercise (Mittelman et al. 1993, Willich et al. 1993, Albert et al. 2000). This may be one explanation for the lower CHD mortality in former endurance athletes who reported regular vigorous activity.

6. Physical activity, serum sex hormones and serum lipids

The serum level of total testosterone or estradiol did not show significant correlations with serum lipids and lipoproteins in healthy men. This supports the idea that serum sex hormones explain only a small percentage of the variations in serum lipids and lipoproteins (Haffner et al. 1992, Alexandersen et al. 1996) and in predicting CHD (Cayley et al. 1987, Contoreggi et al. 1990). Lower serum free testosterone was observed in the CHD-patients when compared with the healthy sedentary men of the same age. Persons with CHD may have significantly lower testosterone levels than persons without heart disease (Lichtenstein et al. 1987). It can be speculated however that the lower levels of testosterone or free testosterone in CHD-patients may not be the cause of the disease but merely that the decrease is induced by the stress related to their disease (Kaufman & Vermeulen 1997). That serum testosterone or free testosterone did not differ significantly between the CHD patients and the physically active men supports this suggestion. One potential confounder is the high level of endurance-type of physical activity, which is known to decrease serum sex hormone levels (Wheeler et al. 1984, Hackney et al. 1988,

DeSouza & Miller 1997). Although an increase in physical activity in the previously sedentary men caused a decrease in serum levels of total and free testosterone levels and an increase in the serum level of HDL-C is in accordance with the finding that the suppression of endogenous testosterone leads to an increase in serum HDL-C in men (Goldberg et al. 1985) it does not explain the low levels of both serum HDL-C and testosterone in CHD patients. Thus, associations between serum sex hormones and serum lipids and lipoproteins may not be causal but may represent a secondary phenomenon like stress related to physical activity or disease rather than a direct atherogenic influence. Therefore, these findings do not support the suggestions that positive associations found between serum levels of testosterone and HDL are clinically relevant. Merely, the results suggest that physical activity-inactivity in the study population should be considered whenever the associations between serum levels of sex hormones, lipids and lipoproteins and cardiovascular risk are investigated.

The concentration of serum free testosterone, that is generally considered to be a parameter of the biologically available testosterone, was low in highly physically active men and the concentraion in serum decreased after exercise in previously sedentary men. The serum levels of estradiol and DHEAS, both associated with cardiovascular risk, did not change due to an increase in physical activity, and they did not show any significant differences between the tertiles of fitness or physical activity. Therefore, the ratio of free testosterone to free estradiol decreased in association with physical activity. The concerted regulation of hepatic triglyceride lipase (HL) activity by both endogenous androgens and estrogens may explain some of the findings in this study. An increase in testosterone-estradiol ratio causes a concomitant increase in HL activity (Sorva et al. 1988). The triglyceride-rich HDL (HDL2) is a preferred substrate for HL, and by depleting triglycerides from HDL2, hepatic lipase remodels HDL towards HDL3 (see Figure 9). In contrast to LPL in skeletal muscle, HL catalyses the degradation of HDL lipids and the activity of HL is inversely related to serum HDL-C (Kuusi et al. 1980, Kuusi et al. 1989). Moreover, the HL activity decreases (Kuusi et al. 1982) but skeletal musce LPL activity increases along with an increase in physical fitness. Thus, skeletal muscle LPL activity and HL activity have opposite effects on serum HDL-C levels. Accordingly, one explanation for an increased serum HDL-C in physically active men may be the decreased free testosterone to free estradiol ratio. In addition, the increased estrogenic effect after training may be enhanced due to the increased ratio of DHEAS to free testosterone which may enhance the estrogenic effects of DHEAS in situations when the androgenic milieu in men has changed (Ebeling & Koivisto 1994). Since estradiol is shown to increase the skeletal muscle LPL activity (Ellis et al. 1994), the increased estrogenic milieu may be one reason for the observed increase in LPL activity. That physiological levels of serum estradiol offer some degree of CHD protection and

persevere serum HDL-C level in men (Bagatell et al. 1994) supports these findings.

Also serum levels of LDL-C are regulated by endogenous estradiol (Tikkanen et al. 1986, Sorva et al. 1988). Men in physical training may have significantly increased serum estrogens and decreased serum LDL-C levels (Frey et al. 1983). The estrogen level was not shown to increase in this study but it is possibly that the estrogenic effect has been enhanced by the decrease in the androgenic milieu due to physical activity by the mechanisms outlined above. This gives an explanation why the most physically active men in the present study had the lowest LDL-C level and training decreased serum LDL-C level.

7. The clustering of physical activity and serum levels of SHBG, apo A-I and insulin

The cluster analysis in healthy men showed that high ST-%, a high level of physical activity, high serum levels of SHBG, HDL-C, apo A-I and a low serum level of insulin are associated. Although the serum level of SHBG correlates positively with HDL-C, the concentration of SHBG is probably of minor importance as a risk factor for cardiovascular disease in men (Barrett-Connor et al. 1988, Hautanen et al. 1994). This association probably reflects the influence of physical activity or inactivity on both variables. Similarly the association between a high level of serum insulin and a low percentage of ST fibers observed in this study and in other studies as well (Lillioja et al. 1987, Marin et al. 1994), may represent physical inactivity merely than being the reason for one or the other.

A low level of physical activity and fitness was associated with a high level of serum insulin. This association may explain a low level of serum SHBG in sedentary men because insulin is an inhibitor of SHBG production (Plymate et al. 1988). When the level of serum insulin is high, the activity of HL is increased (Katzel et al. 1992). Thus, a high level of serum insulin in sedentary men may both decrease SHBG production in the liver and reduce serum HDL-C because the activity of HL is observed to be inversely related to serum HDL-C levels (Kuusi et al. 1980, Kuusi et al. 1989).

Physical exercise increased apo A-I level and physical activity correlated strongly with apo A-I, the main apolipoprotein of HDL. Nascent HDL contains apo A-I and collects free cholesterol from extrahepatic tissues (Figure 9). Apo A-I is an activator of the enzyme lecithin:cholesteryl acyltranferase (LCAT) that esterifies cholesterol enabling further cholesterol uptake for reversed cholesterol transport. A higher HDL-C level in physically active men is associated with increased apo A-I survival (Thompson et al. 1991) and exercise training increases apo A-I level by increasing its

intraplasmic half-life (Thompson et al. 1988). This suggests that the clustering of high levels of SHBG, high levels of apo A-I, high levels of HDL-C and low levels of insulin together is due to their shared association with physical activity, and that high ST-% clustered with these variables is an explanation for high levels of physical activity.

8. Adiposity, physical activity and serum lipids

In the healthy men both BMI and subcutaneous adipose tissue were associated with an atherogenic lipid profile (see Study IV, Table 2), positively with serum TG and LDL-C and inversely with serum HDL-C. A low BMI or adiposity has been suggested to explain favorable lipid levels associated with physical activity (Williams et al. 1983, Marti et al. 1989, Williams et al. 1992). Also Mahaney and co-workers (Mahaney et al. 1995) have observed that physical activity accounts, in part for associations of plasma HDL-C and TG levels with adiposity. Indeed, in healthy men BMI correlated negatively with physical activity in this study. It has been suggested (Sardinha et al. 2000) that especially truncal skinfolds are associated with serum lipid levels independently of fitness. In the previously sedentary men, training induced changes in serum lipoprotein levels without influencing body weight but in these men training resulted in both increase in fitness and in the reduction of subcutaneous adipose tissue in abdominal region. Therefore, training can alter both serum lipoprotein levels and the regional distribution of fat (Schwartz et al. 1991, Houmard et al. 1994) and induce changes in serum lipids without altering body weight (Kiens et al. 1980, Thompson et al. 1988, Thompson 1997b). These findings suggest that factors other than weight loss solely are involved in improvement in the plasma lipid profile in connection to exercise training. Increases in VO_{2max} and in skeletal muscle lipid oxidation capacity suggest that the role of skeletal muscle in inducing changes in serum lipids and body adiposity may be important (Figure 11).

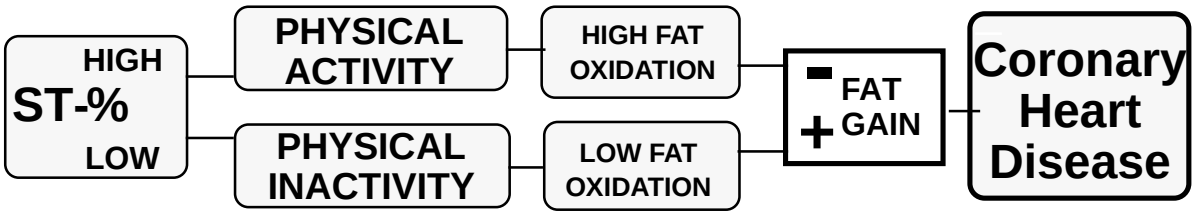

Figure 11. The possible role of ST-% in physical acitivity, fat gain and risk of CHD.

The CHD patients (Study IV and V) had a low ST-%, the highest BMI and the lowest activites of oxidative and lipid oxidising enzymes in their skeletal muscles. In addition, the most sedentary men had low activities of oxidative and lipid oxidising

enzymes in their skeletal muscles, as well as significantly more subcutaneous adipose tissue than the most active and fit men who had the highest lipid oxidising enzyme activities (Study IV). In support of these findings Kim and co-workers have also observed that in obese human skeletal muscles the activities activities of CPT and citrate synthase are reduced (Kim et al. 2000). Thus, a reduced capacity for fat oxidation in skeletal muscle may be an important factor for gaining fat (Figure 11). Indeed, a high oxidative capacity of skeletal muscle has been observed to prevent weight gain over a period of time (Simoneau et al. 1996). It should also be noted, that vigorous physical activity increases oxidative potential more, is more effective for stimulation of fatty acid oxidation and induces a greater loss in adiposity than the moderate intensity program (Tremblay et al. 1994, Chilibeck et al. 1998). It can be concluded that moderate fitness and physical activity levels decrease CHD risk by altering serum lipids without much change in weight or adiposity, but more vigorous physical activity decreases the risk for CHD more by increasing further the fat oxidating capacity of skeletal muscles and reducing body fat and finally preventing weight gain.

BMI of the former endurance athletes was significantly lower than in other athletes and controls and they were physically the most active. An earlier finding in former athletes (Kujala et al. 1994) has revealed that only 2 % of endurance athletes had BMI more than 30 compared with 23 % and 12 % of power athletes and controls, respectively. The differences between the groups of former athletes support the suggestion of the significance of skeletal muscle properties on fat gain. Indeed, an inverse relationship between ST-% and body adiposity was observed in healthy men and a similar kind of relationship has been observed in some other studies as well (Wade et al. 1990, Hickey et al. 1995, Kriketos et al. 1996, Kriketos et al. 1997, Helge et al. 1999). Because in the present study ST-% correlates strongly with fitness and physical activity, the associations between ST-% and body fat indicators may merely be related to the effects of ST-% on the physical activity and fitness level of a person. Thus, it is suggested (Figure 11) that the ST-% may play a role in fat gain and finally in the risk of CHD.

PRACTICAL CONSIDERATIONS AND SUGGESTIONS FOR FUTURE

The skeletal muscle properties seem to be clustered together with several CHD risk factors. Thus, skeletal muscle properties may explain individual differences in health-related fitness phenotypes. One possibility is that the clustering of CHD risk factors is mainly as a result of physical inactivity due to the low ST-% of an individual. On the contrary, a high ST-% seems to make it easier for individuals to achieve high levels of physical activity and fitness, and this may serve to prevent obesity, increase insulin sensitivity and change serum lipid profile to reduce risk of CHD.

One important public health challenge is to move our society from being sedentary to being physically active (Booth et al. 2000). The public health emphasis has shifted from the traditional structured exercise programme to 'active living' (Pate et al. 1995), i.e. an increase in our daily physical activity. However, beneficial effects of physical activity may be mediated through fitness (Blair et al. 1995, McMurray et al. 1998, Strenfeld et al. 1999) but increase in fitness in 'active living' groups (Dunn et al. 1999) are a fraction of the improvement observed in more vigorous interventions (Wood et al. 1983, Wood et al. 1988. Wood et al. 1991). The most intensive exercise intervention seems to be the most effective but long term adherence to exercise is difficult in all participants (Harland et al. 1999). Motivating sedentary people to start exercise, to exercise at the level that improve fitness and to maintain this behaviour is a complex process. It is known that about one third of those to whom exercise programmes are offered will actually start participating, but 3 to 6 months later, only 50 - 60 % of the original participants are still in the programme (Lechner & De Vries 1995). How much skeletal muscle properties or perhaps other inherited characteristics of an individual have effect on these figures has not been studied. Also how these factors can be taken into account when prescribing physical activity to sedentary individuals should be studied. Thus, the influence of ST-% on the results of studies in health-related-fitness and physical activity should be investigated in more detailed.

The present study included only men. Gender-related differences in the metabolic response to exercise (Friedlander et al. 1998, Esbjörnsson-Liljedahl et al. 1999) and differences in effects of physical activity on CHD-risk (Haapanen et al. 1997, Sherman et al. 1999) have been observed. Thus, studies on the influence of skeletal muscle properties on physical activity and health-related fitness in women are needed.

CONCLUSIONS

The key findings of this study are as follows:

1. The percentage of ST fibers in the vastus lateralis muscle associates positively with the serum HDL-C concentration and negatively with the serum triglyceride concentration in middle-aged men with different levels of physical activity and fitness. The association between ST-% and favorable serum lipid and lipoprotein levels may be due to the fact that ST muscle fibers have a high capacity to use fatty acids liberated by LPL from TG-rich lipoproteins, which decreases serum TG concentration and elevates serum HDL-C levels.
2. Long-term, progressive training increases the activities of key enzymes in oxidative metabolism in both FT and ST muscle fibers and especially those of lipid metabolism in ST muscle fibers. Thus, a high ST-% and long-term endurance training contribute to the oxidative and lipid metabolism of skeletal muscle. An increase in oxidative and lipid metabolism of skeletal muscle, may also be achieved by regular and long-term physical activity in those initially unfit individuals who have a low ST-% and these changes favour changes in serum lipids and lipoproteins, especially in serum HDL-C.
3. The percentage of ST fibers has a significant impact on both fitness and physical activity level. A person who has a high ST-% may, by having natural endowment for physical activity, adopt high physical activity characteristics that give protection against CHD by modifying several CHD risk factors. The clustering of ST-%, fitness and physical activity strengthens the argument for selection processes in health-related fitness variables and gives one explanation for observations that more protection against CHD is regularly observed in endurance-trained persons.
4. Previous aptitude for endurance athletic events and continuity of vigorous physical activity associates with protection against CHD, but aptitude for power-speed events does not bestow protection against CHD. Thus, both natural selections to sport at a young age and physical activity in later years are predictors of CHD. Skeletal muscle properties may have influence on both selections to and continuity of regular vigorous physical activity, and ultimately the risk of CHD.
5. Differences in fitness, physical activity and ST-% of the study population have influence on the associations between serum hormones and lipids and lipoproteins as well as associations between body fat distribution and serum lipids and lipoproteins. Therefore, when the variations of these CHD risk factors are studied, the contribution of the effects of fitness, physical activity and skeletal muscle properties on these variables must be taken into account.

SUMMARY

The percentage of slow-twitch (ST) muscle fibers (ST-%) in men correlated positively with a favorable serum lipid profile, especially with the serum HDL-C level. The ST-% also correlated with fitness and leisure-time physical activity (LTPA). In multiple regression analysis ST-%, fitness and LTPA explained about 32 % of the variation in serum HDL-C. In the sedentary men with a low ST-% (a mean of 42 %) the activities of enzymes in lipid and oxidative metabolism within skeletal muscle were similar to those in CHD patients. However, in physically active men the ST-% was as high as (a mean of 65 %) usually observed in endurance athletes and the skeletal muscle enzyme activities were the highest. The men with a high ST-% reported 9 times more leisure-time physical activity than the sedentary men, and two times more than physically active men with a low ST-%. In cluster analysis 72 healthy men fell naturally into three groups; one group was characterised by a low ST-% (mean 42 %), low fitness and low HDL-C but high serum insulin; the second group had a high ST-% (66 %), high fitness and a moderately high HDL-C ; the third group had a high ST-% (66 %) and high fitness and the highest HDL-C but low LDL-C and low serum insulin. More men in the third cluster were physically active (> 3 times per week) compared with those in other clusters. Serum total sex hormone concentrations did not correlate with serum lipids, but serum SHBG associated positively with HDL-C, fitness and LTPA. On the contrary, serum insulin associated with unfavorable serum lipid profile and with indicators of body fat and there was a negative correlation with ST-%, fitness and LTPA. Serum free testosterone was the lowest in physically active men, its level correlated negatively with HDL-C and was decreased after training but was also low in CHD patients. Body adiposity correlated negatively with ST-% and HDL-C. Thus, all associations between serum hormones, serum lipids and obesity may not be causal but may represent secondary phenomenon like physical activity or the influence of ST-% on these variables.

In previously inactive men with a low ST-% (a mean of 42 %), a 12-month home-based moderate to vigorous exercise programme increased fitness and especially the skeletal muscle enzyme activities that before training were similar to those of CHD-patients of the same age. Also the ratio of the activites of enzyme in glycolysis to activites of enzymes in fatty acid metabolism decreased and this was reflected as a decrease in lactate accumulation during exercise indicating tighter control between glycolysis and lipid oxidation in the skeletal muscle after training. The activity of enzymes in fatty acid metabolism correlated negatively with serum triacyglyceride concentration but positively with serum HDL-C concentration and an increase in the activity correlated positively with an increase in serum HDL-C. The effects of progressive, endurance training on Type I (slow-twitch, ST) fibers and Type II (fast-twitch, FT) fibers was studied in rats. Training increased oxidative enzyme activity

in the FT muscle fibers and the activities of enzymes in both oxidative and lipid metabolism in the ST muscle fibers. Thus, endurance exercise training evokes a set of metabolic adaptations in skeletal muscle that may produce significant health-related influences of physical activity on serum lipids.

To investigate the associations between a natural selection to sports, the continuity of physical activity and the occurrence of CHD, a prospective cohort study in male former top-level athletes participating at a young age in different types of sports (endurance, power-speed and 'other') and a healthy control group of 20 year olds was carried out. Data on the occurrence of CHD was obtained by means of death certificates, three nationwide registers and questionnaire studies, and data on physical activity in later life through the questionnaires. All groups of former athletes were more physically active than the control group. Despite a rather similar total volume of physical activity, compared to power-speed athletes, former endurance athletes participated more often in vigorous activity and had less CHD. Both endurance and other than power and speed athletes had less CHD than the control group. The incidence of new CHD was lower among those who participated in vigorous physical activity at the beginning of the nine years follow-up. Thus, both previous aptitude for endurance athletic events and the continuity of vigorous physical activity seem to be associated with protection against CHD, but aptitude for power-speed events does not bestow protection against CHD.

In conclusion, both high physical fitness and physical activity are associated with a lower CHD risk profile and support is given to the findings that regular moderate to vigorous activity provides protection against CHD. However, the most favorable combination seems to be high physical activity in those individuals who have inherited high ST-%. This strengthens the argument for selection processes in health-related fitness variables and explains the better protection against CHD in endurance-trained persons. A low ST-% seems to be a risk factor for CHD but exercise training induces a set of metabolic adaptations in skeletal muscle that increase lipid metabolism in the skeletal muscle and ultimately influence serum lipids and lipoproteins. In addition, skeletal muscle properties, especially ST-%, should be considered as important aspects in studies addressing the influence of fitness and physical activity on other CHD risk factors.

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