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EFFECT OF IL-6 DEFICIENCY ON MYOCARDIAL EXPRESSION OF FATTY ACID TRANSPORTERS AND INTRACELULAR LIPID DEPOSITS

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IL-6 is a biologically active substance and is thought to contribute to the development of obesity. Recent findings suggest that susceptibility to intracellular lipid accumulation is to a large extent determined by changes in the expression of fatty acid transporters such as FAT/CD36, FABPpm and FATP-1. The aim of the present study was to determine the effect of IL-6 deficiency on the expression of fatty acid transporters, as well as, assess the concomitant changes in intracellular lipids. We found that Il-6 deficiency upregulated the myocardial expression of FAT/CD36 (+40%) and did not significantly affect the content of FABPpm and FATP-1 (+15% and +5% respectively). Although no change in the intramyocardial total lipid content was noted, there was a significant increase in the intracellular content of both free fatty acid (FFA), diacylglicerol (DG) and ceramide fractions (+45%, +37% and +48%, respectively) in hearts from IL-6 $^{-/-}$ mice. A trend for IL-6 deficiency to increase in saturated FA species in these fractions was also observed (+8%, +12% and +10%, respectively). In contrast, IL-6 deficiency has no effect on the content of monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) species in each intramyocardial lipid fractions examinated. These findings suggest that IL-6 deficiency results in 1) upregulation of myocardial content of FAT/CD36, 2) the increase in the content of biologically active lipid pools (FFA, DG and ceramide). This lipid accumulation with concomitant trend for increase in the saturation status of these lipid fractions may, at least in part, provide a factor related to the development of intramyocardial lipotoxicity, observed in obese individuals.

Key words: *FAT/CD36, FABPpm, FATP-1, lipids, heart, IL-6*

Recent evidence suggests that IL-6 might be an important component of obesity-related insulin resistance. IL-6 is expressed in many mammalian cells and tissues including adipocytes, cardiac myocytes and skeletal muscles (1,2). A strong link suggesting a possible role of IL-6 in alternations in carbohydrate and lipid metabolism has been provided by studies employing IL-6 deficient mice. Specifically, it has been shown that, mice lacking IL-6 gene develop mature-onset obesity (2). In addition, markers of inflammation, such as TNF-α, IL-1 and IL-6, were shown to be elevated in the serum of patients with type 2 diabetes (3,4). Furthermore, higher plasma IL-6 levels have also been associated with obesity in humans (5-7). Thus, based on these reports (2,5) and because high plasma IL-6 levels coincide with insulin resistance in humans (6), one would expect IL-6 $^{-/-}$ mice to be more insulin sensitive than the wild type.

Considerable evidence has accumulated implicating alterations in lipid metabolism as contributing to the development of insulin resistance (8-10). Recently it has been demonstrated that an excess of lipid accumulation disturbs intracellular insulin signaling in skeletal muscles and cardiac myocytes (11). This lipid accumulation is mainly due to reduced rates of fatty acid (FA) oxidation (12-14). However, increased accumulation of lipids in cytosol depends also on the excessive transmembrane transport of long chain fatty acids (LCFAs) (15-17). Recent findings strongly suggest that susceptibility to increased intracellular fatty acid transport is to a large extent determined by an increase in the expression of fatty acid transporters (16-18). Several fatty acid transporters are known to be involved in regulation of protein-mediated LCFA transport into the cardiac myocytes (19-21). A number of studies have identified FAT/CD36 and FABPpm as the main myocardial fatty acid transporters facilitating LCFA movement across the plasma membrane in health and diseases (20-22). Notably, recent studies have established the role of FAT/CD36 and FABPpm in excessive LCFA transport into the cardiac myocytes in obesity (17) and in type 2 diabetes (16). However, while much is known about adipocyte FATP-1 function and expression, there is little information on its regulation in cardiac myocytes and its relationship to myocardial lipid accumulation (23).

Given that, it is of particular interest to examine whether IL-6 deficiency is associated with any changes in the myocardial expression of fatty acid transporters: FAT/CD36, FABPpm and FATP-1. Furthermore, as the myocardial fatty acid transporter expression may be a key factor in contributing to lipid accumulation in the heart, we examinated intramyocardial content of different lipid fractions in mice lacking IL-6 compared to the wild type littermates. We

determined also the effects of IL-6 $^{-/-}$ genotype on associated changes in the composition of specific myocardial lipid fractions.

MATERIALS AND METHODS

FAT/CD36 and FABPpm were detected using the MO25 antibody (24) and FABPpm antisera (25), respectively. FATP-1 was detected with commercially available antibody (I-20, Santa Cruz, CA). All other chemicals were obtained from Sigma-Aldrich (St. Louis, MO). Female mice (C57B4/6J IL6$^{-/-tm1Kopf}$) were bred on site and maintained at 22°C on a reverse light-dark cycle in approved animal holding facilities. They had unrestricted access to food and water. This study was approved by the local ethics committee on animal care.

The mice were killed by cervical dislocation and immediately samples of the left ventricle were taken. They were cleaned of any visible non-muscle tissue, freezed in liquid nitrogen and finely powdered. The powder was transferred to a glass tube and lipids were extracted using the Folch method (26) as modified according to van der Vusse et al (27). Individual fatty acid methyl esters were identified and quantified according to the retention times of standards by gas liquid chromatography (Hewlett-Packard 5890 Series II gas chromatograph, HP-INNOWax capillary column). Total free fatty acid (FFA), diacyloglicerol (DG), phospholipid (PL), triglyceride (TG) and ceramide content was estimated as the sum of the particular fatty acid species content of the assessed fraction and it was expressed in nanomoles per gram of tissue. We have also calculated the following indices of fatty acid profile of each lipid fractions examined in each heart: saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA).

Routine Western blotting procedures were used to detect proteins as described previously (23,28). The total protein expression of FAT/CD36, FABPpm and FATP-1 was determined in crude membranes of the hearts. Briefly, proteins were separated using 10% SDS-polyacrylamide gel electrophoresis. Membranes were immunoblotted with primary antibodies: MO25 (FAT/CD 36), FABPpm antiserum and FATP-1. Protein content was determined with bicinchonic acid method with BSA serving as a protein standard. Signals obtained by Western blotting were quantified by densitometry (Biorad).

All data are expressed as mean ± SEM. Statistical difference between groups was tested with analyses of variance and appropriate post-hoc tests, or with a Student t-test. Statistical significance was set at $P \leq 0.05$.

RESULTS

No changes were observed in non-fasting blood glucose concentration, serum free fatty acid concentration, the whole body weight and the weight of the heart in between IL-6 $^{-/-}$ and WT mice (data not shown).

Effect of IL-6 deficiency on fatty acid transporter expression (FAT/CD36, FABPpm, FATP-1)

The total myocardial FAT/CD36 protein content was higher in hearts from IL-6 $^{-/-}$ (+40%, $P<0.05$, *Fig. 1A*) compared to the wild type mice. The total

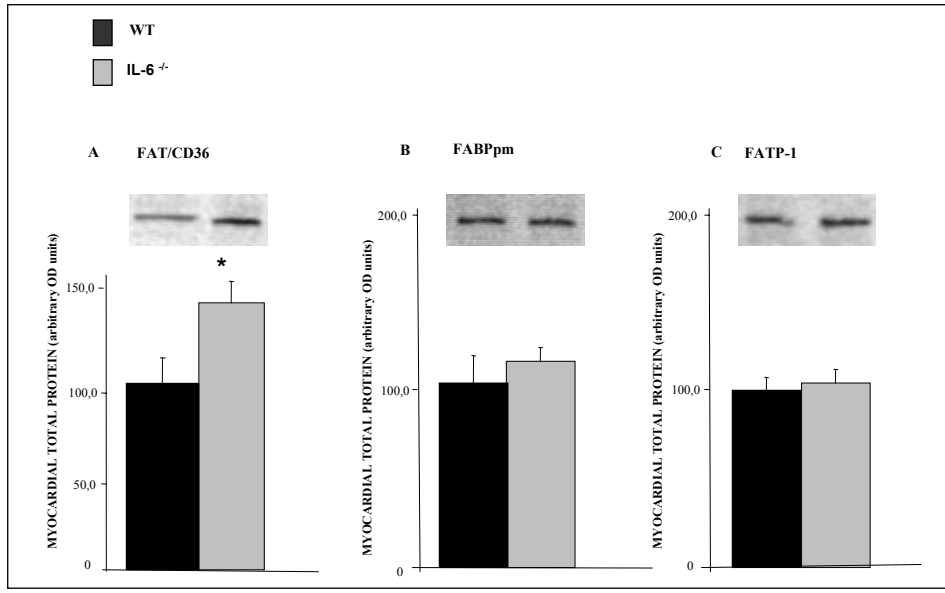

Fig. 1. The effect of IL-6 $^{-/-}$ genotype on myocardial total expression of (A) FAT/CD36, (B) FABPpm and (C) FATP-1. Crude membranes were prepared from left ventricle homogenates as described in Materials and Methods. Data are based on 5 independent determinations for each heart (mean ± SEM). *P<0.05, IL-6 $^{-/-}$ vs WT

FABPpm protein expression was also increased in hearts from IL-6 $^{-/-}$ compared to WT mice, although the change did not reach the level of significance (+15%, P>0.05, *Fig. 1B*). IL-6 deficiency has no effect on myocardial FATP-1 protein expression (P>0.05, *Fig. 1C*).

Effect of IL-6 deficiency on the intramyocardial lipid content

Myocardial content of diacylglicerol, free fatty acids and ceramide was significantly increased in IL-6 deficient mice compared to WT animals (+45%, +37%, +48%, respectively, P<0.05, *Fig. 2*). The content of triacylglycerols and phospholipids remained stable (P>0.05, *Fig. 2*) as well as the total intramyocardial lipid content did not differ between IL-6 $^{-/-}$ and WT mice (P>0.05, *Fig. 2*).

Effects of IL-6 deficiency on the intramyocardial lipid composition

Although no changes in the content of myocardial PUFA as well as MUFA species were observed in between IL-6 $^{-/-}$ mice and WT in all lipid fractions examined, there was a trend for IL-6 deficiency to increase the amount of saturated FFA-FA, DG-FA and ceramide-FA species (8%, 12% and 10%, p=0.06 and p=0.07, respectively, *Fig. 3*).

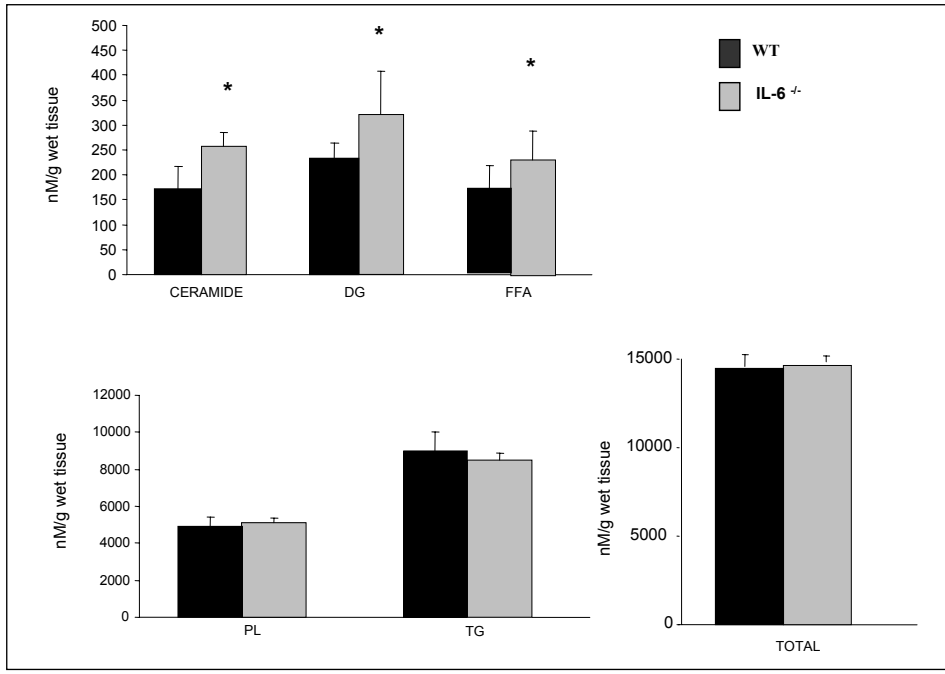

Fig. 2. The effect of IL-6 $^{-/-}$ genotype on the intracellular lipid content in the myocardium. Different lipid pools were extracted from the left ventricle homogenates as described in Materials and Methods. Data are based on 5 independent determinations for each heart (mean ± SEM). DG- diacylglicerols, FFA- free fatty acids, PL - phospholipids, TG - triacylgliceroles, Total - the sum of individual lipid fractions. *P<0.05, IL-6 $^{-/-}$ vs WT

DISCUSSION

The present study revealed the effects of IL-6 deficiency on myocardial a) expression of fatty acid transporters and b) intracellular content of different lipid fractions. To the best of our knowledge, this is the first report presenting the effects of IL-6 $^{-/-}$ deficiency on the myocardial expression of fatty acid transporters. All of examined transporters (FAT/CD36, FABPpm and FATP-1) are expressed in many mammalian tissues, including cardiomyocytes (22,23,29,30). However, based on the present study and others (23,28,31), it appears that, only the myocardial expression of FAT/CD36 is highly regulatable. Notably, it is well recognized that FAT/CD36 plays a major role among fatty acid transport proteins and changes in its expression are highly associated with concomitant alternations in LCFA transport (28,32,33). Recent studies have shown that the increase in sarcolemmal FAT/CD36 expression is the key mechanism promoting the increased rate of LCFA uptake in obesity and type 2 diabetes (16,17,32,33). In humans, an association between FAT/CD36 deficiency and hypertrophic

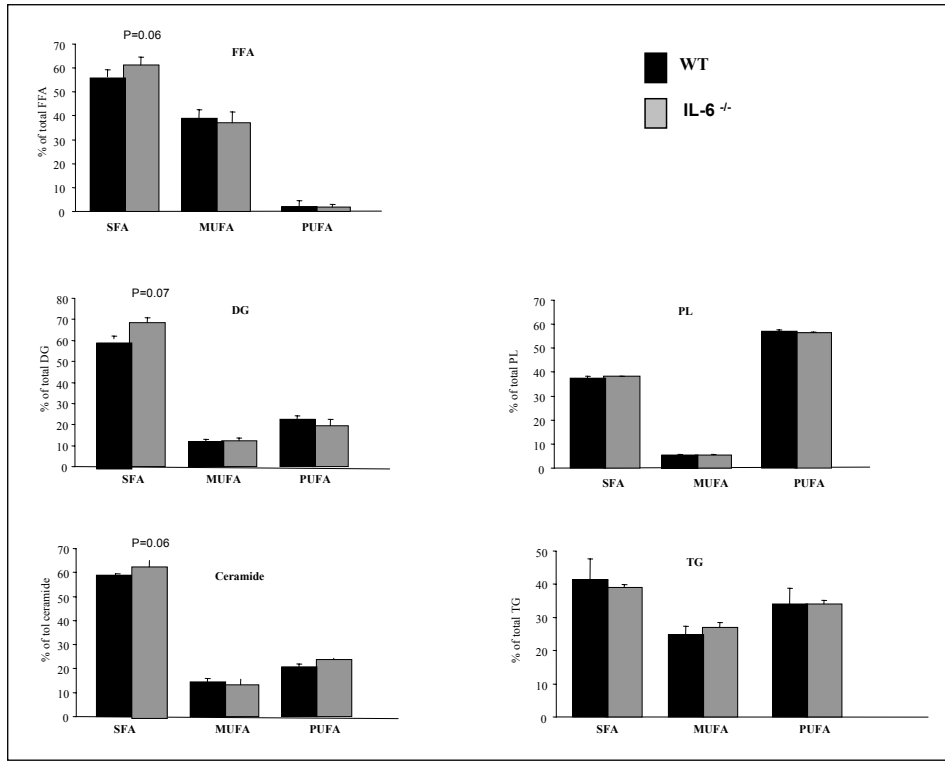

Fig. 3. The effect of IL-6 $^{-/-}$ genotype on the intracellular lipid composition in the myocardium. Different lipid fractions were extracted and the content of their fatty acid residues were summed as SFA-saturated fatty acids, MUFA-monounsaturated fatty acids, PUFA-polyunsaturated fatty acids in all fractions examinated as described in Materials and Methods. Data are based on 5 independent determinations for each muscle (mean ± SEM). FFA- free fatty acids, DG- diacylglicerols, PL - phospholipids, TG - triacylgliceroles.
*P<0.05, IL-6 $^{-/-}$ vs WT

cardiomyopathy has also been reported and linked to impaired uptake of long chain fatty acid by the myocardium (32). Other studies have identified significant defects in myocardial LCFA uptake in CD36-deficient humans (34), although a role for CD36 deficiency in the pathogenesis of alternations in myocardial LCFA metabolism in humans remains to be established.

In marked contrast to myocardial overexpression of FAT/CD36, IL-6 $^{-/-}$ deficiency did not affect the expression of FABPpm and FATP-1 proteins. This may indicate that there are specific responses to IL-6 $^{-/-}$ deficiency (i.e FAT/CD36 *vs* FABPpm and FATP-1). Otherwise, it can be speculated that FABPpm and FATP-1 play a minor role in myocardial LCFA transport, as it has been suggested recently (23,28,31).

An important aspect of our study was to determine whether the IL-6 $^{-/-}$ deficiency, that upregulated myocardial FAT/CD36 expression, also affects

cardiomyocyte lipid content. The obtained results revealed no changes in total myocardial lipid deposits due to lack of changes in the quantitatively major fractions namely, triacylglicerols and phospholipids. Surprisingly at first, as it has been reported that IL-6 exaggerates fatty acid oxidation in isolated soleus muscle (35) and thus, IL-6 deficiency could be expected to promote intracellular lipid accumulation. Several studies have dealt with alternations in fatty acid metabolism in mice lacking IL-6 gene. It has been demonstrated that, IL-6 $^{-/-}$ mice developed maturity onset obesity with disturbed carbohydrate and lipid metabolism (2). In contrast, Di Gregorgio et al. reported that IL-6 $^{-/-}$ mice do not present features of obesity or abnormal lipid metabolism although these mice on HF diet had elevated glucose levels after a GT (36). These discrepancies may be related to observed by van Hall et al. (37) changes in fat metabolism during IL-6 infusion that were probably elicited indirectly by coincidental changes in the content of other hormones such as epinephrine and cortisol rather than by a direct effect of IL-6.

In the present study we reported marked increase in intramyocardial diacylglicerol, free fatty acid and ceramide fractions. This may favor studies presenting IL-6 $^{-/-}$ mice as an animal model correlated with obesity related insulin resistance. In support of this view, there are studies showing plausible mechanistic links between the development of insulin resistance and accumulation of DG and ceramide in muscle without concomitant changes in intramuscular TG stores (38). Others have also pointed to elevated intramyocellular DG levels in a number of animal models of insulin resistance (39-41), while ceramide content was shown to be increased in muscle from obese insulin resistant humans (40,42,43). Recent study demonstrates also that, the changes in composition of DG and ceramide are related to the improvements of insulin sensitivity in obese subjects after endurance training (43). Specifically, in mentioned above study, endurance training reduced both total ceramide content and the content of saturated ceramide species with a trend for training to reduce both the total diacylglcyerol (DG) content and the content of saturated DG-FA species (43). Based on these reports we examined the effect of IL-6 deficiency on fatty acid composition of the myocardial lipid fractions. We found only a tendency in hearts lacking IL-6 gene for accumulation of saturated species in FFA, DG and ceramide fractions. This may be in inverse correlation with insulin resistance in cardiac myocytes, as it has been demonstrated that increase fraction of myocardial polyunsaturated fatty acids (PUFA) content exert the ability to channel fatty acids towards mitochondrial oxidation and thus direct FA away from lipid storage (44,45).

In the present study we have provided several novel observations. Firstly, we have shown in mice lacking IL-6 gene upregulation of the myocardial FAT/CD36 expression and no significant changes in FABPpm and FATP-1. Secondly, we observed that, the increase in FAT/CD36 in heart from IL-6 $^{-/-}$ mice was associated with the increases in the myocardial total content of

diacylglicerol, free fatty acid and ceramide fractions as well as a tendency for accumulation of saturated FA species in these fractions. This lipid accumulation with concomitant trend for increase in saturation status of their fatty acid residues may, at least in part, provide a factor related to the development of intramyocardial lipotoxicity, observed in obese individuals (8-10). However, we also found lack of effects of IL-6 deficiency on myocardial content of triacylglicerol and phospholipid lipid pools.

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Non-alcoholic fatty liver disease (NAFLD), characterized as excess lipid accumulation in the liver which is not due to alcohol use, has emerged as one of the major health problems around the world. The dysregulated lipid metabolism creates a lipotoxic environment which promotes the development of NAFLD, especially the progression from simple steatosis (NAFL) to non-alcoholic steatohepatitis (NASH). Purposeand Aim. This review focuses on the mechanisms of lipid accumulation in the liver, with an emphasis on the metabolic fate of free fatty acids (FFAs) in NAFLD and presents an update on the relevant cellular processes/mechanisms that are involved in lipotoxicity. The biodistribution study obtained by Bridle showed lipid nanoparticles from the vaccine did not stay in the deltoid muscle where they were injected as the vaccine's developers claimed would happen, but circulated throughout the body and accumulated in large concentrations in organs and tissues, including the spleen, bone marrow, liver, adrenal glands and â€" in "quite high concentrations" â€" in the ovaries.Â "If you find lipid nanoparticles in an organ or tissue, that tells you the drug got to that location," Malone explained. According to the data in the Japanese study, lipid nanoparticles were found in the whole blood circulating throughout the body within four hours, and then settled in large concentrations in the ovaries, bone marrow and lymph nodes. This glycerol, along with the fatty acids delivered from the liver, are used to synthesize triglyceride within the adipocyte. By these mechanisms, insulin is involved in further accumulation of triglyceride in fat cells. From a whole body perspective, insulin has a fat-sparing effect.Â Other Notable Effects of Insulin. In addition to insulin's effect on entry of glucose into cells, it also stimulates the uptake of amino acids, again contributing to its overall anabolic effect. When insulin levels are low, as in the fasting state, the balance is pushed toward intracellular protein degradation.Â Type 1 or insulin-dependent diabetes mellitus is the result of a frank deficiency of insulin. The onset of this disease typically is in childhood. In this review, the impact of G6PD deficiency on CVD was critically reconsidered, taking into account the most recent acquisitions on molecular and biochemical mechanisms, namely, antioxidative mechanisms, glutathione recycling, and nitric oxide production, as well as their mutual interactions, which may be impaired by the enzyme defect in the context of the pentose phosphate pathway. Overall, current evidence supports the notion that G6PD downregulation may favor the onset and evolution of atheroma in subjects at risk of CVD.Â The coenzyme is also important for the elongation and desaturation of fatty acids [20Â Consequently, to maintain a reductive environment within myocardial cells during ischemia-reperfusion, it is necessary to counteract myocardial oxidative stress. Mobilization of fatty acids from. triacylglycerols. â€¢ A) During FASTING, during the intensive physical labor and in response to stress condition: release of free FAs from adipose tissue is an adaptation process to provide energy for skeletal and cardiac muscle and also indirectly to the brain via ketone bodies.Â â€“ effects - phenotype â€¢ A decreased flow of acyl CoA derivatives of FAs to mitochondria - lipid accumulation â€“ aching and feeble muscles â€“ heart muscle cell impairment â€¢ Increased utilization of glucose - hypoglykemia. â€“ therapy â€“ high carnitine diet. â€¢ Carnitine palmitoyltransferase I or II deficiency â€“ a phenotype similar to that of carnitine deficiency. Carnitine-mediated transport of activated fatty acids across the inner mitochondrial membrane.